In Vitro Cytotoxicity Test Methods for Estimating Acute Oral Systemic Toxicity

Background Review Document

Volume 2 of 2

Prepared by
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for the Evaluation of Alternative Toxicological Methods (NICEATM)

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LIST OF ACRONYMS AND ABBREVIATIONS

A-CUTE-TOX	A-Cute-Tox Project (EU Research & Development Integrated Project)
ADME	Absorption, distribution, metabolism, and elimination
ANOVA	Analysis of variance
ASTDR	Agency for Toxic Substances and Disease Registry
ASTM	American Society for Testing and Materials
ATC	Acute Toxic Class method
ATCC	American Type Culture Collection
ATWG	Acute Toxicity Working Group
BBB	Blood:brain barrier
BPE	Bovine pituitary extract
BRD	Background Review Document
^{o}C	Degrees Celsius
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CCOHS	Canadian Centre for Occupational Health and Safety (CCOHS)
CDER	U.S. FDA Center for Drug Evaluation and Research
CESARS	Chemical Evaluation Search and Retrieval System
CFU	Colony forming units
CHRIS	Chemical Hazard Response
CI	Confidence interval
CICADS	Concise International Chemical Assessment Documents
CIS	ILO Occupational Safety and Health Information Centre
CNS	Central nervous system
COLIPA	The European Cosmetic Toiletry and Perfumery Association
CPSC	U.S. Consumer Product Safety Commission
CSF	Colony stimulating factor
CTFA	Cosmetic, Toiletries and Fragrance Association
CV	Coefficient of variation
DART®/ETIC	Developmental and Reproductive Toxicology/Environmental Teratology Information Center
DEA	U.S. Drug Enforcement Administration
DHHS	U.S. Department of Health and Human Services
DIMDI	Deutsches Institut fur Medizinische Dokumentation und Information (The German Institute for Medical Documentation and Information)
DNA	Deoxyribose nucleic acid
DMEM	Dulbecco's Modification of Eagle's Medium
DMSO	Dimethyl sulfoxide
D-PBS	Dulbecco's phosphate buffered saline
DOD	U.S. Department of Defense
DOT	U.S. Department of Transportation
EC	European Commission
EC_{50}	Concentration of a substance that produces 50% of the maximum possible response for that substance

ECBC	U.S. Army Edgewood Chemical Biological Center
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EDIT	Evaluation-guided development of new *in vitro* tests
EHC	Environmental Health Criteria
EHS	EPA's Extremely Hazardous Substance list
EPA	U.S. Environmental Protection Agency
ERG	Emergency Response Guidebook
ETOH	Ethanol (Ethyl alcohol)
EU	European Union
EXTONET	The Extension Toxicology Network
FAL	FRAME Alternatives Laboratory
FAO	UN Food and Agriculture Organization
FB1	Fumonisin B1
FDA	U.S. Food and Drug Administration
FDP	Fixed Dose Procedure
FIFRA	U.S. Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register
FRAME	Fund for the Replacement of Animals in Medical Experiments
GABA	Gamma amino butyric acid
GCCP	Good cell culture practices
GHS	Globally Harmonized System (of Classification and Labeling of Chemicals)
GLP	Good Laboratory Practices
gm	Grams
HBSS	Hanks' balanced salt solution
HPV	High Production Volume
hr	Hour(s)
HSDB	Hazardous Substances Data Bank
HSG	Health and Safety Guides
HTD	Highest tolerated dose
IARC	International Agency for Research on Cancer
IC_{20}	Concentration producing 20% inhibition of the endpoint measured
IC_{50}	Concentration producing 50% inhibition of the endpoint measured
IC_{80}	Concentration producing 80% inhibition of the endpoint measured
ICCVAM	Interagency Coordinating Committee for the Validation of Alternative Methods
ICSC	International Chemical Safety Cards
ID	Insufficient data
ID_{50}	Index of cytotoxicity; dose producing a 50% reduction in protein value
IIVS	Institute for *In Vitro* Sciences
ILO	International Labour Organisation
i.m.	Intramuscular
INVITOXX	*In Vitro* Techniques in Toxicology (ERGATT FRAME ECVAM Data bank)

IOM	Institute of Medicine
i.p.	Intraperitoneal
IPCS	International Programme on Chemical Safety
IRAG	Interagency Regulatory Alternatives Group
IRPTC	International Register of Potentially Toxic Chemicals
ISO	International Standards Organization
IUCLID	International Uniform Chemical Information Database
i.v.	Intravenous
JECFA	Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
KBM®	Keratinocyte basal medium
kg	Kilogram
K_{ow}	Octanol-water partition coefficient
L	Liter
LC	Lethal blood concentration
LD_{50}	Dose that produces lethality in 50% of test animals
LDH	Lactate dehydrogenase
MAS	Maximum average Draize score
MEIC	Multicentre Evaluation of *In Vitro* Cytotoxicity
MeSH®	Medical Subject Heading
µL	Microliters
µm	Micrometers
µM	Micromoles
mg	Milligram
MIT	Metabolic inhibition test
mL	Milliliter
mM	Millimolar
MMAS	Modified maximum average score
mmol	Millimoles
MPE	Mean photo effect
MSDS	Material Safety Data Sheets
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
N	Number (of substances)
NA	Not applicable
NADH	Nicotine adenine dinucleotide (reduced)
NC	Not calculated
NCS	Newborn calf serum
NCTR	U.S. FDA National Center for Toxicological Research
n.d.	Not detectable
NHK	Normal human epidermal keratinocytes
NICEATM	National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods
NIEHS	U.S. National Institute of Environmental Health Sciences
NIH	U.S. National Institutes of Health
NIOSH	U.S. National Institute for Occupational Safety and Health
NLM	National Library of Medicine

NR	Neutral red
NRU	Neutral red uptake
NTP	U.S. National Toxicology Program
OAT	Organic anionic transporters
OD	Optical density
OD_{540}	Optical density (absorbance) at a wavelength of 540 nm
OECD	Organisation for Economic Co-operation and Development
OHM/TADS	EPA Oil and Hazardous Materials/Technical Assistance Data System
OPP	U.S. EPA Office of Pesticide Programs
OPPTS	EPA Office of Prevention, Pesticides and Toxic Substances
ORD	U.S. EPA Office of Research and Development
OSHA	U.S. Occupational Safety and Health Administration
OTA	Ochratoxin A
PBS	Phosphate buffered saline
PC	Positive control
PDS	Pesticide Data Sheets
pg	Picogram
PG	Packing group
PIF	Photoinhibition factor
PIMS	Poisons Information Monographs
pK	Acid/base dissociation constant
PLS	Partial Least Squares (analysis)
PPIS	EPA Pesticide Product Information System
PPT	Precipitate
QA	Quality assurance
QC	Quality control
R^2	Coefficient of determination
r_s	Spearman correlation coefficient
RC	Registry of Cytotoxicity
REACH	Registration, evaluation, authorisation and restriction of chemicals
RTECS®	Registry of Toxic Effects of Chemical Substances
RTK NET	The Right-to-Know Network
SD	Standard deviation
SIDS	OECD Screening Information Data Sets
SIS	Scientific Information Service
SLS	Sodium lauryl sulfate
SMT	Study management team
SOP	Standard operating procedure
3T3	BALB/c mouse fibroblasts, clone A31 (ATCC # CCL-163)
TESS	Toxic Exposure Surveillance System
TG	Test guideline
TRI	U.S. EPA Toxics Release Inventory
TSCA	Toxic Substances Control Act
UDP	Up-and-Down Procedure
UN	United Nations

UNEP	United Nations Environment Programme
USP	U.S. Pharmacopoeia
UV	Ultraviolet (light)
VC	Vehicle control
WHO	World Health Organization
XTT	2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
ZEBET	Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch (German Center for Documentation and Evaluation of Alternative Methods to Animal Experiments)

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Appendix A

NICEATM/ECVAM Validation Study Management

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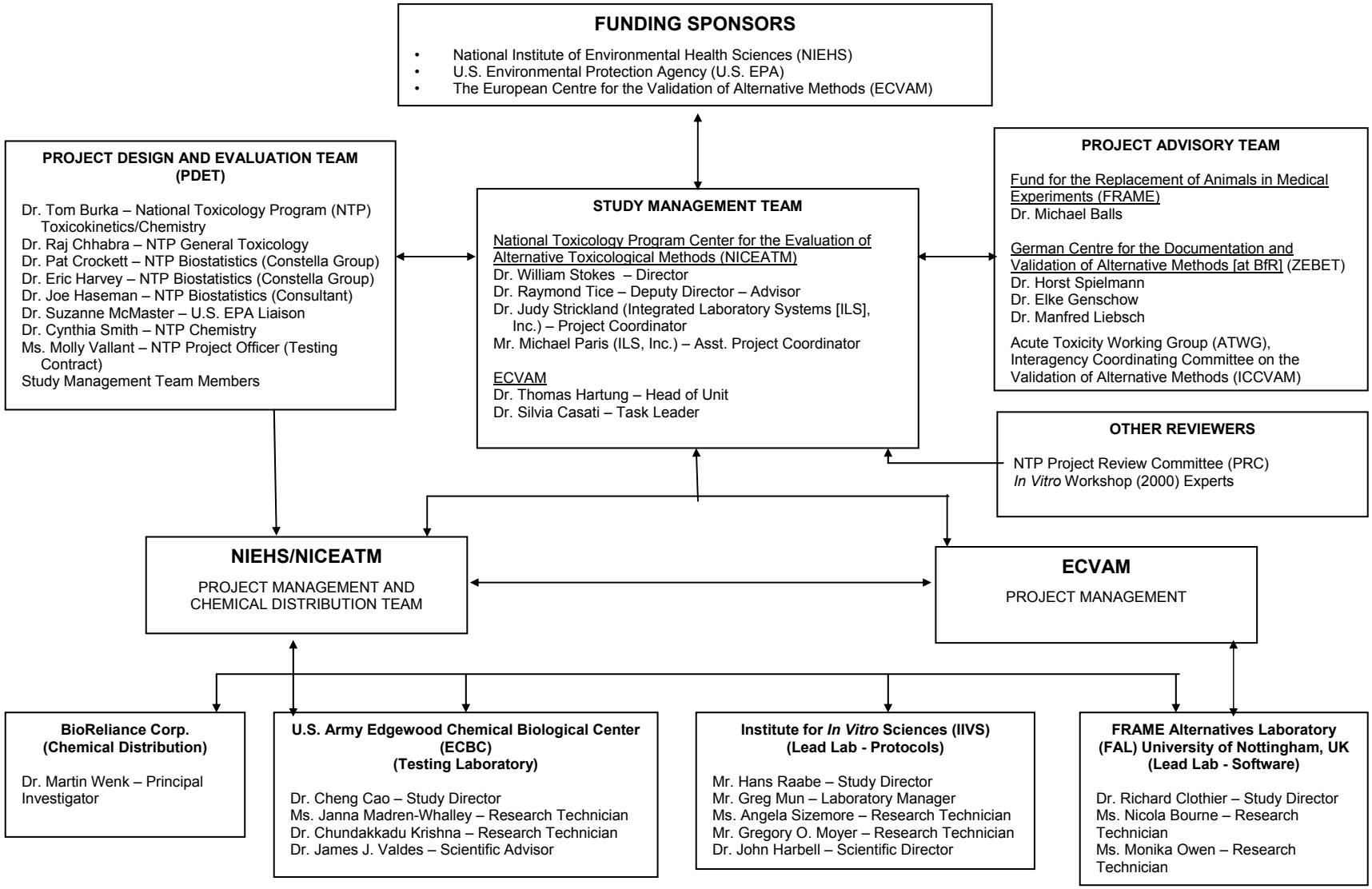
NICEATM/ECVAM Validation Study Management

NICEATM and ECVAM staff managed the study as shown in **Figure A-1**. The NICEATM-ECVAM Study Management Team (SMT), in consultation with the Project Design and Evaluation Team and other advisors shown in **Figure A-1**, designed the study, selected the reference substances (see **Section 3**), and selected the laboratories that would purchase and distribute chemicals and perform solubility and cytotoxicity testing. BioReliance Corporation (Rockville, MD) purchased the reference substances, tested the solubility, and distributed the coded reference substances to the laboratories that performed the cytotoxicity testing. The Institute for *In Vitro* Sciences (IIVS; Gaithersburg, MD), U.S. Army Edgewood Chemical Biological Center (ECBC; Edgewood, MD), and Fund for the Replacement of Animals in Medical Experiments (FRAME) Alternatives Laboratory, University of Nottingham, Queen's Medical Center (FAL; Nottingham, UK) were the participating laboratories that performed the solubility and cytotoxicity testing.

Figure A-1 Study Management Chart



Appendix B

Validation Study Test Method Protocols
(Phase III)

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Appendix B1

Test Method Protocol for the BALB/c 3T3 Neutral Red Uptake (NRU) Cytotoxicity Test

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TEST METHOD PROTOCOL
for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an *In Vitro* Validation Study
Phase III

November 4, 2003

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test
A Test for Basal Cytotoxicity
Phase III

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the BALB/c 3T3 Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and supports the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

The 3T3 NRU test will be performed to analyze the *in vitro* toxicity of 60 blinded/coded test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for the predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals: *Blinded Chemicals (60)*

B. Controls:

Positive:	Sodium Lauryl Sulfate
Vehicle (Negative):	Assay medium (DMEM containing 5% NBCS, 4 mM L-Glutamine, 100 IU/mL Penicillin, 100 µg/mL Streptomycin)
Solvent:	Assay medium, DMSO, or ethanol directed by the Study Management Team, for preparation of test chemicals

IV. TESTING FACILITY AND KEY PERSONNEL

A. Facility Information

1) Name:
2) Address:
3) Study Director:
4) Laboratory Technician(s):
5) Scientific Advisor:
6) Quality Assurance Director:
7) Safety Manager:
8) Facility Management:

B. Test Schedule

1) Proposed Experimental Initiation Date:
2) Proposed Experimental Completion Date:
3) Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{logIC50} - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test chemical preparation, incubator function); all optical density data obtained from the spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

1. Cell Lines

BALB/c 3T3 cells, clone 31
CCL-163, LGC Reference Materials, Customer Service, Queens Road, Teddington, Middlesex, TW110LY, UK
CCL-163, American Type Culture Collection [ATCC], Manassas, VA, USA)

2. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

a) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
b) Laminar flow clean bench/cabinet (standard: "biological hazard")
c) Water bath: 37ºC ± 1ºC
d) Inverse phase contrast microscope
e) Sterile glass tubes with caps (e.g., 5 mL)
f) Centrifuge (optionally: equipped with microtiter plate rotor)
g) Laboratory balance
h) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
i) Shaker for microtiter plates
j) Cell counter or hemocytometer
k) Pipetting aid
l) Pipettes, pipettors (multi-channel and single channel; multichannel repeater pipette), dilution block
m) Cryotubes
n) Tissue culture flasks (e.g., 75 - 80 cm^2, 25 cm^2)
o) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Falcon tissue culture-treated)
p) pH paper (wide and narrow range)
q) Multichannel reagent reservoir
r) Waterbath sonicator
s) Magnetic stirrer
t) Antistatic bar ionizer/antistatic gun (optional for neutralizing static on 96-well plates)
u) Dry heat block (optional)
v) Adhesive film plate sealers (e.g., Excel Scientific SealPlate™,Cat # STR-SEAL-PLT or equivalent)
w) Vortex mixer
x) Filters/filtration devices

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of 3T3 cells. Multi-channel repeater pipettes may be used for plating cells in the 96-well plates, dispensing plate rinse solutions, NR medium, and desorb solution. Do not use the repeater pipette for dispensing test chemicals to the cells.]

3. Chemicals, Media, and Sera

a) Dulbecco's Modification of Eagle's Medium (DMEM) without L-Glutamine; should have high glucose [4.5gm/l] (e.g., ICN-Flow Cat. No. 12-332-54)
b) L-Glutamine 200 mM (e.g., ICN-Flow # 16-801-49)
c) New Born Calf Serum (NBCS or NCS) (e.g., Biochrom # SO 125)
d) 0.05 % Trypsin/0.02 % EDTA solution (e.g., SIGMA T 3924, ICN-Flow, # 16891-49)
e) Phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}(for trypsinization)
f) Hanks' Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+}(CMF-HBSS)
g) Dulbecco's Phosphate Buffered Saline (D-PBS) [formulation containing calcium and magnesium cations; glucose optional] (for rinsing)
h) Penicillin/streptomycin solution (e.g. ICN-Flow # 16-700-49)
i) Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
j) Dimethyl sulfoxide (DMSO), U.S.P. analytical grade (Store under nitrogen @ -20ºC)
k) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
l) Glacial acetic acid, analytical grade
m) Distilled H_2O or any purified water suitable for cell culture and NR desorb solution (sterile)
n) Sterile/non-sterile paper towels (for blotting 96-well plates)

[Note: Due to lot variability of NBCS/NCS, first check a lot for growth stimulating properties with 3T3 cells (approximately 20-24 h doubling time) and then reserve a sufficient amount of NBCS/NCS. May use pre-tested serum lot from Phases Ia, Ib, and II of the validation study if the serum has been stored under appropriate conditions and shelf-life has not expired.]

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard). All methods and procedures will be adequately documented.]

1. Media

DMEM (buffered with sodium bicarbonate) supplemented with (final concentrations in DMEM are quoted):

a) for freezing (Freeze Medium); contains 2X concentration of NBCS/NCS and DMSO of final freezing solution

40 %	NBCS/NCS
20 %	DMSO

b) for routine culture (Routine Culture Medium)

10 %	NBCS/NCS
4 mM	Glutamine

c) for test chemical dilution (Chemical Dilution Medium)

4 mM	Glutamine
200 IU/mL	Penicillin
200 µg/mL	Streptomycin

d) for dilution of NR stock solution (NR Dilution Medium)

5 %	NBCS/NCS
4 mM	Glutamine
100 IU/mL	Penicillin
100 µg/mL	Streptomycin

[Note: The Chemical Dilution Medium with test chemical will dilute the serum concentration of the Routine Culture Medium in the test plate to 5 %. Serum proteins may mask the toxicity of the test substance, but serum cannot be totally excluded because cell growth is markedly reduced in its absence.]

Completed media formulations should be kept at approximately 2-8° C and stored for no longer than two weeks.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay (e.g., SIGMA #N2889, 3.3 mg/mL). Store liquid tissue culture-grade NR Stock Solution at the storage conditions and shelf-life period recommended by the manufacturer.

If the liquid form is not available, the following formulation can be prepared.

EXAMPLE: 0.25 g NR Dye powder in 100 mL H_2O

The NR Stock Solution (powder in water) should be stored in the dark at room temperature for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

0.758 mL (3.3 mg NR dye/mL solution)	NR Stock Solution
99.242 mL	NR Dilution Medium (pre-warmed to 37° C)

The final concentration of the NR Medium is **25 µg NR dye/mL** and aliquots will be prepared on the day of application.

[Note: The NR Medium shall be filtered (e.g., Millipore filtering, 0.2 – 0.45 μm pore size) to reduce NR crystals. Aliquots of the NR Medium should be maintained at 37° C (e.g., in a waterbath) before adding to the cells and used within 30 min of preparation but also used within 15 min after removing from 37° C storage.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

BALB/c 3T3 cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 75 - 80 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells should be examined on a daily (i.e., on workdays) basis under a phase contrast microscope, and any changes in morphology or their adhesive properties noted in a Study Workbook.

2. Receipt of Cryopreserved BALB/c 3T3 Cells

Upon receipt of cryopreserved BALB/c 3T3 cells, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells

Thaw cells by putting ampules into a water bath at 37°C ± 1ºC. Leave for as brief a time as possible.

a) Resuspend the cells in pre-warmed Routine Culture Medium and transfer into pre-warmed Routine Culture Medium in a tissue-culture flask.

b) Incubate at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air.

c) When the cells have attached to the bottom of the flask (within 4 to 24 h), decant the supernatant and replace with fresh pre-warmed (37ºC) medium. Culture as described above.

d) Passage at least two times before using the cells in a cytotoxicity test.

A fresh batch of frozen cells from the stock lot of cells should be thawed out and cultured approximately every two months. This period resembles a sequence of about 18 passages.

4. Routine Culture of BALB/C 3T3 Cells

When cells exceed 50 % confluence (but less than 80 % confluent) they should be removed from the flask by trypsinization:

a) Decant medium, briefly rinse cultures with 5 mL PBS or Hanks' BSS (without Ca^{2+}, Mg^{2+}) per 25 cm^2 flask (15 mL per 75 cm^2 flask). Wash cells by gentle agitation to remove any remaining serum that might inhibit the action of the trypsin.

b) Discard the washing solution. Repeat the rinsing procedure and discard the washing solution.

c) Add 1-2 mL trypsin-EDTA solution per 25 cm^2 to the monolayer for a few seconds (e.g., 15-30 seconds).

d) Remove excess trypsin-EDTA solution and incubate the cells at room temperature.

e) After 2-3 minutes (min), lightly tap the flask to detach the cells into a single cell suspension.

5. Cell Counting

After detaching the cells, add 0.1-0.2 mL of pre-warmed (37ºC) Routine Culture Medium/cm^2 to the flask (e.g., 2.5 mL for a 25 cm^2 flask). Disperse the monolayer by gentle trituration. It is important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension obtained using a hemocytometer or cell counter (e.g., Coulter counter).

6. Subculture of Cells

After determination of cell number, the culture can be sub-cultured into other flasks or seeded into 96-well microtiter plates. BALB/c 3T3 cells are routinely passaged at suggested cell densities as listed in the table (approximate doubling time is 20-24 h). The individual laboratories will need to determine and adjust the final density to achieve appropriate growth.

Table 1. Cell Density Guidelines for Subculturing

Days in Culture	Seeding Density (cells/cm^2)	Total Cells per 25 cm^2 flask	Total Cells per 75 cm^2 flask
2	16800	4.2×10^5	1.26×10^6
3	8400	2.1×10^5	6.3×10^5
4	4200	1.05×10^5	3.15×10^5

[Note: It is important that cells have overcome the lag growth phase when they are used for the test.]

7. Freezing Cells (procedure required only if current stock of cells is depleted)

Stocks of BALB/c 3T3 cells can be stored in sterile, freezing tubes in a liquid nitrogen freezer. DMSO is used as a cryoprotective agent.

a) Centrifuge trypsinized cells at approximately 200 x g.

b) Suspend the cells in cold Routine Culture Medium (half the final freezing volume) so a final concentration of 1-5x10^6 cells/mL can be attained.

c) Slowly add cold Freeze Medium to the cells so that the solvent will equilibrate across the cell membranes. Bring the cell suspension to the final freezing volume. The final cell suspension will be 10 % DMSO. Aliquot the cell suspension into freezing tubes and fill to 1.8 mL.

d) Place the tubes into an insulated container (e.g., styrofoam trays) and place in a freezer (-70 to -80°C) for 24 h. This gives a freezing rate of approximately 1°C/min. The laboratory needs to ensure that the freezing protocol is applicable to the 3T3 cells and that the cells are viable when removed from cryopreservation.

e) Place the frozen tubes into liquid nitrogen for storage.

8. Preparation of Cells for Assays

a) Cultured cells that are going to be used in seeding the 96-well plates should be fed fresh medium the day before subculturing to the plates. On the day of plate seeding, prepare a cell suspension of 2.0 – 3.0x10^4 cells/mL in Routine Culture Medium. Using a multi-channel pipette, dispense 100 µl Routine Culture Medium only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate (See **Section VII.F.1**). In the remaining wells, dispense 100 µl of a cell suspension of 2.0 – 3.0x10^4 cells/mL (= 2.0 – 3.0x10^3 cells/well). The seeding density should be noted to ensure that the cells in the control wells are not overgrown after three days (i.e., 24 h incubation in step **b** and 48 h exposure to test chemicals). Prepare one plate per chemical to be tested.

b) Incubate cells for 24 ± 2 h (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air) so that cells form a less than half (< 50%) confluent monolayer. This incubation period assures cell recovery and adherence and progression to exponential growth phase.

c) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

9. Determination of Doubling Time

a) A cell doubling time procedure was performed on the initial lot of cells that was used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined in Phase III if there is a change in the lot of cells used. Establish cells in culture and trypsinize cells as per **Section VII.C.4** for subculture. Resuspend cells in NR Dilution Medium (5 % NBCS/NCS). Seed cells at 4200 cells/cm^2.

b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).

c) After 4 - 6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue; Nigrosin) if Study Director sees a need. Use appropriate size exclusion limits if using a Coulter counter. Determine the total number of cells and document. Repeat sampling at 24 h, 48 h, 72 h, and 96 h post inoculation. Change culture medium at 72 h or sooner in remaining dishes if indicated by pH drop.

d) Plot cell concentration (per mL of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Preparation of Test Chemicals

The Study Management Team will provide direction on the solvent to be used for each test chemical. [Note: Preparation under red or yellow light is recommended to preserve chemicals that degrade upon exposure to light.]

1. Test Chemicals in Solution

a) Allow test chemicals to equilibrate to room temperature before dissolving and diluting.

b) Prepare test chemical immediately prior to use. Test chemical solutions should not be prepared in bulk for use in subsequent tests. Ideally, the solutions must not be cloudy nor have noticeable precipitate. Each stock dilution should have at least 1-2 mL total volume to ensure adequate solution for the test wells in a single 96-well plate. The SMT may direct the Study Director to store an aliquot (e.g., 1 mL) of the highest 2X stock solution (e.g., low solubility chemicals) in a freezer (e.g., -70°C) for use in future chemical analyses.

c) For chemicals dissolved in DMSO or ethanol, the final DMSO or ethanol concentration for application to the cells must be 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations.

d) The stock solution for each test chemical should be prepared at the highest concentration found to be soluble in the solubility test conducted per the *Test Method Protocol for Solubility Determination*. Thus, the highest test concentration applied to the cells in each range finding experiment is:
   - 0.5 times the highest concentration found to be soluble in the solubility test, if the chemical was soluble in Chemical Dilution Medium, or

- 1/200 the highest concentration found to be soluble in the solubility test if the chemical was soluble in ethanol or DMSO.

e) The seven lower concentrations in the range finding experiment would then be prepared by successive dilutions that decrease by one log unit each. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in Chemical Dilution Medium before application to 3T3 cells.

Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme

If DMSO was determined to be the preferred solvent at Tier 2 of the solubility test (i.e., 200,000 μg/mL), dissolve the chemical in DMSO at 200,000 μg/mL for the chemical stock solution.

1) Label eight tubes 1 – 8. Add 0.9 mL solvent (e.g., DMSO) to tubes 2 -- 8.

2) Prepare stock solution of 200,000 μg test chemical/mL solvent in tube # 1.

3) Add 0.1 mL of 200,000 μg/mL dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 20,000 μg/mL).

4) Add 0.1 mL of 20,000 μg/mL dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 2,000 μg/mL)

5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.

6) Since each concentration is 200 fold greater than the concentration to be tested, make a 1:100 dilution by diluting 1 part dissolved chemical in each tube with 99 parts of Chemical Dilution Medium (e.g., 0.1 mL test chemical in DMSO + 9.9 mL Chemical Dilution Medium) to derive the eight 2X concentrations for application to 3T3 cells. Each 2X test chemical concentration will then contain 1 % v/v solvent. The 3T3 cells will have 0.05 mL Routine Culture Medium in the wells prior to application of the test chemical. By adding 0.05 mL of the appropriate 2X test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 1,000 μg/mL) in a total of 0.1 mL and the solvent concentration in the wells will be 0.5% v/v.

7) A test article prepared in Chemical Dilution Medium, DMSO, or ethanol may precipitate upon transfer into the Routine Culture Medium. The 2X dosing solutions should be evaluated for precipitates and the results recorded in the workbook. It will be permissible to test all of the dosing solutions in the dose range finding assay and main experiments. However, doses containing test article precipitates should be avoided and generally will not be used in the ICx determinations for the definitive tests. Precipitates in 2X dosing solutions are permissible for range finder tests but not for definitive tests.

Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Prior to or immediately after application of the test chemical to the 96-well plate, measure the pH of the highest 2X dosing concentration of the test chemical (i.e., C1 in the test plate, see Figure 1) in culture medium. Use pH paper (e.g., pH 0 - 14 to estimate and pH 5 – 10 to determine more precise value; or Study Director's discretion) for measurements. The pH paper should be in contact with the solution for approximately one minute. Document the pH and note the color of the 2X concentration medium (i.e., in the EXCEL® template). Medium color for all dosing dilutions should be noted in the workbooks. Do not adjust the pH.

3. Concentrations of Test Chemical

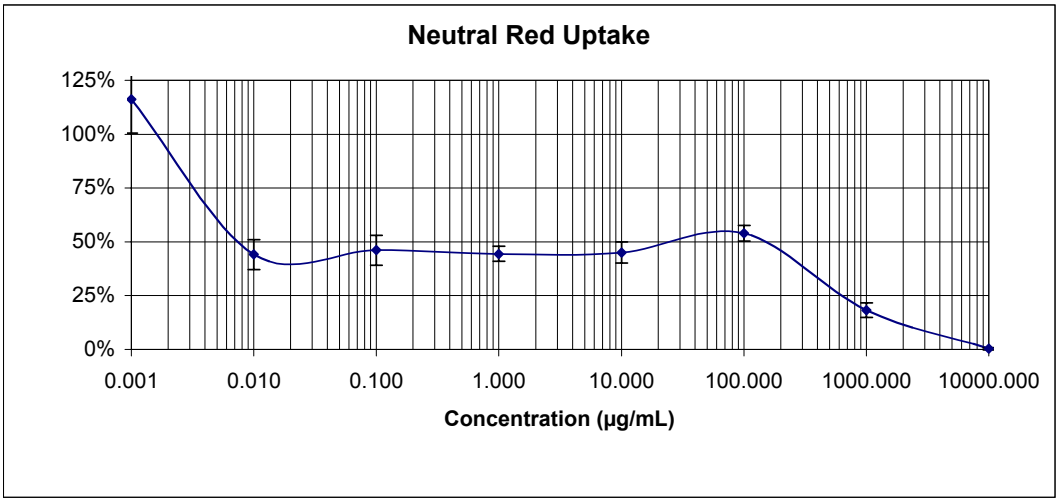
a) Range Finder Experiment

Test eight concentrations of the test chemical by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

If a range finder experiment does not generate enough cytotoxicity, then higher doses should be attempted. If cytotoxicity is limited by solubility, then more stringent solubility procedures to increase the stock concentration (to the maximum concentration specified in Section VII.D.3.b.) should be employed. Place the test chemical concentration into an incubator (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air) and stir or rock for up to 3 hours, if necessary, to facilitate dissolution. For stocks prepared in medium, vessel caps should be loose to allow for CO_2 exchange. Proceed with dosing solution preparation and dosing.

- If a range finding test produces a biphasic curve, then the doses selected for the subsequent main experiments should cover the most toxic dose-response range (see Example 1 – the most toxic range is 0.001 – 0.1 µg/mL).

Example 1 – Biphasic Curve



b) Main Experiment

[Note: After the range finding assay is completed, the definitive concentration-response experiment shall be performed three times on three different days for each chemical (i.e., one plate per day per chemical.]

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller (e.g., dilution factor of $^{6}\sqrt{10} = 1.47$). Cover the relevant concentration range around the IC_{50} (> 0 % and < 100 % effect) preferably with several points of a graded effect, but with a minimum of two points, one on each side of the estimated IC_{50} value, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than one cytotoxic concentration on each side of the IC_{50} value shall be repeated, where possible, with a smaller dilution factor (see **Section VII.E.5.a.4**). Each experiment should have at least one cytotoxicity value > 0 % and ≤ 50.0 % viability and at least one cytotoxicity value > 50.0 % and < 100 % viability. A progression factor of 1.21 [$^{12}\sqrt{10}$] is regarded the smallest factor achievable and will be the lowest dosing interval required.)

Determine which test chemical concentration is closest to the IC_{50} value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

Maximum Doses to be Tested in the Main Experiments
If minimal or no cytotoxicity was measured in the dose range finding assay, a maximum dose for the main experiments will be established as follows:

- For test chemicals prepared in Chemical Dilution Medium, the highest test article concentration that may be applied to the cells in the main experiments will be either 100 mg/mL, or the maximum soluble dose. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of Chemical Dilution Medium will be added to the vessel so that the concentration is 200,000 µg/mL (200 mg/mL). The solution is mixed using the mechanical procedures that produced solubility when performing the solubility test specified in *Test Method Protocol for Solubility Determination*. If complete solubility is achieved in medium, then 7 additional serial stock dosing solutions may be prepared from the 200 mg/mL 2X stock. If the test chemical is insoluble in medium at 200 mg/ml, proceed by adding medium, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mechanical procedures specified in *Test Method Protocol for Solubility Determination*. More stringent solubility procedures may be employed if needed based on results from the range finder experiment (**Section VII.D.3.a.**). The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- For test chemicals prepared in either DMSO or ethanol, the highest test article concentration that may be applied to the cells in the main experiments will be either 2.5 mg/mL, or less, depending upon the maximum solubility in solvent. Weigh the test chemical into a glass tube and document the weight. Add the appropriate solvent (determined from the original solubility test) to the vessel so

that the concentration is 500,000 μg/mL (500 mg/mL). Mix the solution using the sequence of mechanical procedures specified in *Test Method Protocol for Solubility Determination*. If complete solubility is achieved in the solvent, then 7 additional serial stock dosing solutions may be prepared from the 500 mg/mL 200X stock. If the test chemical is insoluble in solvent at 500 mg/ml, proceed by adding solvent, in small incremental amounts, to attempt to dissolve the chemical by again using the sequence of mixing procedures. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- If precipitates are observed in the 2X dilutions, continue with the experiment, make the appropriate observations and documentation, and report data to the SMT.

c) Test Chemical Dilutions

The dosing factor of 3.16 ($= \sqrt[2]{10}$) divides a log into two equidistant steps, a factor of 2.15 ($= \sqrt[3]{10}$) divides a decade into three steps. The factor of 1.47 ($= \sqrt[6]{10}$) divides a log into six equidistant steps, the factor of 1.78 ($\sqrt[4]{10}$) divides a log into four equidistant steps, and the factor of 1.21 ($= \sqrt[12]{10}$) divides the log into 12 steps.

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

E. Test Procedure

1. 96-Well Plate Configuration

The 3T3 NRU assay for test chemicals will use the 96-well plate configuration as shown in **Figure 1**.

Figure 1. 96-Well Plate Configuration for Positive Control (PC) and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb
B	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
C	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
D	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
E	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
F	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
G	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
H	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb

VC1 and VC2 = VEHICLE CONTROL
$C_1 – C_8$ = Test Chemicals or PC (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (Test chemical or PC, but contain **no** cells)
VCb = VEHICLE CONTROL BLANK (contain **no** cells)

2. Application of Test Chemical

a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized.

1) The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs; or Corning/Transtar model 4878 disposable reservoir liners, 8-channel; or other multichannel reservoirs).

2) The second method utilizes a "dummy" plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate (with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the plate containing cells. More volume than needed for the test plate (i.e. greater than 50 µl/well) should be in the wells of the dummy plate.

At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing.

Do not use a multichannel repeater pipette for dispensing test chemical to the plates.

b) After 24 h ± 2 h incubation of the cells, remove Routine Culture Medium from the cells by careful inversion of the plate (i.e., "dump") over an appropriate receptacle. Gently blot the plate on a sterile paper towel so that the monolayer is minimally disrupted. Do not use automatic plate washers for this procedure nor vacuum aspiration.

c) Immediately add 50 µL of fresh pre-warmed Routine Culture Medium to all of the wells, including the blanks. Fifty microliters (50 µL) of dosing solution will be rapidly transferred from the 8-channel reservoir (or dummy plate) to the appropriate wells of the test plate using a single delivery multi-channel pipettor. For example, the VC may be transferred first (into columns 1, 2, 11, and 12), followed by the test article dosing solutions from lowest to highest dose, so that the same pipette tips on the multi-channel pipettor can be used for the whole plate. [The Vehicle Control blank (VCb) wells (column 1, column 12, wells A2, A11, H2, H11) will receive the Vehicle Control dosing solutions (which should include any solvents used). Blanks for wells A3 – A10 and H3 – H10 shall receive the appropriate test chemical solutions for each concentration (e.g., wells A3 and H3 receive C_1 solution).

d) Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).

e) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in the development of the positive control database in Phase I of the Validation Study. If multiple sets of test chemical plates are set up, then clearly designate the positive control plates for each set; each set will be an individual entity. The Study Director will decide how many test chemical plates will be run with a positive control plate. The mean IC_{50} ± two and a half standard deviations (SD) for the SLS acceptable tests from Phases Ia, Ib, and II (after the removal of outliers) are the values that will be used as an acceptance criterion for test sensitivity for the 3T3 NRU assay. This plate will follow the same schedule and procedures as used for the test chemical plates (including appropriate chemical concentrations in the appropriate wells and meeting test acceptance criteria – see **sections VII.E.1, E.2, and E.5**).

3. Microscopic Evaluation

After at least 46 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions. Numerical scoring of the cells (see **Section VII.E.3**) should be determined and documented in the Study Workbook and in the appropriate section of Addendum II of the EXCEL® study template.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

a) Carefully remove (i.e., "dump") the medium with test chemical and rinse the cells very carefully with 250 µL pre-warmed D-PBS. Remove the rinsing solution by dumping and remove excess by gently blotting on paper towels. Add 250 µL NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3±0.1 h. Observe the cells briefly during the NR incubation (e.g., between 2 and 3 h – Study Director's discretion) for NR crystal formation. Record observations in the Study Workbook. Study Director can decide to reject the experiment if excessive NR crystallization has occurred.

b) After incubation, remove the NR medium, and carefully rinse cells with 250 µl pre-warmed D-PBS.

c) Decant and blot D-PBS from the plate.

d) Add exactly 100 µl NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.

e) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution. Plates should be protected from light by using a cover during shaking.

f) Plates should be still for at least five minutes after removal from the plate shaker (or orbital mixer). If any bubbles are observed, assure that they have been ruptured prior to reading the plate. Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader (spectrophotometer), using the blanks as a reference. [Note: Phases Ia and Ib data show the mean OD value for the plate blanks to be 0.057 ± 0.043 for 3T3 cells (± 2.5 standard deviations; data from 3 labs; N = 189). Use this range as a **guide** for assessment of the blank values.] Save raw data in the Excel format as provided by the SMT.

5. Quality Check of 3T3 NRU Assay

a) Test Acceptance Criteria

All acceptance criteria (i.e., criteria 1, 2, and 3) must be met for a test to be acceptable.

1) The PC (SLS) IC_{50} must be within ± two and a half (2.5) standard deviations of the historical mean established by the Test Facility (as per **VII.E.2.e**), and must meet criteria 2 and 3, and must have an r^2 (coefficient of determination) value calculated for the Hill model fit (i.e., from PRISM® software) ≥ 0.85.

2) The left and right mean of the VCs do not differ by more than 15% from the mean of all VCs.

3) At least one calculated cytotoxicity value > 0 % and ≤ 50.0 % viability and at least one calculated cytotoxicity value > 50.0 % and < 100 % viability must be present.

Exception: If a test has only one point between 0 and 100 % **and** the smallest dilution factor (i.e., 1.21) was used **and** all other test acceptance criteria were met, then the test will be considered acceptable.

Stopping Rule for Insoluble Chemicals: If the most rigorous solubility procedures have been performed and the assay cannot achieve adequate toxicity to meet the test acceptance criteria after three definitive trials, then the Study Director may end all testing for that particular chemical.

[Note: A corrected mean $OD_{540 \pm 10nm}$ of 0.103 - 0.813 for the VCs is a target range but will not be a test acceptance criterion. Range determined from Phase Ib VC OD values from 3 laboratories (mean ± 2.5 standard deviations, N = 98).]

b) Checks for Systematic Cell Seeding Errors

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay. If volatility is suspected, then proceed to **Section VII.E.6**.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

6. Volatility of Test Chemicals

Highly volatile test chemicals may generate vapors from the treatment medium during the test chemical treatment incubation period. These vapors may become resorbed into the treatment medium in adjacent wells, such that culture wells nearest the highest doses may become contaminated by exposure to resorbed test article vapors. If the test chemical is particularly toxic at the doses tested, the cross contamination may be evident as a

significant reduction in viability in the vehicle control cultures (i.e., VC1) adjacent to the highest test chemical doses.

If potential test article volatility is suspected (e.g., for low density liquids) or if the initial range finder test (non-sealed plate) results show evidence of toxic effects in the control cultures (i.e., > 15 % difference in viability between VC1 [column 2] and VC2 [column 11]), then seal the subsequent test plates by the following procedure.

a) Plate Sealer Method

1) Plates and chemicals will be prepared as usual according to **Sections VII.D and VII.E**.
2) Immediately after the 96-well culture plate has been treated with the suspected volatile chemical (**Section VII.E.2.b**), apply the adhesive plate sealer (e.g., using a hand, microplate roller, etc.) directly over the culture wells. Assure that the sealer adheres to each culture well (well tops should be dry). Place the 96-well plate cover over the sealed plate and incubate the plate under specified conditions (**Section VII.E.2.b**). [Note: Do not jam the plate lid over the film to avoid deforming the sealer and causing the sealer to detach from culture wells. Loose fit of the plate lid is acceptable.]
3) At the end of the treatment period, the plate sealer should be carefully removed to avoid spillage. Continue with the NRU assay as per **Section VII.E.4**.

F. Data Analysis

The Study Director will use good biological/scientific judgment for determining "unusable" wells that will be excluded from the data analysis and provide explanations for the removal of any data from the analysis.

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicate well) per test concentration (blanks will be subtracted). This value is compared with the mean NRU of all VC values. Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet template provided by the SMT. The template will automatically determine cell viability, IC_{50} values by linear interpolation, and perform statistical analyses (including statistical identification of outliers). The template will also calculate the concentrations associated with 20 %, 50 %, and 80 % viability using the Hill slope and EC_{50} (i.e., IC_{50}) from the Hill function analysis.

The Hill function analysis shall be performed using statistical software (e.g., GraphPad PRISM® 3.0) and a template specified by the SMT to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical.

The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the SMT through the designated contacts in electronic format and hard copy upon completion of testing. The SMT will be directly responsible for the statistical analyses of the Validation Study data.

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IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**
(Print or type name)

_____ _____

Test Facility STUDY DIRECTOR **DATE**
(Print or type name)

Appendix B2

Test Method Protocol for the Normal Human Epidermal Keratinocyte (NHK) Neutral Red Uptake (NRU) Cytotoxicity Test

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TEST METHOD PROTOCOL
for the NHK Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an In Vitro Validation Study
Phase III

November 4, 2003

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The Normal Human Keratinocyte (NHK) Neutral Red Uptake Cytotoxicity Test A Test for Basal Cytotoxicity Phase III

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the Normal Human Keratinocyte (NHK) Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and is in support of the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. NHK Neutral Red Uptake Cytotoxicity Test

The NHK NRU test will be performed to analyze the *in vitro* toxicity of 60 blinded/coded test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for the predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals: *Blinded chemicals (60)*

B. Controls:

Positive:	Sodium Lauryl Sulfate
Vehicle (Negative):	Assay medium
Solvent (as directed):	Assay medium, DMSO, or ethanol as directed by the Study Management Team, for preparation of test chemicals

IV. TESTING FACILITY AND KEY PERSONNEL

A. Facility Information

1) Name:
2) Address:
3) Study Director:
4) Laboratory Technician(s):
5) Scientific Advisor:
6) Quality Assurance Director:
7) Safety Manager:
8) Facility Management:

B. Test Schedule

1) Proposed Experimental Initiation Date:
2) Proposed Experimental Completion Date:
3) Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A.. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{logIC50} - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test chemical preparation, incubator function); all optical density data obtained from the

spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used unless otherwise noted.]

1. Cell Lines

Normal Human Epidermal Keratinocytes (NHK)

Non-transformed cells; from cryopreserved primary or secondary cells (**Clonetics #CC-2507 or equivalent).** Cells will be Clonetics NHK cells.

Cambrex [Cambrex Bio Science, 8830 Biggs Ford Road, Walkersville, MD 21793-0127

Cambrex Europe [Cambrex Bio Science Verviers, S.P.R.L. Parc Industriel de Petit Rechain, B-4800 Verviers, BELGIUM]

2. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

a) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
b) Laminar flow clean bench (standard: "biological hazard")
c) Water bath: 37ºC ± 1ºC
d) Inverse phase contrast microscope
e) Sterile glass tubes with caps (e.g., 5mL)
f) Centrifuge (optionally: equipped with microtiter plate rotor)
g) Laboratory balance
h) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
i) Shaker for microtiter plates
j) Cell counter or hemocytometer
k) Pipetting aid
l) Pipettes, pipettors (multi-channel and single channel; multichannel repeater pipette), dilution block
m) Cryotubes
n) Tissue culture flasks (75 - 80 cm^2, 25 cm^2)
o) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Corning/COSTAR tissue culture-treated)
p) pH paper (wide and narrow range)
q) Multichannel reagent reservoir
r) Waterbath sonicator
s) Magnetic stirrer

t) Antistatic bar ionizer/antistatic gun (optional for neutralizing static on 96-well plates)
u) Dry heat block (optional)
v) Adhesive film plate sealers (e.g., Excel Scientific SealPlate™,Cat # STR-SEAL-PLT or equivalent)
w) Vortex mixer
x) Filters/filtration devices

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of NHK. Multi-channel repeater pipettes may be used for plating cells in the 96-well plates, dispensing plate rinse solutions, NR medium, and desorb solution. Do not use the repeater pipette for dispensing test chemicals to the cells.]

3. Chemicals, Media, and Sera

a) Keratinocyte Basal Medium without Ca^{++} (KBM®, Clonetics CC-3104) that is completed by adding the KBM® SingleQuots® (Clonetics CC-4131) to achieve the proper concentrations of epidermal growth factor, insulin, hydrocortisone, antimicrobial agents, bovine pituitary extract, and calcium (e.g., Clonetics Calcium SingleQuots®, 300 mM $CaCl_2$, Clonetics # CC-4202).
b) HEPES Buffered Saline Solution (HEPES-BSS) (e.g., Clonetics # CC-5022)
c) 0.025 % Trypsin/EDTA solution (e.g., Clonetics # CC-5012)
d) Trypsin Neutralizing Solution (TNS) (e.g., Clonetics # CC-5002)
e) Phosphate Buffered Saline (PBS)
f) Dulbecco's Phosphate Buffered Saline (D-PBS) [formulation containing calcium and magnesium cations; glucose optional] (for rinsing)
g) Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
h) Dimethyl sulfoxide (DMSO), U.S.P analytical grade (Store under nitrogen @ -20ºC)
i) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
j) Glacial acetic acid, analytical grade
k) Hanks' Balanced Salt Solution without Ca^{2+} or Mg^{2+} (CMF-HBSS) (e.g., Invitrogen # 14170)
l) Distilled H_2O or any purified water suitable for cell culture and NR desorb solution (sterile)
m) Sterile/non-sterile paper towels (for blotting 96-well plates)

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard). All methods and procedures will be adequately documented.]

1. Media

a) Routine Culture Medium/Treatment Medium

KBM® (Clonetics CC-3104) supplemented with KBM® SingleQuots® (Clonetics CC-4131) and Clonetics Calcium SingleQuots® (CC-4202) to make 500 mL medium. Final concentration of supplements in medium are:

0.0001 ng/mL	Human recombinant epidermal growth factor
5 μg/mL	Insulin
0.5 μg/mL	Hydrocortisone
30 μg/mL	Gentamicin
15 ng/mL	Amphotericin B
0.10 mM	Calcium
30 μg/mL	Bovine pituitary extract

Complete media should be kept at 2-8°C and stored for no longer than two weeks.

NOTE:
KBM® SingleQuots® contain the following stock concentrations and volumes:

0.1 ng/mL	hEGF	0.5 mL
5.0 mg/mL	Insulin	0.5 mL
0.5 mg/mL	Hydrocortisone	0.5 mL
30 mg/mL	Gentamicin, 15 ug/mL Amphotericin-B	0.5 mL
7.5 mg/mL	Bovine Pituitary Extract (BPE)	2.0 mL

Clonetics Calcium SingleQuots® are 2 mL of 300mM calcium.

165 μl of solution per 500 mL calcium-free medium equals 0.10 mM calcium in the medium.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay (e.g., SIGMA #N2889, 3.3 mg/mL). Store liquid tissue culture-grade NR Stock Solution at the storage conditions and shelf-life period recommended by the manufacturer.

If the liquid form is not available, the following formulation can be prepared.

EXAMPLE: 0.33 g NR Dye powder in 100 mL H_2O

The NR Stock Solution (powder in water) should be stored in the dark at room temperature for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

1.0 mL (3.3 mg NR dye/mL)	NR Stock Solution
99.0 mL	Routine Culture Medium (pre-warmed to 37° C.)

The final concentration of the NR Medium is **33 µg NR dye/mL** and aliquots will be prepared on the day of application.

[Note: The NR Medium shall be filtered (e.g., Millipore filtering, 0.2 – 0.45 µm pore size) used to reduce NR crystals. Aliquots of the NR Medium should be maintained at 37° C (e.g., in a waterbath) before adding to the cells and used within 30 min of preparation but also used within 15 min after removing from 37° C storage.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

NHK cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 25 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells should be examined on a daily (i.e., on workdays) basis under a phase contrast microscope, and any changes in morphology or their adhesive properties must be noted in a Study Workbook.

2. Receipt of Cryopreserved Keratinocytes

Upon receipt of cryopreserved keratinocytes, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells and Establishing Cell Cultures

a) Thaw cells by putting ampules into a water bath at 37°C for as brief a time as possible. Do not thaw cells at room temperature or by hand. Seed the thawed cells into culture flasks as quickly as possible and with minimal handling.

b) Slowly (taking approximately 1-2 min) add 9 mL of pre-warmed Routine Culture Medium to the cells suspended in the cryoprotective solution and transfer cells into flasks containing pre-warmed Routine Culture Medium (See Table 1).

c) Incubate the cultures at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air until the cells attach to the flask (within 4 to 24 h), at which time the Routine Culture Medium should be removed and replaced with fresh Routine Culture Medium.

d) Unless otherwise specified, the cells should be incubated at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air and fed every 2-3 days until they exceed 50 % confluence (but less than 80 % confluent).

Table 1. Guidelines for Establishing Cell Cultures

Cells/25 cm^2 flask (in approximately 5 mL) 1 flask each cell concentration	6.25×10^4 ($2500/cm^2$)	1.25×10^5 ($5000/cm^2$)	2.25×10^5 ($9000/cm^2$)
Approximate Time to Subculture	96+ hours	72 - 96 hours	48 - 72 hours
Cells to 96-Well Plates	6 – 8 plates	6 – 8 plates	6 – 8 plates

Cell growth guidelines – actual growth of individual cell lots may vary.

4. Subculture of NHK Cells to 96-Well Plates

[Note: It is important that cells have overcome the lag growth phase when they are used for the test. Keratinocytes will be passaged only into the 96-well plates and will not be subcultured into flasks for use in later assays]

a) When the keratinocyte culture in a 25 cm^2 flask exceeds 50 % confluence (but less than 80 % confluent), remove the medium and rinse the culture twice with 5 mL HEPES-BSS. The first rinse may be left on the cells for up to 5 minutes and the second rinse should remain on the cells for approximately 5 minutes. Discard the washing solutions.

b) Add 2 mL trypsin/EDTA solution to each flask and remove after 15 to 30 seconds. Incubate the flask at room temperature for 3 to 7 min. When more than 50 % of the cells become dislodged, rap the flask sharply against the palm of the hand.

c) When most of the cells have become detached from the surface, rinse the flask with 5 mL of room temperature TNS. If more than one flask is subcultured, the same 5 mL of TNS may be used to rinse a total of up to two flasks.

d) Then rinse the flask with 5 mL CMF-HBSS and transfer the cell suspension to a centrifuge tube.

e) Pellet the cells by centrifugation for 5 min at approximately 220 x g. Remove the supernatant by aspiration.

f) Resuspend the keratinocyte pellet by gentle trituration (to have single cells) in Routine Culture Medium. It is important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension using a hemocytometer or cell counter.

g) Prepare a cell suspension –1.6 – 2.0 $x10^4$ cells/mL in Routine Culture Medium. Using a multi-channel pipette, dispense 125 µl Routine Culture Medium only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate. In the remaining wells, dispense 125 µl of the cell suspension ($2x10^3 – 2.5x10^3$ cells/well). Prepare one plate per chemical to be tested (see **Figure 1**, **Section VII.E.1**).

h) Incubate cells (37ºC ± 1ºC, 90 % ± 5.0 % humidity, and 5 % ± 1 % CO_2/air) so that cells form a 20+ % monolayer (~48-72 h). This incubation period assures cell recovery and adherence and progression to exponential growth phase.

i) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

5. Determination of Doubling Time

a) A cell doubling time procedure was performed on the initial lot of cells that was used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined in Phase III if there is a change in the lot of cells used. Establish cells in culture and trypsinize cells as per **Section VII.C.4** for subculture. Resuspend cells in appropriate culture medium. Use **Table 1** to determine seeding densities.

b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).

c) After 4-6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue; Nigrosin). Determine the total number of cells and document. Repeat sampling at 24 hr, 48 hr, 72 hr, and 96 hr post inoculation. Change culture medium at 72 hr or sooner in remaining dishes if indicated by pH drop.

d) Plot cell concentration (per mL of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. The doubling time will be in the log (exponential) phase of the growth curve. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Preparation of Test Chemicals

The Study Management Team will provide direction on the solvent to be used for each test chemical. [Note: Preparation under red or yellow light is recommended to preserve chemicals that degrade upon exposure to light.]

1. Test Chemical in Solution

a) Allow test chemicals to equilibrate to room temperature before dissolving and diluting.

b) Prepare test chemical immediately prior to use. Test chemical solutions should not be prepared in bulk for use in subsequent tests. Ideally, the solutions must not be cloudy nor have noticeable precipitate. Each stock dilution should have at least 1-2 mL total volume to ensure adequate solution for the test wells in a single 96-well plate. The SMT may direct the Study Director to store an aliquot (e.g., 1 mL) of the

highest 2X stock solution (e.g., low solubility chemicals) in a freezer (e.g., -70°C) for use in future chemical analyses.

c) For chemicals dissolved in DMSO or ethanol, the final DMSO or ethanol concentration for application to the cells must be 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations.

d) The stock solution for each test chemical should be prepared at the highest concentration found to be soluble in the solubility test (*Test Method Protocol for Solubility Determination*). Thus, the highest test concentration applied to the cells in each range finding experiment is:
   - 0.5 times the highest concentration found to be soluble in the solubility test, if the chemical was soluble in medium, or
   - 1/200 the highest concentration found to be soluble in the solubility test if the chemical was soluble in ethanol or DMSO.

e) The seven lower concentrations in the range finding experiment would then be prepared by successive dilutions that decrease by one log unit each. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in medium before application to NHK cells.

   Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme

   If DMSO was determined to be the preferred solvent at Tier 2 of the solubility test (i.e., 200,000 μg/mL), dissolve the chemical in DMSO at 200,000 μg/mL for the chemical stock solution.

   1) Label eight tubes 1 – 8. Add 0.9 mL solvent (e.g., DMSO) to tubes 2 -- 8.

   2) Prepare stock solution of 200,000 μg test chemical/mL solvent in tube # 1.

   3) Add 0.1 mL of 200,000 μg/mL dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 20,000 μg/mL).

   4) Add 0.1 mL of 20,000 μg/mL dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 2,000 μg/mL)

   5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.

   6) Since each concentration is 200 fold greater than the concentration to be tested, make a 1:100 dilution by diluting 1 part dissolved chemical in each tube with 99 parts of culture medium (e.g., 0.1 mL of test chemical in DMSO + 9.9 mL culture medium) to derive the eight 2X concentrations for application to NHK cells. Each 2X test chemical concentration will then contain 1 % v/v solvent. The NHK cells will have 0.125 mL of culture medium in the wells prior to application of the test chemical. By adding 0.125 mL of the appropriate 2X test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 1,000 μg/mL) in a total of 0.250 mL and the solvent concentration in the wells will be 0.5% v/v.

7) A test article prepared in DMSO or ethanol may precipitate upon transfer into the Routine Culture Medium. The 2X dosing solutions should be evaluated for precipitates and the results recorded in the workbook. It will be permissible to test all of the dosing solutions in the dose range finding assay and main experiments. However, doses containing test article precipitates should be avoided and generally will not be used in the ICx determinations for the definitive tests. Precipitates in 2X dosing solutions are permissible for range finder tests but not for definitive tests.

Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Prior to or immediately after application of the test chemical to the 96-well plate, measure the pH of the highest 2X dosing concentration of the test chemical (i.e., C1 in the test plate, see Figure 1) in culture medium. Use pH paper (e.g., pH 0 – 14 to estimate and pH 5 – 10 to determine more precise value; or Study Director's discretion). The pH paper should be in contact with the solution for approximately one minute. Document the pH and note the color of the 2X concentration medium (i.e., in the EXCEL® template). Medium color for all dosing dilutions should be noted in the workbooks. Do not adjust the pH.

3. Concentrations of Test Chemical

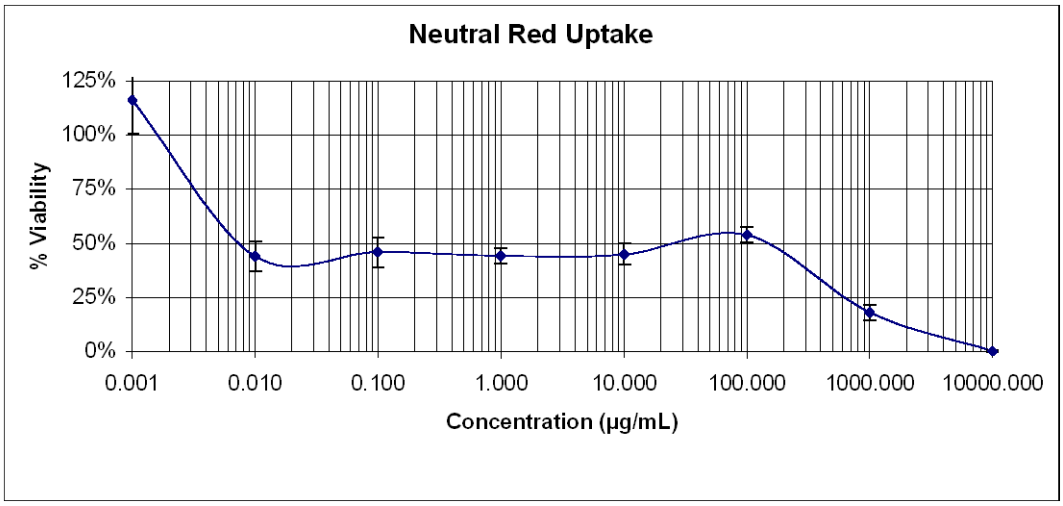
a) Range Finder Experiment

Test eight concentrations of the test chemical by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

If a range finder experiment does not generate enough cytotoxicity, then higher doses should be attempted. If cytotoxicity is limited by solubility, then more stringent solubility procedures to increase the stock concentration (to the maximum concentration specified in Section VII.D.3.b.) should be employed. Place the highest test chemical concentration into an incubator (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air) and stir or rock for up to 3 hours, if necessary, to facilitate dissolution. For stocks prepared in medium, vessel caps should be loose to allow for CO_2 exchange. Proceed with dosing solution preparation and dosing.

- If a range finding test produces a biphasic curve, then the doses selected for the subsequent main experiments should cover the most toxic dose-response range (see Example 1 – the most toxic range is 0.001 – 0.1 μg/mL).

Example 1 – Biphasic Curve



b) Main Experiment

[Note: After the range finding assay is completed, the definitive concentration-response experiment shall be performed three times on three different days for each chemical (i.e., one plate per day per chemical).]

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller (e.g., dilution factor of $^{6}\sqrt{10} = 1.47$). Cover the relevant concentration range around the IC_{50} (> 0 % and < 100 % effect) preferably with several points of a graded effect, but with a minimum of two points, one on each side of the estimated IC_{50} value, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than one cytotoxic concentration on each side of the IC_{50} value shall be repeated, where possible, with a smaller dilution factor (see **Section VII.E.5.a.4**). Each experiment should have at least one cytotoxicity value > 0 % and ≤ 50.0 % viability and at least one cytotoxicity value > 50.0 % and < 100 % viability. A progression factor of 1.21 [$^{12}\sqrt{10}$] is regarded the smallest factor achievable and will be the lowest dosing interval required.)

Determine which test chemical concentration is closest to the IC_{50} value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

Maximum Doses to be Tested in the Main Experiments
If minimal or no cytotoxicity was measured in the dose range finding assay, a maximum dose for the main experiments will be established as follows:

- For test chemicals prepared in Routine Culture Medium, the highest test article concentration that may be applied to the cells in the main experiments will be either 100 mg/mL, or the maximum soluble dose. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of Routine

Culture Medium will be added to the vessel so that the concentration is 200,000 μg/mL (200 mg/mL). The solution is mixed using the mechanical procedures specified in *Test Method Protocol for Solubility Determination*. If complete solubility is achieved in medium, then 7 additional serial stock dosing solutions may be prepared from the 200 mg/mL 2X stock. If the test chemical is insoluble in medium at 200 mg/ml, proceed by adding medium, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in *Test Method Protocol for Solubility Determination*. More stringent solubility procedures may be employed if needed based on results from the range finder experiment (**Section VII.D.3.a.**). The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- For test chemicals prepared in either DMSO or ethanol, the highest test article concentration that may be applied to the cells in the main experiments will be either 2.5 mg/mL, or less, depending upon the maximum solubility in solvent. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of the appropriate solvent (determined from the original solubility test) will be added to the vessel so that the concentration is 500,000 μg/mL (500 mg/mL). The solution is mixed as specified in *Test Method Protocol for Solubility Determination*. If complete solubility is achieved in the solvent, then 7 additional serial stock dosing solutions may be prepared from the 500 mg/mL 200X stock. If the test chemical is insoluble in solvent at 500 mg/ml, proceed by adding solvent, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- If precipitates are observed in the 2X dilutions, continue with the experiment, make the appropriate observations and documentation, and report data to the SMT.

c) Test Chemical Dilutions

The dosing factor of 3.16 ($= \sqrt[2]{10}$) divides a log into two equidistant steps, a factor of 2.15 ($= \sqrt[3]{10}$) divides a decade into three steps. The factor of 1.47 ($= \sqrt[6]{10}$) divides a log into six equidistant steps, the factor of 1.78 ($\sqrt[4]{10}$) divides a log into four equidistant steps, and the factor of 1.21 ($= \sqrt[12]{10}$) divides the log into 12 steps.

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

E. Test Procedure

1. 96-Well Plate Configuration

The NHK NRU assay for test chemicals will use the 96-well plate configuration shown in **Figure 1**.

Figure 1. 96-Well Plate Configuration for Positive Control (PC) and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb
B	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
C	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
D	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
E	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
F	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
G	VCb	VC1	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC2	VCb
H	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb

VC1 and VC2	=	VEHICLE CONTROL
$C_1 - C_8$	=	Test Chemicals or PC (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b	=	BLANKS (Test chemical or PC, but contain **no** cells)
VCb	=	VEHICLE CONTROL BLANK (contain **no** cells)

2. Application of Test Chemical

a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized.

1) The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs or Corning/Transtar model 4878 disposable reservoir liners, 8-channel; or other multichannel reservoirs).

2) The second method utilizes a "dummy" plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate

(with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the plate containing cells. More volume than needed for the test plate (i.e. greater than 125 µl/well) should be in the wells of the dummy plate.

At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing. Do not use a multichannel repeater pipette for dispensing test chemical to the plates.

b) After 48 - 72 h (i.e., after cells attain 20+ % confluency [see **Section VII.C.4(h)**]) incubation of the cells, add 125 µl of the appropriate concentration of test chemical, the PC, or the VC (see **Figure 1** for the plate configuration) directly to the test wells. Do not remove Routine Culture Medium for re-feeding the cells. The dosing solutions will be rapidly transferred from the 8-channel reservoir (or dummy plate) to the test plate using a single delivery multi-channel pipettor. For example, the VC may be transferred first (into columns 1, 2, 11, and 12), followed by the test article dosing solutions from lowest to highest dose, so that the same pipette tips on the multi-channel pipettor can be used for the whole plate. [The Vehicle Control blank (VCb) wells (column 1, column 12, wells A2, A11, H2, H11) will receive the Vehicle Control dosing solutions (which should include any solvents used). Blanks for wells A3 – A10 and H3 – H10 shall receive the appropriate test chemical solution for each concentration (e.g., wells A3 and H3 receive C_1 solution).] Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).

c) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in the development of the positive control database in Phase I of the Validation Study. If multiple sets of test chemical plates are set up, then clearly designate the positive control plates for each set; each set will be an individual entity. The Study Director will decide how many test chemical plates will be run with a positive control plate. The mean IC_{50} ± two and a half standard deviations (SD) for the SLS acceptable tests from Phases Ia, Ib, and II (after the removal of outliers) are the values that will be used as an acceptance criterion for test sensitivity for the NHK NRU assay. This plate will follow the same schedule and procedures as used for the test chemical plates (including appropriate chemical concentrations in the appropriate wells and meeting test acceptance criteria see **Sections VII.E.1, E.2, and E.5**).

3. Microscopic Evaluation

After at least 46 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions. Numerical scoring of the cells (see **Section**

VII.E.3) should be determined and documented in the Study Workbook and in the appropriate section of Addendum II of the EXCEL® study template.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

a) Carefully remove (i.e., "dump") the Routine Culture Medium (with test chemical) and rinse the cells very carefully with 250 µL pre-warmed D-PBS. Remove the rinsing solution by dumping and remove excess by gently blotting on paper towels. Add 250 µL NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3±0.1 h. Observe the cells briefly during the NR incubation (e.g., between 2 and 3 h – Study Director's discretion) for NR crystal formation. Record observations in the Study Workbook. Study Director can decide to reject the experiment if excessive NR crystallization has occurred.

b) After incubation, remove the NR medium, and carefully rinse cells with 250 µL pre-warmed D-PBS.

c) Decant and blot D-PBS from the plate. (Optionally: centrifuge the reversed plate.)

d) Add exactly 100 µL NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.

e) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution. Plates should be protected from light by using a cover during shaking.

f) Plates should be still for at least five minutes after removal from the plate shaker (or orbital mixer). If any bubbles are observed, assure that they have been ruptured prior to reading the plate. Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader (spectrophotometer), using the blanks as a reference. [Phases Ia and Ib data show the mean OD value for the plate blanks to be 0.055 ± 0.035 for NHK cells (± 2.5 standard deviations; data from 3 labs; N = 156). Use this range as a **guide** for assessment of the blank values.] Save raw data in the Excel format as provided by the SMT.

5. Quality Check of Assay

a) Test Acceptance Criteria

All acceptance criteria (i.e., criteria 1, 2, and 3) must be met for a test to be acceptable.

1) The PC (SLS) IC_{50} must be within two and a half (2.5) standard deviations of the historical mean established by the Test Facility (as per **VII.E.2.c**), and must meet criteria 2 and 3, and must have an r^2 (coefficient of determination) value calculated for the Hill model fit (i.e., from PRISM® software) ≥ 0.85.

2) The left and the right mean of the VCs do not differ by more than 15 % from the mean of all VCs.

3) At least one calculated cytotoxicity value > 0 % and ≤ 50.0 % viability and at least one calculated cytotoxicity value > 50.0 % and < 100 % viability must be present.

Exception: If a test has only one point between 0 and 100 % **and** the smallest dilution factor (i.e., 1.21) was used **and** all other test acceptance criteria were met, then the test will be considered acceptable.

Stopping Rule for Insoluble Chemicals: If the most rigorous solubility procedures have been performed and the assay cannot achieve adequate toxicity to meet the test acceptance criteria after three definitive trials, then the Study Director may end all testing for that particular chemical.

[Note: A corrected mean $OD_{540 \pm 10nm}$ of 0.205 - 1.645 for the VCs is a target range but will not be a test acceptance criterion. Range determined from Phase Ib VC OD values from 3 laboratories (mean ± 2.5 standard deviations, N = 69).]

b) Checks for Systematic Cell Seeding Errors

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay. If volatility is suspected, then proceed to **Section VII.E.6**.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

6. Volatility of Test Chemicals

Highly volatile test chemicals may generate vapors from the treatment media during the test chemical treatment incubation period. These vapors may become resorbed into the treatment medium in adjacent wells, such that culture wells nearest the highest doses may become contaminated by exposure to resorbed test article vapors. If the test chemical is

particularly toxic at the doses tested, the cross contamination may be evident as a significant reduction in viability in the vehicle control cultures (i.e., VC1) adjacent to the highest test chemical doses.

If potential test article volatility is suspected (e.g., for low density liquids) or if the initial range finder test (non-sealed plate) results show evidence of toxic effects in the control cultures (i.e., > 15 % difference in viability between VC1 [column 2] and VC2 [column 11]), then seal the subsequent test plates by the following procedure.

a) Plate Sealer Method

1) Plates and chemicals will be prepared as usual according to **Sections VII.D and VII.E**.
2) Immediately after the 96-well culture plate has been treated with the suspected volatile chemical (**Section VII.E.2.b**), apply the adhesive plate sealer (e.g., using a hand, microplate roller, etc.) directly over the culture wells. Assure that the sealer adheres to each culture well (well tops should be dry). Place the 96-well plate cover over the sealed plate and incubate the plate under specified conditions (**Section VII.E.2.b**). [Note: Do not jam the plate lid over the film to avoid deforming the sealer and causing the sealer to detach from culture wells. Loose fit of the plate lid is acceptable.]
3) At the end of the treatment period, the plate sealer should be carefully removed to avoid spillage. Continue with the NRU assay as per **Section VII.E.4**.

F. Data Analysis

The Study Director will use good biological/scientific judgment for determining "unusable" wells that will be excluded from the data analysis and provide explanations for the removal of any data from the analysis.

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicates wells) per test concentration. This value is compared with the mean NRU of all VC values. Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet template provided by the SMT. The template will automatically determine cell viability, IC_{50} values by linear interpolation, and perform statistical analyses (including statistical identification of outliers). The template will also calculate the concentrations associated with 20 %, 50 %, and 80 % viability using the Hill slope and EC_{50} (i.e., IC_{50}) from the Hill function analysis.

The Hill function analysis shall be performed using statistical software (e.g., GraphPad PRISM® 3.0) and a template specified by the SMT to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical.

The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the SMT through the designated contacts in electronic format and hard copy upon completion of testing. The SMT will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

Clonetics Normal Human Keratinocyte Systems Instructions for Use, AA-1000-4-Rev.03/00. (http://www.clonetics.com).

Hackenberg, U. and H. Bartling. 1959. Messen und Rechnen im pharmakologischen Laboratorium mit einem speziellen Zahlensystem (WL24-System). Arch. Exp. Pathol. Pharmakol. 235: 437-463.

Triglia, D., P.T. Wegener, J. Harbell, K. Wallace, D. Matheson, and C. Shopsis. 1989. Interlaboratory validation study of the keratinocyte neutral red bioassay from Clonetics Corporation. In *Alternative Methods in Toxicology*, Volume 7. A.M. Goldberg, ed., pp. 357-365. Mary Ann Liebert, Inc., New York.

Test Method Protocol for Solubility Determination. In Vitro Cytotoxicity Validation Study. Phase III. August 29, 2003. Prepared by The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**

(Print or type name)

_____ _____

Testing Facility STUDY DIRECTOR **DATE**

(Print or type name)

Appendix B3

Test Method Protocol for Solubility Determination (Phase III)

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TEST METHOD PROTOCOL
for Solubility Determination

In Vitro Cytotoxicity Validation Study
Phase III

September 24, 2003

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

Solubility Determination
Phase III

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the BALB/c 3T3 Neutral Red Uptake (NRU) and normal human keratinocyte (NHK) cytotoxicity tests. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing solubility determinations for the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the solubility testing.

A. Solubility Test

The solubility tests will be performed to determine the best solvent to use for each of the 60 blinded/coded test chemicals to be tested in the 3T3 and NHK NRU cytotoxicity tests

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST SUBSTANCES AND SOLVENTS

A. Test Chemicals: *60 Coded Chemicals (60)*

B. Solvents: Chemical Dilution Medium for 3T3 assay (See **Section VII.B.1**)
Treatment Medium for NHK assay (See **Section VII.B.2**)

IV. TESTING FACILITY AND KEY PERSONNEL

A. Facility Information

1) Name:
2) Address:
3) Study Director:
4) Laboratory Technician(s):
5) Scientific Advisor:
6) Quality Assurance Director:

7) Safety Manager:
8) Facility Management:

B. Test Schedule

1) Proposed Experimental Initiation Date:
2) Proposed Experimental Completion Date:
3) Proposed Report Date:

V. TEST SYSTEM

The solubility test procedure is based on attempting to dissolve chemicals in various solvents with a increasingly rigorous mechanical techniques. The solvents to be used, in the order of preference, are cell culture media, DMSO, and ethanol. Solubility shall be determined in a step-wise procedure that involves attempting to dissolve a test chemical in the solvents (in the order of preference) at relatively high concentrations using the sequence of mechanical procedures (**Section VII.C.2.a**). If the chemical does not dissolve, the volume of solvent is increased so as to decrease the concentration by a factor of 10, and then the sequence of mechanical procedures are repeated in an attempt to solubilize the chemical at the lower concentrations.

Determination of whether a chemical has dissolved is based entirely on visual observation. A chemical has dissolved if the solution is clear and shows no signs of cloudiness or precipitation.

VI. DEFINITIONS

A. *Soluble:* Chemical exists in a clear solution without visible cloudiness or precipitate.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, solubility testing, laboratory balance calibration); solubility reports will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

1. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

a) Water bath: 37ºC ± 1ºC
b) Glass tubes with caps (e.g., 5 mL)
c) Laboratory balance
d) Pipetting aid
e) Pipettes, pipettors (multi-channel and single channel; multichannel repeater pipette), dilution block
f) Waterbath sonicator
g) Dry heat block (optional)

2. Chemicals, Media, and Sera

a) Dulbecco's Modification of Eagle's Medium (DMEM) without L-Glutamine; should have high glucose [4.5gm/l] (e.g., ICN-Flow Cat. No. 12-332-54)
b) L-Glutamine 200 mM (e.g., ICN-Flow # 16-801-49)
c) Penicillin/streptomycin solution (e.g. ICN-Flow # 16-700-49)
d) Dimethyl sulfoxide (DMSO), U.S.P. analytical grade (Store under nitrogen @ -20ºC)
e) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
f) Keratinocyte Basal Medium without Ca^{++} (KBM®, Clonetics CC-3104) that is completed by adding the KBM® SingleQuots® (Clonetics CC-4131) to achieve the proper concentrations of epidermal growth factor, insulin, hydrocortisone, antimicrobial agents, bovine pituitary extract, and calcium (e.g., Clonetics Calcium SingleQuots®, 300 mM $CaCl_2$, Clonetics # CC-4202).

B. Preparations of Media and Solutions

[Note: All solutions glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard). All methods and procedures will be adequately documented. Completed media formulations should be kept at approximately 2-8° C and stored for no longer than two weeks.]

1. 3T3 Chemical Dilution Medium

DMEM (buffered with sodium bicarbonate) supplemented with (final concentrations in DMEM are quoted):

4 mM	Glutamine
200 IU/mL	Penicillin
200 μg/mL	Streptomycin

2. NHK Treatment Medium

KBM® (Clonetics CC-3104) supplemented with KBM® SingleQuots® (Clonetics CC-4131) and Clonetics Calcium SingleQuots® (CC-4202) to make 500 mL medium. Final concentration of supplements in medium are:

0.0001 ng/mL	Human recombinant epidermal growth factor
5 μg/mL	Insulin
0.5 μg/mL	Hydrocortisone
30 μg/mL	Gentamicin
15 ng/mL	Amphotericin B
0.10 mM	Calcium
30 μg/mL	Bovine pituitary extract

NOTE:
KBM® SingleQuots® contain the following stock concentrations and volumes:

0.1 ng/mL	hEGF	0.5 mL
5.0 mg/mL	Insulin	0.5 mL
0.5 mg/mL	Hydrocortisone	0.5 mL
30 mg/mL	Gentamicin, 15 ug/mL Amphotericin-B	0.5 mL
7.5 mg/mL	Bovine Pituitary Extract (BPE)	2.0 mL

Clonetics Calcium SingleQuots® are 2 mL of 300 mM calcium.

165 µl of solution per 500 mL calcium-free medium equals 0.10 mM calcium in the medium.

C. Determination of Solubility

The preference of solvent for dissolving test chemicals is medium, DMSO, and then ethanol. Solubility shall be determined in a step-wise procedure that involves attempting to dissolve a test chemical at a relatively high concentration with the sequence of mechanical procedures specified in **Section VII.C.2.a.** If the chemical does not dissolve, the volume of solvent is increased so as to decrease the concentration by a factor of 10, and then the sequence of mechanical procedures in **Section VII.C.2.a** are repeated in an attempt to solubilize the chemical at the lower concentrations. For testing solubility in medium, the starting concentration is 20,000 µg/ml (i.e., 20 mg/mL) in Tier 1, but for DMSO and ethanol the starting concentration is 200,000 µg/ml (i.e., 200 mg/mL) in Tier 2. Weighing out chemical for each solvent (i.e., medium, DMSO, ethanol) can be done all at once, if convenient, but solubility testing (at each tier that calls for more than one solvent) is designed to be sequential - medium, then DMSO, then ethanol – in accordance with the solvent hierarchy (see **Figure 1**). This allows for testing to stop, rather than continue testing with less preferred solvents, if the test chemical dissolves in a more preferred solvent. For example, if a chemical is soluble in medium at a particular tier, testing may stop. Likewise, if a chemical is soluble in DMSO at any tier, testing need not continue with ethanol. However, since the issue of primary importance is testing the solvents and concentrations of test chemical required by any one tier, sequential testing of solvents may be abandoned if the lab can test more efficiently in another way.

1. Method

a) Tier 1 begins with testing 20 mg/mL each in Chemical Dilution Medium and Treatment Medium (see **Table 1**). For each medium, weigh approximately 10 mg (10,000 µg) of the test chemical into glass tubes. Document the chemical weight. Add approximately 0.5 mL of each medium into its respective tube so that the concentration is 20,000 µg/ml (20 mg/mL). Mix the solution as specified in **Section VII.C.2.a.** If complete solubility is achieved in each medium, then additional solubility procedures are not needed.

b) If the test chemical is insoluble in either Chemical Dilution Medium or Treatment Medium, proceed to Tier 2 by adding enough medium, approximately 4.5 mL, to attempt to dissolve the chemical at 2 mg/mL by using the sequence of mixing procedures specified in **Section VII.C.2.a**. If the test chemical dissolves in medium at 2 mg/mL, no further procedures are necessary. If the test chemical does NOT dissolve in one medium or the other (if both are tested in this tier), weigh out approximately 100 mg test chemical in a second glass tube and add enough DMSO to

make the total volume approximately 0.5 mL (for 200 mg/mL) and attempt to dissolve the chemical as specified in **Section VII.C.2.a**. If the test chemical does not dissolve in DMSO, weigh out approximately 100 mg test chemical in another glass tube and add enough ethanol to make the total volume approximately 0.5 mL (for 200 mg/mL) and attempt to dissolve the chemical as specified in **Section VII.C.2.a**. If the chemical is soluble in either solvent, no additional solubility procedures are needed.

c) If the chemical is NOT soluble in one or both media, DMSO, or ethanol at Tier 2, then continue to Tier 3 in Table 1 by adding enough solvent to increase the volume of the three (or four) Tier 2 solutions by 10 and attempt to solubilize again using the sequence of mixing procedures in **Section VII.C.2.a.** If the test chemical dissolves, no additional solubility procedures are necessary. If the test chemical does NOT dissolve, continue with Tier 4 and, if necessary, Tier 5 using DMSO and ethanol. Tier 4 begins by diluting the Tier 3 samples with DMSO or ethanol to bring the total volume to 50 mL. The mixing procedures in **Section VII.C.2.a** are again followed to attempt to solubilize the chemical. Tier 5 is performed, if necessary, by weighing out another two samples of test chemical at ~10 mg each and adding ~50 mL DMSO or ethanol for a 200 μg/mL solution, and following the mixing procedures in **Section VII.C.2.a**.

Example: If complete solubility is not achieved at 20,000 μg/mL in either Chemical Dilution Medium or Treatment Medium at Tier 1 using the mixing procedures specified in **Section VII.C.2.a,** then the procedure continues to Tier 2 by diluting the solution to 5 mL (with either of the appropriate media) and mixing again as specified in **Section VII.C.2.a.** If the chemical is not soluble in Chemical Dilution Medium or Treatment Medium, two samples of ~ 100 mg test chemical are weighed to attempt to solubilize in DMSO and ethanol at 200,000 μg/mL (i.e., 200 mg/mL). Solutions are mixed following the sequence of procedures prescribed in **Section VII.C.2.a** in an attempt to dissolve. If solubility is not achieved at Tier 2, then the solutions (Chemical Dilution Medium and/or Treatment Medium, DMSO, and ethanol) prepared in Tier 2 are diluted by 10 so as to test 200 μg/mL in media, and 20,000 μg/mL in DMSO and ethanol. This advances the procedure to Tier 3. Solutions are again mixed as prescribed in **Section VII.C.2.a** in an attempt to dissolve. If solubility is not achieved in Tier 3, the procedure continues to Tier 4, and to 5 if necessary (see **Figure 1** and **Table 1**).

Table 1. Determination of Solubility in Chemical Dilution Medium, Treatment Medium, DMSO, or Ethanol

TIER	1	2	3	4	5
Total Volume Chemical Dilution Medium/Treatment Medium	0.5 mL	5 mL	50 mL		
Concentration of Test Chemical (Add ~10 mg to a tube. Add enough medium to equal the first volume. Dilute to subsequent volumes if necessary.)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	200 µg/mL (0.20 mg/mL)		
Total Volume DMSO/Ethanol		0.5 mL	5 mL	50 mL	
Concentration of Test Chemical (Add ~100 mg to a large tube. Add enough DMSO or ethanol to equal the first volume. Dilute with subsequent volumes if necessary.)		200,000 µg/mL (200 mg/mL)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	
Total Volume DMSO/Ethanol					50 mL
Concentration of Test Chemical (Add ~10 mg to a large tube. Add enough DMSO or ethanol to equal 50 mL.)					200 µg/mL (0.2 mg/mL)
Equivalent Concentration on Cells	10,000 µg/mL (10 mg/mL)	1000 µg/mL (1 mg/mL)	100 µg/mL (0.1 mg/mL)	10 µg/mL (0.01 mg/mL)	1 µg/mL (0.001 mg/mL)

[NOTE: The amounts of test chemical weighed and Chemical Dilution Medium and Treatment Medium added may be modified from the amounts given above, provided that the targeted concentrations specified for each tier are tested.]

Figure 1. Solubility Flow Chart

TIER 1

STEP 1:	20 mg/mL test chemical (TC) in 0.5 mL Chemical Dilution Medium and Treatment Medium: • if TC soluble in both media, then **STOP**. • if TC insoluble in one medium, then go to STEP 2.

TIER 2

STEP 2:	2 mg/mL TC in medium (one or both) – increase volume from STEP 1 by 10 (i.e., to 5 mL) • if TC soluble, then **STOP**. • if TC insoluble in one medium, then go to STEP 3.

STEP 3:	200 mg/mL TC in DMSO
	• if TC soluble, then **STOP**.
	• if TC insoluble, test at 200 mg/mL in ETOH.
	  • if TC soluble, then **STOP.**
	  • If TC insoluble, go to STEP 4.

TIER 3

STEP 4:	0.2 mg/mL TC in medium (one or both) – increase volume from STEP 2 by 10 (i.e., to 50 mL)
	• if TC soluble in both media, then **STOP**.
	• if TC insoluble in one medium, test at 20 mg/mL in DMSO – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, test at 20 mg/mL in ETOH – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 5.

TIER 4

STEP 5:	2 mg/mL TC in DMSO – increase volume from STEP 4 by 10 (i.e., to 50 mL)
	• if TC soluble, then **STOP.**
	• if TC insoluble, test at 2 mg/mL in ETOH – increase volume from STEP 4 by 10 (i.e., to 50 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 6.

TIER 5

STEP 6:	0.2 mg/mL TC in 50 mL DMSO • if TC soluble, then **STOP.** • if TC insoluble, test at 0.2 mg/mL in 50 mL ETOH • **STOP**

2. Mechanical Procedures

a) The following hierarchy of mixing procedures will be followed to dissolve the test chemical:

1) Add test chemical to solvent as in Tier 1 of **Table 1**. (Test chemical and solvent should be at room temperature.)

2) Gently mix at room temperature. Vortex the tube (1 –2 minutes).

3) If test chemical hasn't dissolved, use waterbath sonication for up to 5 minutes.

4) If test chemical is not dissolved after sonication, then warm solution to 37°C for 5 - 60 min. This can be performed by warming tubes in a 37°C water bath or in a CO_2 incubator at 37°C. The solution may be stirred during warming (stirring in a CO_2 incubator will help maintain proper pH).

5) Proceed to Tier 2 (and Tiers 3-5, if necessary of Table 1 and repeat procedures 2-4).

b) The preference of solvent for dissolving test chemicals is Chemical Dilution Medium or Treatment Medium, DMSO, and then ethanol. Thus, if all solvents for a particular tier are tested simultaneously and a test chemical dissolves in more than one solvent, then the choice of solvent follows this hierarchy. For example, if, at any tier, a chemical is soluble in Chemical Dilution Medium and DMSO, but not in Treatment Medium or ethanol, the choice of solvent would be medium for the 3T3 assay and DMSO for the NHK assay. If the chemical were insoluble in both media, but soluble in DMSO and ethanol, the choice of solvent would be DMSO for both assays.

After the lab has determined the preferred solvent for the test chemical and before proceeding to the cytotoxicity testing, the Study Director will submit the solubility test results (laboratory worksheets are preferable), and discuss the solvent selection with the Study Management Team (SMT) of the validation study. <u>The SMT will provide direction on the solvent to be used in each assay for each chemical prior to cytotoxicity testing</u>. If the laboratory has attempted all solubility testing without success, then the SMT will provide additional guidance for achieving test chemical solubility. The SMT anticipates that all validation study test chemicals will be tested in the NRU assays.

The Testing Facility shall forward the results from the solubility tests assay to the SMT through the designated contacts in electronic format and hard copy upon completion of testing. The SMT will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

U. S. Environmental Protection Agency. 1996. Product Properties Test Guidelines. OPPTS 803.7840. Water Solubility: Column Elution Method; Shake Flask Method. EPA712-C-96-041, Prevention, Pesticides and Toxic Substances, Washington DC.

IX. APPROVAL

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SPONSOR REPRESENTATIVE	**DATE**
(Print or type name)	
_____	_____
Test Facility STUDY DIRECTOR	**DATE**
(Print or type name)	

Appendix B4

Test Method Procedure for Prequalification of Normal Human Epidermal Keratinocyte Growth Medium (Phase III)

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TEST METHOD PROCEDURE
Prequalification of Normal Human Epidermal Keratinocyte Growth Medium

In Vitro Cytotoxicity Validation Study
Phase III

January 28, 2004

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

I. PROPOSAL

The following document provides the guidelines and testing requirements for qualifying lots of Keratinocyte Basal Medium without Ca^{++} (KBM® [CAMBREX/Clonetics # CC-3104]) and the medium supplements (SingleQuots® [CAMBREX/Clonetics # CC-4131]) for use in the normal human epidermal keratinocyte (NHK) neutral red uptake (NRU) assays for Phase III of the In Vitro Cytotoxicity Validation Study. The medium and supplements will be tested so as to demonstrate their ability to perform adequately in the NHK NRU assay prior to purchase by the validation study laboratories for use in Phase III.

The Testing Facility will request the quality control test data from CAMBREX/Clonetics for each potential lot of medium and supplements. Based upon the QC test data, the Testing Facility will purchase and test the one or two most current lots of medium and supplements in stock with CAMBREX/Clonetics that appear to have the potential to support NHK cultures according to the requirements of the In Vitro Cytotoxicity Validation Study NHK neutral red uptake assay.

This test method procedure is based on the Phase III NHK NRU protocol (IIVS Protocol No. SP100066) and outlines the procedures needed for performing the cytotoxicity test specifically for prequalifying NHK culture medium. The test method procedure and NHK NRU protocol support the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method procedure applies to all personnel involved with performing media/supplement testing.

A. NHK Neutral Red Uptake Cytotoxicity Test

The NHK NRU test will be performed to analyze NHK growth characteristics and the *in vitro* toxicity of Sodium Lauryl Sulfate (SLS), as measured by the IC_{50}, with each NHK medium/supplement being tested.

The Testing Facility will select the lots of medium/supplements and combinations based on the maximum available quantity and shelf life, as well as growth test results provided by Cambrex. Potential medium testing/supplement combinations are:

- One lot of medium/one lot of SingleQuots®: Test the lot of medium using the lot of SingleQuots® (one test of three plates).
- Two or more lots of medium/one lot of SingleQuots®: Test each lot of medium using the one lot of SingleQuots® (one test of three plates for each lot of medium)
- One lot of medium/two or more lots of SingleQuots®: Test the lot of medium using each lot of SingleQuots® (one test of three plates for each lot of SingleQuots®).

NHK cultures will be established using each medium/supplement combination, and will be subcultured on 3 different days into 96-well plates for three subsequent SLS cytotoxicity tests using each appropriate test medium/supplement combination.

B. Testing Conditions

The work will be performed in the IIVS Good Laboratory Practice (GLP)-compliant laboratories, but will not be performed in full compliance with national and international GLP guidelines, and neither a protocol nor an audited report will be generated.

The Study Director will provide recommendations and appropriate test data for acceptance/rejection of the tested media/supplements to the Study Management Team (SMT).

The Testing Facility will maintain the following documentation: study workbooks noting all methods and procedures; logs for general laboratory procedures and equipment (e.g., media preparation, SLS preparation, incubator function); electronic and paper formats of all optical density data obtained from the spectrophotometer plate reader; electronic and paper format of all calculations of ICx values and other derived data.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: Molly Vallant, Project Officer, NIEHS

D. Study Management Team Representatives: William Stokes, Silvia Casati, Raymond Tice, Judy Strickland, Michael Paris

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Substances: Keratinocyte Basal Medium without Ca^{++} (KBM®, Clonetics CC-3104)

KBM® SingleQuots® (Clonetics CC-4131)

B. Controls: Positive: Sodium Lauryl Sulfate
Vehicle (Negative): Assay medium

IV. TESTING FACILITY AND KEY PERSONNEL

- Name: Institute for In Vitro Sciences, Inc.
- Address: 21 Firstfield Road, Suite 220
  Gaithersburg, Maryland 20878
- Study Director: Hans Raabe, M.S.
- Laboratory Technician(s): Greg Mun, B.A., Laboratory Manager
  Robin Anderson, B.S.
  Filomena Diaco, B.S.
  Gregory Moyer, B.S.
  Massod Rahimi, B.S.
  Angela Sizemore, B.S.
  Teri Beth Wallace, B.S.

Nathan Wilt, B.S.

V. PROCEDURES

A. Materials

NHK cells used for this procedure will come from the same lot of NHK cells used in Phases I and II of the validation study. Equipment, chemicals, and other media will be the same as in IIVS Protocol No. SP100066.

B. Preparations of Media and Solutions

All media and solutions will be prepared as in IIVS Protocol No. SP100066.

C. Methods

All culture procedures will be performed as in IIVS Protocol No. SP100066..

NHK cultures will be established with cryopreserved cells seeded into individual tissue culture flasks using the existing medium/supplement combination (the "control" medium) and each test medium/supplement combination. It may be acceptable to suspend freshly-thawed cells initially into 9 mL of control medium. The cell suspension will then be added to culture flasks containing pre-warmed control or test medium. The cells will be subcultured on three different days into 96-well plates for three subsequent NRU tests using each appropriate test medium/ supplement combination and control.

D. Preparation of SLS

The preparation of SLS (IIVS code 02AD92) will follow the procedures in Sections VII.D.1.a, b, and d of IIVS Protocol No. SP100066. SLS will be dissolved only in Routine Culture Medium. Determination of the pH will follow Section VII.D.2.

Preparation of SLS concentrations/dilutions will follow the main experiment procedures specifically for testing compounds in Routine Culture Medium as outlined in Section VII.D.3.b of IIVS Protocol No. SP100066. The concentrations/dilutions should be the same or similar to those used for SLS as a positive control in Phase II of the validation study.

E. Test Procedure

The 96-well plate configuration will be the same as that outlined in Section VII.E.1 of IIVS Protocol No. SP100066. The C_1 test concentration will be the highest SLS concentration. Application of the SLS, subsequent toxicity testing, and measurement of NRU will follow procedures outlined in Sections VII.E.2.a and b and Section VII.4 of IIVS Protocol No. SP100066.

Cells cultured in control medium and in each test medium/supplement combination will be tested in parallel for their sensitivity to SLS.

F. Microscopic Evaluation

Observations of the cell cultures in the culture flasks, as well as in the 96-well plates will be performed and documented and should include cell morphology (e.g., overall appearance, colony formation and proliferation, presence of mitotic figures, and distribution). Representative observations of the cultures in the culture flasks will be performed every working day. Representative observations of the cultures in the 96-well plates will be performed daily prior to treatment with SLS; at the end of the 48 hour treatment incubation;

and during the neutral red incubation period (to evaluate relative neutral red uptake in the vehicle control cultures).

Changes in morphology of the cells due to cytotoxic effects of the SLS (prior to measurement of NRU) should be recorded as per procedures outlined in Section VII.E.3 of IIVS Protocol No. SP100066.

G. Data Analysis and Test Evaluation

Data analysis will be performed as in Section VII.F of IIVS Protocol No. SP100066. The following parameters will be evaluated to determine whether the NHK media and supplements are adequate to support the NHK NRU assay:

1) SLS IC_{50}

2) r^2 (coefficient of determination) value calculated for the Hill model fit (i.e., from PRISM® software.

3) Difference between the mean of all vehicle controls (VC) and (a) the left mean VC, and (b) the right mean VC.

4) Number of points between 0 % and 50.0 % viability and between 50.0 % 100 % viability.

5) Mean corrected $OD_{540\text{-}550}$ of the VCs.

6) Cell morphology and confluence of the VCs at the end of the 48 h treatment

The Study Director will utilize all observed growth characteristics and test results to determine whether the media/supplements perform adequately, and provide the test data and a recommendation for the use or rejection of the media/supplements to the SMT. IIVS will request CAMBREX/Clonetics reserve a portion of an acceptable lot based on estimates of media needed by the three laboratories.

V. REFERENCES

IIVS Protocol No. SP100066. Test Method Protocol for the NHK Neutral Red Uptake Cytotoxicity Test. A Test for Basal Cytotoxicity for an In Vitro Validation Study. November 11, 2003. Prepared by the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

VI. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**

(Print or type name)

_____ _____

Testing Facility STUDY DIRECTOR **DATE**
(Print or type name)

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Appendix C

Validation Study Test Method Protocols
(Phases Ia, Ib, and II)

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Appendix C1

Test Method Protocol for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test (Phase Ia)

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TEST METHOD PROTOCOL
for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an *In Vitro* Validation Study

June 14, 2002

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test
A Test for Basal Cytotoxicity

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the BALB/c 3T3 Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and supports the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. Determination of Positive Control Database

An historical database of IC_{50} values for the positive control chemical (Sodium Lauryl [dodecyl] Sulfate {SLS}) must be established and maintained by performing 10 concentration-response assays on the 3T3 cells before performing the NRU assay on test chemicals. Once the mean IC_{50} and the 95 % confidence interval (CI) of the IC_{50} of SLS are established, the values will be used as an acceptance criterion for test sensitivity for the 3T3 NRU assay.

B. BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

After acceptable positive control mean IC_{50} and 95 % CI values have been established, the 3T3 NRU test will be performed to analyze the *in vitro* toxicity of test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for a predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals: *Blinded Chemicals*

B. Controls:

Positive:	Sodium Lauryl Sulfate
Vehicle (Negative):	Assay medium
Solvent (as needed):	Assay medium with appropriate solvent used to prepare the test chemicals (**Section VII.E**)

IV. TESTING FACILITY AND KEY PERSONNEL

- Name:
- Address:
- Study Director:
- Laboratory Technician(s):
- Scientific Advisor:
- Quality Assurance Director:
- Safety Manager:
- Facility Management:

A. Test Schedule

- Proposed Experimental Initiation Date:
- Proposed Experimental Completion Date:
- Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed

as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\log IC50 - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test chemical preparation, incubator function); all optical density data obtained from the spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

1. Cell Lines

BALB/c 3T3 cells, clone 31
CCL-163, LGC Reference Materials, Customer Service, Queens Road, Teddington, Middlesex, TW110LY, UK
CCL-163, American Type Culture Collection [ATCC], Manassas, VA, USA)

2. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

a) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
b) Laminar flow clean bench/cabinet (standard: "biological hazard")
c) Water bath: 37ºC ± 1ºC
d) Inverse phase contrast microscope
e) Sterile glass tubes with caps (e.g., 5 ml)
f) Centrifuge (optionally: equipped with microtiter plate rotor)
g) Laboratory balance
h) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
i) Shaker for microtiter plates
j) Cell counter or hemocytometer

k) Pipetting aid
l) Pipettes, pipettors (multi-channel and single channel), dilution block
m) Cryotubes
n) Tissue culture flasks (e.g., 75 - 80 cm^2, 25 cm^2)
o) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Falcon tissue culture-treated)
p) pH paper (wide and narrow range)

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of 3T3 cells.]

3. Chemicals, Media, and Sera

a) Dulbecco's Modification of Eagle's Medium (DMEM) without L-Glutamine; should have high glucose [4.5gm/l] (e.g., ICN-Flow Cat. No. 12-332-54)
b) L-Glutamine 200 mM (e.g., ICN-Flow # 16-801-49)
c) New Born Calf Serum (NBCS or NCS) (e.g., Biochrom # SO 125)
d) 0.05 % Trypsin/0.02 % EDTA solution (e.g., SIGMA T 3924, ICN-Flow, # 16891-49)
e) Phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}(for trypsinization)
f) Hanks' Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+}(CMF-HBSS)
g) Dulbecco's Phosphate Buffered Saline (D-PBS) with glucose) formulation containing calcium and magnesium cations, and supplemented with 1000mg/L glucose) (for rinsing)
h) Penicillin/streptomycin solution (e.g. ICN-Flow # 16-700-49)
i) Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
j) Dimethyl sulfoxide (DMSO), U.S.P. analytical grade (Store under nitrogen @ -20ºC)
k) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
l) Glacial acetic acid, analytical grade
m) Distilled H_2O or any purified water suitable for cell culture (sterile)
n) Sterile paper towels (for blotting 96-well plates)

[Note: Due to lot variability of NBCS/NCS, first check a lot for growth stimulating properties with 3T3 cells (approximately 20-24 h doubling time) and then reserve a sufficient amount of NBCS/NCS.]

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard). All methods and procedures will be adequately documented.]

1. Media

DMEM (buffered with sodium bicarbonate) supplemented with (final concentrations in DMEM are quoted):

a) for freezing (Freeze Medium); contains 2X concentration of NBCS/NCS and DMSO of final freezing solution

40 %	NBCS/NCS
20 %	DMSO

b) for routine culture (Routine Culture Medium)

10 %	NBCS/NCS
4 mM	Glutamine

c) for treatment with Test Chemicals (Treatment Medium)

5 %	NBCS/NCS
4 mM	Glutamine
100 IU	Penicillin
100 µg/ml	Streptomycin

[Note: The serum concentration of treatment medium is reduced to 5 %, since serum proteins may mask the toxicity of the test substance. Serum cannot be totally excluded because cell growth is markedly reduced in its absence.]

Complete media should be kept at approximately 4° C and stored for no longer than two weeks.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay. If the liquid form is not available, the following formulation can be prepared.

0.4 g NR Dye powder in 100 ml of H_2O

Make up prior to use and store dark at room temperature. May store for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

1 ml (4mg NR dye/ml)	NR Stock Solution
79 ml	DMEM

The final concentration of the NR Medium is 50 µg NR dye/ml.
[Note: The NR medium should be incubated overnight at 37ºC ± 1ºC and centrifuged at approximately 600 x g for 10 min (to remove NR crystals) before adding to the cells. Alternative procedures (e.g., Millipore filtering) can be used as long as they guarantee that NR medium is free of crystals.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

BALB/c 3T3 cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 75 - 80 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells should be examined on a daily basis under a phase contrast microscope, and any changes in morphology or their adhesive properties noted in a Study Workbook (see **Section VII.F.3**).

2. Receipt of Cryopreserved BALB/c 3T3 Cells

Upon receipt of cryopreserved BALB/c 3T3 cells, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells

Thaw cells by putting ampules into a water bath at 37°C ± 1ºC. Leave for as brief a time as possible.

a) Resuspend the cells and transfer into Routine Culture Medium in a tissue-culture flask (see **Section 6**).
b) Incubate at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air.
c) When the cells have attached to the bottom of the flask (this may take up to 4 h), decant the supernatant and replace with fresh medium. Culture as described above.
d) Passage two to three times before using the cells in a cytotoxicity test.

A fresh batch of frozen cells from the stock lot of cells should be thawed out and cultured approximately every two months. This period resembles a sequence of about 18 passages.

4. Routine Culture of BALB/C 3T3 Cells

When cells exceed 50 % confluence (but less than 80 % confluent) they should be removed from the flask by trypsinization:

a) Decant medium, rinse cultures with 5 ml PBS or Hanks' BSS (without Ca^{2+}, Mg^{2+}) per 25 cm^2 flask (15 ml per 75 cm^2 flask). Wash cells by gentle agitation to remove any remaining serum that might inhibit the action of the trypsin.
b) Discard the washing solution.
c) Add 1-2 ml trypsin-EDTA solution per 25 cm^2 to the monolayer for a few seconds (e.g., 15-30 seconds).
d) Remove excess trypsin-EDTA solution and incubate the cells at room temperature.
e) After 2-3 minutes (min), lightly tap the flask to detach the cells into a single cell suspension.

5. Cell Counting

After detaching the cells, add 0.1-0.2 ml of Routine Culture Medium/cm^2 to the flask (e.g., 2.5 ml for a 25 cm^2 flask). Disperse the monolayer by gentle trituration. It is

important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension obtained using a hemocytometer or cell counter (e.g., Coulter counter).

6. Subculture of Cells

After determination of cell number, the culture can be sub-cultured into other flasks or seeded into 96-well microtiter plates. BALB/c 3T3 cells are routinely passaged at suggested cell densities as listed in the table (approximate doubling time is 20-24 h). The individual laboratories will need to determine and adjust the final density to achieve growth as outlined in **Section VII.C.1**.

Table 1. Cell Densities for Subculturing

Days in Culture	Seeding Density (cells/cm^2)	Total Cells per 25 cm^2 flask	Total Cells per 75 cm^2 flask
2	16800	4.2×10^5	1.26×10^6
3	8400	2.1×10^5	6.3×10^5
4	4200	1.05×10^5	3.15×10^5

[Note: It is important that cells have overcome the lag growth phase when they are used for the test.]

7. Freezing Cells

Stocks of BALB/c 3T3 cells can be stored in sterile, freezing tubes in a liquid nitrogen freezer. DMSO is used as a cryoprotective agent.

a) Centrifuge trypsinized cells at approximately 200 x g.
b) Suspend the cells in cold Routine Medium (half the final freezing volume) so a final concentration of $1\text{-}5 \times 10^6$ cells/ml can be attained.
c) Slowly add cold Freeze Medium to the cells so that the solvent will equilibrate across the cell membranes. Bring the cell suspension to the final freezing volume. The final cell suspension will be 10 % DMSO. Aliquot the cell suspension into freezing tubes and fill to 1.8 ml.
d) Place the tubes into an insulated container (e.g., styrofoam trays) and place in a freezer (-70 to -80°C) for 24 h. This gives a freezing rate of approximately 1°C/min. The laboratory needs to ensure that the freezing protocol is applicable to the 3T3 cells and that the cells are viable when removed from cryopreservation.
e) Place the frozen tubes into liquid nitrogen for storage.

8. Preparation of Cells for Assays

a) Cultured cells that are going to be used in seeding the 96-well plates should be fed fresh medium the day before subculturing to the plates. On the day of plate seeding, prepare a cell suspension of 2.5×10^4 cells/ml in Routine Culture Medium. Using a multi-channel pipette, dispense 100 µl Routine Culture Medium only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate (See **Section IV.F**). In the remaining wells, dispense 100 µl of a cell suspension of 2.5×10^4

cells/ml (= $2.5x10^3$ cells/well). The seeding density should be noted to ensure that the cells in the control wells are not overgrown after three days (i.e., 24 h incubation in **b** and 48 h exposure to test chemicals). Prepare one plate per chemical to be tested.

b) Incubate cells for 24 h (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air) so that cells form a less than half confluent monolayer. This incubation period assures cell recovery and adherence and progression to exponential growth phase.

c) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

9. Determination of Doubling Time

a) Establish cells in culture and trypsinize cells as per **Section C.4** for subculture. Resuspend cells in about 5ml Treatment Medium (5 % NBCS/NCS). Seed cells at 4200 cells/cm^2.

b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).

c) After 4 - 6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue) if Study Director sees a need. Use appropriate size exclusion limits if using a Coulter counter. Determine the total number of cells and document. Repeat sampling at 24 h, 48 h, 72 h, and 96 h post inoculation. Change culture medium at 72 h or sooner in remaining dishes if indicated by pH drop.

d) Plot cell concentration (per ml of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Establishing the Positive Control Database

An historical database of IC_{50} values for the positive control chemical (Sodium Lauryl [dodecyl] Sulfate {SLS}) must be established and maintained by performing 10 concentration-response assays on the 3T3 cells.

1. Positive Control Chemical Preparation

The positive control chemical (SLS) is prepared in the same manner as the test chemical (**Sections E.1 and E.2**) by following the instructions and substituting "test chemical" with "SLS."

2. Range Finder Experiment

Before initiating the 10 concentration-response assays, a range finder experiment will be performed using eight concentrations of SLS by diluting the stock solution with a constant factor as per **Sections E.3.a** and **E.3.b**. The eight chemical concentrations will

be tested as per the test procedure outlined in **Section F** and analyzed as per procedures outlined in **Section G**.

3. Test Procedure

Once a range has been determined that satisfies the criteria in **Section E.3.b**, the definitive concentration-response assays shall use a $^{6}\sqrt{10} = 1.47$ dilution scheme centered on the IC_{50}. The Test Facility will perform two tests per day on five different days. The 95 % CI of the IC_{50} of SLS will be established and defined as an acceptance criterion for test sensitivity for the 3T3 NRU assay. The confidence intervals shall be calculated using the average of the individual IC_{50} values from each positive control assay performed. An example of an historical mean IC_{50} of SLS in mammalian cultures is **93 µg/ml** and the 95 % CI is **70 - 116 µg/ml** (Spielmann et. al., 1991). All testing will follow the instructions in **Section F** using the 96-well plate configuration in Figure 1. The test meets acceptance criteria if the conditions in **Sections F.5.a.2** and **F.5.a.3** are met.

Figure 1. 96-Well Plate Configuration for Positive Control and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	b	b	b	b	b	b	b	b	b	b	b	b
B	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
C	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
D	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
E	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
F	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
G	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
H	b	b	b	b	b	b	b	b	b	b	b	b

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 – C_8$ = Test Chemicals or Positive Control (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)

E. Preparation of Test Chemicals

[Note: Test chemical must be freshly prepared immediately prior to use. Each stock dilution should have at least 1-2 ml total volume to ensure adequate solution for the test wells in a single 96-well plate. The solutions must not be cloudy nor have noticeable precipitate. Test chemicals must be at room temperature before dissolving and diluting. Preparation under red or yellow light may be necessary, if rapid photodegradation is likely to occur.]

1. Dissolving Test Chemical

a) Approximately 200,000 µg (200 mg) of the test chemical will be weighed into a glass tube and the weight will be documented. Assay-specific culture medium will be added to the vessel so that the concentration is 2,000,000 µg/ml (2000 mg/ml) and mixed using the mixing procedures outlined in **Section E.1.c**. If complete solubility is achieved, then additional solubility procedures are not needed. The test chemical can then be prepared and diluted for use in an assay. If only partial solubility is achieved, then add additional medium in the steps outlined in Table 1 until the concentration is a minimum of 200,000 µg /ml. If complete solubility at 200,000 µg/ml in culture medium can't be attained, then repeat the solubility steps in Table 1 using the other solvent(s) in the solubility hierarchy outlined in **Section E.1.c**. Test chemicals that are only soluble in DMSO or ethanol will be prepared at 2,000,000 µg/ml as the highest concentration of stock solution.

Table 2 Determination of Solubility

Solubility Data	Step 1	Step 2	Step 3
Total volume of medium added (ml)	0.1	0.5	1.0
Total volume of DMSO or ethanol added (ml)	0.1	0.5	1.0
Approximate solubility (µg/ml)	≥ 2,000,000	400,000	200,000

Example: If complete solubility is not achieved in 0.1 ml medium (Step 1), then 0.4 ml must be added to obtain a total volume of 0.5 ml (Step 2). No additional weighing of chemical is needed. Chemical and medium are again mixed in an attempt to dissolve.

b) Each test chemical will be prepared such that the highest test concentration applied to the cells in each range finding experiment is 100,000 µg/ml in culture medium (10,000 µg/ml if DMSO or ethanol is used). If 100,000 µg/ml in culture medium cannot be achieved, then the highest concentration attainable will be used. If the range finding experiment shows that 10,000 µg/ml is not high enough for the range of chemicals dissolved in DMSO or ethanol to meet the acceptance criteria, then higher concentrations will be used for the definitive experiment.

c) The following mixing and solvent hierarchy will be followed in dissolving the test chemical:

1) Dissolve test chemical in Treatment Medium.
2) Gently mix. Vortex the tube (1 – 2 minutes).
3) If test chemical hasn't dissolved, use sonication for up to 5 minutes.
4) If sonication doesn't work, then warm solution to 37°C.

If the test chemical doesn't dissolve (i.e., solution is cloudy or has precipitate) in the Treatment Medium, then follow the steps 1) through 4) using DMSO instead of Treatment Medium.

If the test chemical doesn't dissolve in DMSO, then follow steps 1) through 4) using ethanol instead of DMSO.

d) For the range finding experiments, the highest 2x concentration of test chemical dissolved only in culture medium will be 200,000 µg/ml (200 mg/ml). The highest 2x concentration of test chemical first dissolved in DMSO or ethanol then transferred to culture medium will be 20,000 µg/ml (20 mg/ml). Dissolve test chemical in appropriate medium/solvent (at 200-fold the desired final test concentration in the case of DMSO or ethanol solvents, i.e., 20,000µg/ml). The final solvent (DMSO or ethanol) concentration for application to the cells should be kept at a constant level of 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in medium before application to 3T3 cells.

Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme

1) Label eight tubes 1 – 8. Add 0.9 ml solvent (e.g., DMSO or ethanol) to tubes 2 -- 8.
2) Prepare stock solution of 2,000,000 µg test chemical/ml solvent in tube # 1.
3) Add 0.1 ml of 2,000,000 µg/ml dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 200,000 µg/ml).
4) Add 0.1 ml of 200,000 µg/ml dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 20,000 µg/ml)
5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.
6) Since each concentration is 200 fold greater than the concentration to be tested, dilute 1 part dissolved chemical in each tube with 99 parts of culture medium (e.g., 0.1 ml of test chemical in DMSO + 9.9 ml culture medium) to derive the 8 2x concentrations for application to 3T3 cells. Each test chemical concentration will then contain 1 % v/v solvent. The 3T3 cells will have 0.05 ml Treatment Medium in the wells prior to application of the test chemical. By adding 0.05 ml of the appropriate 2x test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 10,000 µg/ml) in a total of 0.100 ml and the solvent concentration in the wells will be 0.5% v/v.

Check carefully to determine whether the chemical is still dissolved after the transfer from solvent stock solution to medium, and reduce the highest test concentration, if necessary. Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Measure the pH of the highest concentration of the test chemical in culture medium using pH paper. Document the pH and note the color of the medium for all dilutions. Do not adjust the pH.

3. Concentrations of Test Chemical

a) Range Finder Experiment

Test eight concentrations of the test chemical/PC by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

b) Main Experiment

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller (e.g., $^{6}\sqrt{10} = 1.47$; NOTE: this dilution factor will be used for the definitive positive control assays [**Section VII.D.3**]). Cover the relevant concentration range (≥10 % and ≤ 90 % effect) with at least three points of a graded effect, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than three cytotoxic concentrations in the relevant range shall be repeated, where possible, with a smaller dilution factor. (Taking into account pipetting errors, a progression factor of 1.21 is regarded the smallest factor achievable.)

c) Test Chemical Dilutions

- A factor of $^{2}\sqrt{10} = 3.16$ could be used for covering a large range: (e.g., 1 ⇒3.16 ⇒10 ⇒31.6 ⇒100 ⇒316 ⇒1000 ⇒3160 µg/ml).

- The simplest geometric concentration series (i.e., constant dilution / progression factor) are dual geometric series (e.g., a factor of 2). These series have the disadvantage of numerical values that permanently change between logs of the series: (e.g., *log0*-2, 4, 8; *log1*- 16, 32, 64; *log2*- 128, 256, 512; *log3*- 1024, 2048,).

- The decimal geometric series, first described by Hackenberg and Bartling (1959) for use in toxicological and pharmacological studies, has the advantage that independent experiments with wide or narrow dose factors can be easily compared because they share identical concentrations. Furthermore, under certain circumstances, experiments can even be merged together:

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The dosing factor of 3.16 (= $^{2}\sqrt{10}$) divides a log into two equidistant steps, a factor of 2.15 (= $^{3}\sqrt{10}$) divides a decade into three steps. The factor of 1.47 (= $6\sqrt{10}$) divides a log into six equidistant steps, and the factor of 1.21 (= $^{12}\sqrt{10}$) divides the log into 12 steps.

For an easier biometrical evaluation of several related concentration response experiments use decimal geometric concentration series rather than dual geometric series. The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

- Determine which test chemical concentration is closest to the IC50 value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

F. Test Procedure

1. 96-Well Plate Configuration

The 3T3 NRU assay for test chemicals will use the 96-well plate configuration shown in Figure 1.

2. Application of Test Chemical

a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized. The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs and/or Corning/Transtar model 4878 disposable reservoir liners, 8-channel). The second method utilizes a "dummy" plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate (with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the plate containing cells. More volume than needed for the test plate (i.e. greater than 50 µl/well) should be in the wells of the dummy plate. At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing. A third option, though not a recommended option, is to transfer test chemical solutions well by well using a single channel pipettor or repeat pipettor. This option will increase the amount of time needed for test chemical application. The use of a repeat pipettor increases the risk of dislodging cells from the culture plate.

b) After 24 h ± 1 h incubation of the cells, remove Routine Culture Medium from the cells by careful inversion of the plate (i.e., "dump") over an appropriate receptacle. Gently blot the plate on a sterile paper towel so that the monolayer is minimally disrupted. Do not use automatic plate washers for this procedure nor vacuum aspiration.

c) Immediately add 50 µl of Treatment Medium to each well. Then add 50 µl Treatment Medium containing either the appropriate concentration of test chemical, the PC, or the VC (see Figure 1 for the plate configuration). The solutions will be transferred from the dummy plate to the test plate by adding the vehicle control first then lowest to highest dose so that the same pipette tips on the eight channel pipettor can be used for the whole plate.

d) Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).

e) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in developing the positive control database. This plate will follow the same schedule and procedures as used for the test chemical plates.

3. Microscopic Evaluation

After at least 46h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

a) Carefully remove (i.e., "dump") the Treatment Medium and rinse the cells very carefully with 250 µl pre-warmed D-PBS. Remove the rinsing solution by gentle tapping. Add 250 µl NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3 h.
b) After incubation, remove the NR medium, and carefully rinse cells with 250 µl D-PBS.
c) Decant and blot D-PBS from the plate. (Optionally: centrifuge the reversed plate.)
d) Add exactly 100 µl NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.
e) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution.
f) Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader

(spectrophotometer), using the blanks as a reference. Save raw data in the Excel format as provided by the Study Management Team.

5. Quality Check of 3T3 NRU Assay

a) *Test Acceptance Criteria*

1) A test meets acceptance criteria, if the IC_{50} for SLS is within the 95 % CI of the historical mean established by the Test Facility (as per **Section D**).

2) A test meets acceptance criteria if the mean OD_{540} of VCs is ≥ 0.3 and ≤ 1.1.

3) A test meets acceptance criteria if the left and the right mean of the VCs do not differ by more than 15 % from the mean of all VCs.

b) Checks for Systematic Cell Seeding Errors

The absolute value of optical density (OD_{540} of NRU) obtained in the untreated vehicle control may indicate whether the 2.5×10^3 cells seeded per well have grown exponentially with normal doubling time during the two days of the assay. If doubling time experiments were performed using the NRU assay, then the historical optical densities observed during the doubling time experiments can be used for comparison to determine exponential growth.

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

c) *Quality Check of Concentration-Response*

The IC_{50} derived from the concentration-response of the test chemicals will be backed by at least three responses ≥ 10 % and ≤ 90 % inhibition of NRU. If this is not the case, and the concentration progression factor can be easily reduced, reject the experiment and repeat it with a smaller progression factor. Numerical scoring of the cells (see **Section F.3**) should be determined and documented in the Study Workbook.

G. Data Analysis

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicate well) per test concentration (blanks will be subtracted). This value is compared with the mean NRU of all VC values (provided VC values have met the VC acceptance criteria). Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet provided by the Study Management Team for determining cell viability and

performing statistical analyses.

The concentration of a test chemical reflecting a 20 %, 50 %, and 80 % inhibition of cell viability (i.e., the IC_{20}, IC_{50}, and IC_{80}) is determined from the concentration-response by applying a Hill function to the concentration-response data. It will not be necessary for the Testing Facilities to derive the equation since statistical software (e.g., GraphPad PRISM® 3.0) specified by the Study Management Team shall be used to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical. In addition, the Study Management Team shall provide guidelines for calculating ICx values and confidence limits. The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the Study Management Team/biostatistician through the designated contacts in electronic format and hard copy upon completion of testing. The Study Management Team will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

Hackenberg, U. and H. Bartling. 1959. Messen und Rechnen im pharmakologischen Laboratorium mit einem speziellen Zahlensystem (WL24-System). Arch. Exp. Pathol. Pharmakol. 235: 437-463.

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IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**
(Print or type name)

_____ _____

Test Facility STUDY DIRECTOR **DATE**
(Print or type name)

Appendix C2

Test Method Protocol for the Normal Human Epidermal Keratinocyte (NHK) Neutral Red Uptake Cytotoxicity Test (Phase Ia)

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TEST METHOD PROTOCOL
for the NHK Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an In Vitro Validation Study

June 14, 2002

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The Normal Human Keratinocyte (NHK) Neutral Red Uptake Cytotoxicity Test
A Test for Basal Cytotoxicity

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the Normal Human Keratinocyte (NHK) Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and is in support of the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. Determination of Positive Control Database

An historical database of IC_{50} values for the positive control chemical (Sodium Lauryl [dodecyl] Sulfate {SLS}) must be established and maintained by performing 10 concentration-response assays on the NHK cells before performing the NRU assay on test chemicals. Once the mean IC_{50} and the 95 % confidence interval (CI) of the IC_{50} of SLS are established, the values will be used as an acceptance criterion for test sensitivity for the NHK NRU assay.

B. NHK Neutral Red Uptake Cytotoxicity Test

After acceptable positive control mean IC_{50} and 95 % CI values have been established, the NHK NRU test will be performed to analyze the *in vitro* toxicity of test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for a predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals: *Blinded chemicals 1*

B. Controls: Positive: Sodium Lauryl Sulfate
Vehicle (Negative): Assay medium
Solvent (as needed): Assay medium with appropriate solvent used to prepare the test chemicals (**Section VII.E**)

IV. TESTING FACILITY AND KEY PERSONNEL

- Name:
- Address:
- Study Director:
- Laboratory Technician(s):
- Scientific Advisor:
- Quality Assurance Director:
- Safety Manager:
- Facility Management:

A. Test Schedule

- Proposed Experimental Initiation Date:
- Proposed Experimental Completion Date:
- Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A.. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\log IC50 - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test chemical preparation, incubator function); all optical density data obtained from the spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used unless otherwise noted.]

1. Cell Lines

Normal Human Epidermal Keratinocytes (NHK)

Non-transformed cells; from cryopreserved primary or secondary cells (**Clonetics #CC-2507 or equivalent).** Cells will be Clonetics NHK cells.

Clonetics/BioWhittaker [BioWhittaker, 8830 Biggs Ford Road, Walkersville, MD 21793-0127

BioWhittaker Europe [BioWhittaker Europe, S.P.R.L. Parc Industriel de Petit Rechain, B-4800 Verviers, BELGIUM]

2. Technical Equipment

a) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
b) Laminar flow clean bench (standard: "biological hazard")
c) Water bath: 37ºC ± 1ºC
d) Inverse phase contrast microscope
e) Sterile glass tubes with caps (e.g., 5ml)
f) Centrifuge (optionally: equipped with microtiter plate rotor)
g) Laboratory balance
h) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
i) Shaker for microtiter plates
j) Cell counter or hemocytometer
k) Pipetting aid
l) Pipettes, pipettors (multi-channel and single channel), dilution block
m) Cryotubes
n) Tissue culture flasks (75 - 80 cm^2, 25 cm^2)
o) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Corning/COSTAR tissue culture-treated)
p) pH paper (wide and narrow range)

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of NHK.]

3. Chemicals, Media, and Sera

a) Keratinocyte Basal Medium without Ca^{++} (KBM®, Clonetics CC-3104) that is completed by adding the KBM® SingleQuots® (Clonetics CC-4131) to achieve the proper concentrations of epidermal growth factor, insulin, hydrocortisone, antimicrobial agents, bovine pituitary extract, and calcium (e.g., Clonetics Calcium SingleQuots®, CC-4202).
b) HEPES Buffered Saline Solution (HEPES-BSS) (e.g., Clonetics # CC-5022)
c) 0.025 % Trypsin/EDTA solution (e.g., Clonetics # CC-5012)
d) Trypsin Neutralizing Solution (TNS) (e.g., Clonetics # CC-5002)
e) Phosphate Buffered Saline (PBS)
f) Dulbecco's Phosphate Buffered Saline (D-PBS) with glucose) formulation containing calcium and magnesium cations, and supplemented with 1000 mg/L glucose)
g) Fetal bovine serum (FBS)
h) Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
i) Dimethyl sulfoxide (DMSO), U.S.P analytical grade (Store under nitrogen @ -20ºC)
j) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
k) Glacial acetic acid, analytical grade
l) Hanks' Balanced Salt Solution without Ca^{2+} or Mg^{2+} (CMF-HBSS) (e.g., Invitrogen # 14170)
m) Distilled H_2O or any purified water suitable for cell culture (sterile)
n) Sterile paper towels (for blotting 96-well plates)

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard).]

1. Media

a) Routine Culture Medium/Treatment Medium

KBM® (Clonetics CC-3104) supplemented with KBM® SingleQuots® (Clonetics CC-4131) and Clonetics Calcium SingleQuots® (CC-4202) to make 500ml of medium. Final concentration of supplements in medium are:

0.0001 ng/ml	Human recombinant epidermal growth factor
5 μg/ml	Insulin
0.5 μg/ml	Hydrocortisone
30 μg/ml	Gentamicin
15 ng/ml	Amphotericin B
0.10 mM	Calcium
30 μg/ml	Bovine pituitary extract

Complete media should be kept at 4°C and stored for no longer than two weeks.

NOTE:
KBM® SingleQuots® contain the following stock concentrations and volumes:

0.1 ng/ml	hEGF	0.5 ml
5.0 mg/ml	Insulin	0.5 ml
0.5 mg/ml	Hydrocortisone	0.5 ml
30 mg/ml	Gentamicin, 15 ug/ml Amphotericin-B	0.5 ml
7.5 mg/ml	Bovine Pituitary Extract (BPE)	2.0 ml

Clonetics Calcium SingleQuots® are 2 ml of 300mM concentration of calcium.

165 ul of solution per 500 ml calcium-free medium equals 0.10 mM calcium in the medium.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay. If the liquid form is not available, the following formulation can be prepared.

0.4 g NR Dye powder in 100 ml of H_2O

Make up prior to use and store dark at room temperature. May store for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

1 ml (4mg NR dye/ml)	NR Stock Solution
79 ml	KGM

The final concentration of the NR Medium is 50 μg NR dye/ml.

[Note: The NR medium should be incubated overnight at 37ºC ± 1ºC and centrifuged at approximately 600 x g for 10 min (to remove NR crystals) before adding to the cells. Alternative procedures (e.g., Millipore filtering) can be used as long as they guarantee that NR medium is free of crystals.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

NHK cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 25 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells should be

examined on a daily basis under a phase contrast microscope, and any changes in morphology or their adhesive properties must be noted in a Study Workbook (See **Section VII.F.3**)

2. Receipt of Cryopreserved Keratinocytes

Upon receipt of cryopreserved keratinocytes, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells and Establishing Cell Cultures

a) Thaw cells by putting ampules into a water bath at 37°C for as brief a time as possible. Do not thaw cells at room temperature or by hand. Seed the thawed cells into culture flasks as quickly as possible and with minimal handling.
b) Slowly (taking approximately 1-2 min) add 9 ml of Routine Culture Medium to the cells suspended in the cryoprotective solution and transfer cells into flasks containing pre-warmed Routine Culture Medium (See Table 1).
c) Incubate the cultures at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air until the cells attach to the flask, at which time the Routine Culture Medium should be removed and replaced with fresh Routine Culture Medium.
d) Unless otherwise specified, the cells should be incubated at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air and fed every 2-3 days until they exceed 50 % confluence (but less than 80 % confluent).

Table 1. Establishing Cell Cultures

Cells/25 cm^2 flask (in approximately 5 ml) 1 flask each cell concentration	6.25×10^4 (2500 cm^2)	1.25×10^5 (5000 cm^2)	2.25×10^5 (9000 cm^2)
Approximate Time to Subculture	96+ hours	72 - 96 hours	48 - 72 hours
Cells to 96-Well Plates	6 – 8 plates	6 – 8 plates	6 – 8 plates

Cell growth guidelines – actual growth of individual cell lots may vary.

4. Subculture of NHK Cells to 96-Well Plates

[Note: It is important that cells have overcome the lag growth phase when they are used for the test. Keratinocytes will be passaged only into the 96-well plates and will not be subcultured into flasks for use in later assays]

(a) When the keratinocyte culture in a 25 cm^2 flask exceeds 50 % confluence (but less than 80 % confluent), remove the medium and rinse the culture twice with 5 ml HEPES-BSS. The second rinse should be left on the cells for approximately 5 minutes. Discard the washing solution.
(b) Add 2 ml trypsin/EDTA solution to each flask and remove after 15 to 30 seconds. Incubate the flask at room temperature for 3 to 7 min. When more than 50 % of the cells become dislodged, rap the flask sharply against the palm of the hand.
(c) When most of the cells have become detached from the surface, rinse the flask with 5 ml of room temperature TNS.
(d) Then rinse the flask with 5 ml CMF-HBSS and transfer the cell suspension to a centrifuge tube.

(e) Pellet the cells by centrifugation for 5 min at approximately 220 x g. Remove the supernatant by aspiration.
(f) Resuspend the keratinocyte pellet by gentle trituration (to have single cells) in Routine Culture Medium. It is important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension using a hemocytometer or cell counter.
(g) Prepare a cell suspension of 0.8 - 1x10^4 cells/ml in Routine Culture Medium. Using a multi-channel pipette, dispense 250 µl PBS only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate. In the remaining wells, dispense 250 µl of the cell suspension (2x10^3 – 2.5x10^3 cells/well). Prepare one plate per chemical to be tested.
(h) Incubate cells (37ºC ± 1ºC, 90 % ± 5.0 % humidity, and 5 % ± 1 % CO_2/air) so that cells form a 30+ % monolayer (~48-72 h). This incubation period assures cell recovery and adherence and progression to exponential growth phase.
(i) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

5. Determination of Doubling Time

a) Establish cells in culture and trypsinize cells as per **Section C.4** for subculture. Resuspend cells in appropriate culture medium. Use Table 1 to determine seeding densities.
b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).
c) After 4-6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue). Determine the total number of cells and document. Repeat sampling at 24 hr, 48 hr, 72 hr, and 96 hr post inoculation. Change culture medium at 72 hr or sooner in remaining dishes if indicated by pH drop.
d) Plot cell concentration (per ml of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. The doubling time will be in the log (exponential) phase of the growth curve. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Establishing the Positive Control Database

An historical database of IC_{50} values for the positive control chemical (Sodium Lauryl [dodecyl] Sulfate {SLS}) must be established and maintained by performing 10 concentration-response assays on the NHK cells.

1. Positive Control Chemical Preparation

The positive control chemical (SLS) is prepared in the same manner as the test chemical (**Sections E.1 and E.2**) by following the instructions and substituting "test chemical" with "SLS."

2. Range Finder Experiment

Before initiating the 10 concentration-response assays, a range finder experiment will be performed using eight concentrations of SLS by diluting the stock solution with a constant factor as per **Section E.3.a** and **E.3.b**. The eight chemical concentrations will be tested as per the test procedure outlined in **Section F** and analyzed as per procedures outlined in **Section G**.

3. Test Procedure

Once a range has been determined that satisfies the criteria in **Section E.3.b**, the definitive concentration-response assays shall use a $^{6}\sqrt{10} = 1.47$ dilution scheme centered on the IC_{50}. The Test Facility will perform two tests per day on five different days. The 95 % CI of the IC_{50} of SLS will be established and defined as an acceptance criterion for test sensitivity for the NHK NRU assay. The confidence intervals shall be calculated using the average of the individual IC_{50} values from each positive control assay performed. An example of an historical mean IC_{50} of SLS in NHK cultures is **4.4 µg/ml ± 0.97 µg/ml [**two standard deviations] (Triglia, 1989). All testing will follow the instructions in **Section F** using the 96-well plate configuration in Figure 1. The test meets acceptance criteria if the conditions in **Sections F.5.a.2** and **F.5.a.3** are met.

Figure 1. 96-Well Plate Configuration for Positive Control and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	b	b	b	b	b	b	b	b	b	b	b	b
B	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
C	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
D	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
E	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
F	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
G	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
H	b	b	b	b	b	b	b	b	b	b	b	b

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 – C_8$ = Test Chemicals or Positive Control (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)

E. Preparation of Test Chemicals

[Note: Test chemical must be freshly prepared immediately prior to use. Each stock dilution should have at least 1-2 ml total volume to ensure adequate solution for the test wells in a

single 96-well plate. The solutions must not be cloudy nor have noticeable precipitate. Test chemicals must be at room temperature before dissolving and diluting. Preparation under red or yellow light may be necessary, if rapid photodegradation is likely to occur.]

1. Dissolving Test Chemical

a) Approximately 200,000 μg (200 mg) of the test chemical will be weighed into a glass tube and the weight will be documented. Assay-specific culture medium will be added to the vessel so that the concentration is 2,000,000 μg/ml (2000 mg/ml) and mixed using the mixing procedures outlined in **Section E.1.c**. If complete solubility is achieved, then additional solubility procedures are not needed. The test chemical can then be prepared and diluted for use in an assay. If only partial solubility is achieved, then add additional medium in the steps outlined in Table 1 until the concentration is a minimum of 200,000 μg /ml. If complete solubility at 200,000 μg/ml in culture medium can't be attained, then repeat the solubility steps in Table 1 and **Section E.1.c** using the other solvent(s) in the solubility hierarchy. Test chemicals that are only soluble in DMSO or ethanol will be prepared at 2,000,000 μg/ml as the highest concentration of stock solution.

Table 2 Determination of Solubility

Solubility Data	Step 1	Step 2	Step 3
Total volume of medium added (ml)	0.1	0.5	1.0
Total volume of DMSO or ethanol added (ml)	0.1	0.5	1.0
Approximate solubility (μg /ml)	≥ 2,000,000	400,000	200,000

Example: If complete solubility is not achieved in 0.1 ml medium (Step 1), then 0.4 ml is added to obtain a total volume of 0.5 ml (Step 2). No additional weighing of chemical is needed. Chemical and medium are again mixed in an attempt to dissolve.

b) Each test chemical will be prepared such that the highest test concentration applied to the cells in each range finding experiment is 100,000 μg/ml in culture medium (10,000 μg/ml if DMSO or ethanol is used). If 100,000 μg/ml in culture medium cannot be achieved, then the highest concentration attainable will be used. If the range finding experiment shows that 10,000 μg/ml is not high enough for the range of chemicals dissolved in DMSO or ethanol to meet the acceptance criteria, then higher concentrations will be used for the definitive experiment.

c) The following mixing and solvent hierarchy will be followed in dissolving the test chemical:

1) Dissolve test chemical in Treatment Medium.
2) Gently mix. Vortex the tube (1 –2 minutes).
3) If test chemical hasn't dissolved, use sonication for up to 5 minutes.
4) If sonication doesn't work, then warm solution to 37°C.

If the test chemical doesn't dissolve (i.e., solution is cloudy or has precipitate) in the Treatment Medium, then follow the steps 1) through 4) using DMSO instead of Treatment Medium.

If the test chemical doesn't dissolve in DMSO, then follow steps 1) through 4) using ethanol instead of DMSO.

d) For the range finding experiments, the highest 2x concentration of test chemical dissolved only in culture medium will be 200,000 μg/ml (200 mg/ml). The highest 2x concentration of test chemical first dissolved in DMSO or ethanol then transferred to culture medium will be 20,000 μg/ml (20 mg/ml). Dissolve test chemical in appropriate medium/solvent (at 200-fold the desired final test concentration in the case of DMSO or ethanol solvents, i.e., 20,000 μg/ml). The final solvent (DMSO or ethanol) concentration for application to the cells should be kept at a constant level of 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in medium before application to NHK cells.
Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme
1) Label eight tubes 1 – 8. Add 0.9 ml solvent (e.g., DMSO or ethanol) to tubes 2 -- 8.
2) Prepare stock solution of 2,000,000 μg test chemical/ml solvent in tube # 1.
3) Add 0.1 ml of 2,000,000μg/ml dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 200,000 μg/ml).
4) Add 0.1 ml of 200,000 μg/ml dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 20,000 μg/ml)
5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.
6) Since each concentration is 200 fold greater than the concentration to be tested, dilute 1 part dissolved chemical in each tube with 99 parts of culture medium (e.g., 0.1 ml of test chemical in DMSO + 9.9 ml culture medium) to derive the 8 2x concentrations for application to NHK cells. Each test chemical concentration will then contain 1 % v/v solvent. The NHK cells will have 0.125 ml of culture medium in the wells prior to application of the test chemical. By adding 0.125 ml of the appropriate 2x test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 10,000 μg/ml) in a total of 0.250 ml and the solvent concentration in the wells will be 0.5% v/v.

Check carefully to determine whether the chemical is still dissolved after the transfer from solvent stock solution to medium, and reduce the highest test concentration, if necessary. Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Measure the pH of the highest concentration of the test chemical in culture medium using pH paper. Document the pH and note the color of the medium for all dilutions. Do not adjust the pH.

3. Concentrations of Test Chemical

a) Range Finder Experiment

Test eight concentrations of the test chemical/PC by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

b) Main Experiment

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller (e.g., $\sqrt[6]{10} = 1.47$; NOTE: this dilution factor will be used for the definitive positive control assays [**Section VII.D.3]**). Cover the relevant concentration range (≥10 % and ≤ 90 % effect) with at least three points of a graded effect, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than three cytotoxic concentrations in the relevant range shall be repeated, where possible, with a smaller dilution factor. (Taking into account pipetting errors, a progression factor of 1.21 is regarded the smallest factor achievable.)

c) Test Chemical Dilutions

- A factor of $\sqrt[2]{10} = 3.16$ could be used for covering a large range: (e.g., 1 ⇒3.16 ⇒10 ⇒31.6 ⇒100 ⇒316 ⇒1000 ⇒3160 µg/ml).

- The simplest geometric concentration series (i.e., constant dilution / progression factor) are dual geometric series (e.g., a factor of 2). These series have the disadvantage of numerical values that permanently change between logs of the series: (e.g., *log0*-2, 4, 8; *log1*- 16, 32, 64; *log2*- 128, 256, 512; *log3*- 1024, 2048,).

- The decimal geometric series, first described by Hackenberg and Bartling (1959) for use in toxicological and pharmacological studies, has the advantage that independent experiments with wide or narrow dose factors can be easily compared because they share identical concentrations. Furthermore, under certain circumstances, experiments can even be merged together:

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The dosing factor of 3.16 ($= \sqrt[2]{10}$) divides a log into two equidistant steps, a factor of 2.15 ($= \sqrt[3]{10}$) divides a decade into three steps. The factor of 1.47 ($= \sqrt[6]{10}$) divides a log into six equidistant steps, and the factor of 1.21 ($= \sqrt[12]{10}$) divides the log into 12 steps.

For an easier biometrical evaluation of several related concentration response experiments use decimal geometric concentration series rather than dual geometric series. The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

- Determine which test chemical concentration is closest to the IC50 value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

F. Test Procedure

1. The NHK NRU assay for test chemicals will use the 96-well plate configuration shown in Figure 1.

2. Application of Test Chemical

   a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized. The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs and/or Corning/Transtar model 4878 disposable reservoir liners, 8-channel). The second method utilizes a "dummy" plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate (with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the plate containing cells. More volume than needed for the test plate (i.e. greater than 125 µl/well) should be in the wells of the dummy plate. At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing. A third option, though not a recommended option, is to transfer test chemical solutions well by well using a single channel pipettor or repeat pipettor. This option will increase the amount of time needed for test chemical application. The use of a repeat pipettor increases the risk of dislodging cells from the culture plate.
   b) After 24 - 72 h incubation of the cells, remove Routine Culture Medium from the cells by careful inversion of the plate (i.e., "dump") over an appropriate receptacle. Gently blot the plate on a sterile paper towel so that the monolayer is minimally disrupted. Do not use automatic plate washers for this procedure nor vacuum aspiration.
   c) Immediately add 125 µl of fresh Routine Culture Medium to each well. Add 125 µl of the appropriate concentration of test chemical, the PC, or the VC (see Figure 1 for the plate configuration).
   d) Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).
   e) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in developing the positive control database. This plate will follow the same schedule and procedures as used for the test chemical plates.

3. Microscopic Evaluation

After at least 46 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

a) Carefully remove (i.e., "dump") the Routine Culture Medium (with test chemical) and rinse the cells very carefully with 250 µl pre-warmed D-PBS. Remove the rinsing solution by gentle tapping and blot the plate. Add 250 µl NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3 h.
b) After incubation, remove the NR medium, and carefully rinse cells with 250 µl D-PBS.
c) Decant and blot D-PBS from the plate. (Optionally: centrifuge the reversed plate.)
d) Add exactly 100 µl NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.
e) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution.
f) Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader (spectrophotometer), using the blanks as a reference. Save raw data in the Excel format as provided by the Study Management Team.

5. Quality Check of Assay

a) *Test Acceptance Criteria*

1) A test meets acceptance criteria, if the IC_{50} for SLS is within the 95 % CI of the historical mean established by the Test Facility (as per **Section D**).
2) A test meets acceptance criteria if the mean OD_{540} of VCs is ≥ 0.3 and ≤ 1.1.
3) A test meets acceptance criteria if the left and the right mean of the VCs do not differ by more than 15 % from the mean of all VCs.

b) Checks for Systematic Cell Seeding Errors

The absolute value of optical density (OD_{540} of NRU) obtained in the untreated vehicle control may indicate whether the $2x10^3 – 2.5x10^3$ cells seeded per well have grown exponentially with normal doubling time during the assay. Historical optical densities observed during doubling time experiments can be used for comparison to determine exponential growth.

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

c) *Quality Check of Concentration-Response*

The IC_{50} derived from the concentration-response of the test chemicals should be backed by at least three responses between 10 and 90 % inhibition of NRU. If this is not the case, and the concentration progression factor can be easily reduced, reject the experiment and repeat it with a smaller progression factor. Numerical scoring of the cells (see **Section F.3**) should be determined and documented in the Study Workbook.

G. Data Analysis

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicates wells) per test concentration. This value is compared with the mean NRU of all VC values (provided VC values have met the VC acceptance criteria). Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet provided by the Study Management Team for determining cell viability and performing statistical analyses.

The concentration of a test chemical reflecting a 20 %, 50 %, and 80 % inhibition of cell viability (i.e., the IC_{20}, IC_{50}, and IC_{80}) is determined from the concentration-response by applying a Hill function to the concentration-response data. It will not be necessary for the Testing Facilities to derive the equation since statistical software (e.g., GraphPad PRISM® 3.0) specified by the Study Management Team shall be used to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical. In addition, the Study Management Team shall provide guidelines for calculating ICx values and confidence limits. The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the Study Management Team/biostatistician through the designated contacts in electronic format and hard copy upon completion of testing. The Study Management Team will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

Clonetics Normal Human Keratinocyte Systems Instructions for Use, AA-1000-4-Rev.03/00. (http://www.clonetics.com).

Hackenberg, U. and H. Bartling. 1959. Messen und Rechnen im pharmakologischen Laboratorium mit einem speziellen Zahlensystem (WL24-System). Arch. Exp. Pathol. Pharmakol. 235: 437-463.

Triglia, D., P.T. Wegener, J. Harbell, K. Wallace, D. Matheson, and C. Shopsis. 1989. Interlaboratory validation study of the keratinocyte neutral red bioassay from Clonetics Corporation. In *Alternative Methods in Toxicology*, Volume 7. A.M. Goldberg, ed., pp. 357-365. Mary Ann Liebert, Inc., New York.

IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**

(Print or type name)

_____ _____

Testing Facility STUDY DIRECTOR **DATE**

(Print or type name)

Appendix C3

Test Method Protocol for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test (Phase Ib)

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TEST METHOD PROTOCOL
for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an *In Vitro* Validation Study
Phase Ib

November 15, 2002
Revised November 22, 2002
Revised by IIVS Nov. 26, 2002

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test
A Test for Basal Cytotoxicity
Phase Ib

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the BALB/c 3T3 Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and supports the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

The 3T3 NRU test will be performed to analyze the *in vitro* toxicity of three (3) blinded/coded test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for the predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals: *Blinded Chemicals (3)*

B. Controls:

Positive:	Sodium Lauryl Sulfate
Vehicle (Negative):	Assay medium (DMEM containing 5% NBCS, 4 mM L-Glutamine, 100 IU/mL Penicillin, 100 µg/mL Streptomycin)
Solvent (as needed):	Assay medium with appropriate solvent used to prepare the test chemicals (**Section VII.E**)

IV. TESTING FACILITY AND KEY PERSONNEL

1) Name:
2) Address:
3) Study Director:
4) Laboratory Technician(s):
5) Scientific Advisor:
6) Quality Assurance Director:
7) Safety Manager:
8) Facility Management:

A. Test Schedule

1) Proposed Experimental Initiation Date:
2) Proposed Experimental Completion Date:
3) Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{logIC50} - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test chemical preparation, incubator function); all optical density data obtained from the

spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

1. Cell Lines

BALB/c 3T3 cells, clone 31
CCL-163, LGC Reference Materials, Customer Service, Queens Road, Teddington, Middlesex, TW110LY, UK
CCL-163, American Type Culture Collection [ATCC], Manassas, VA, USA)

2. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

1) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
2) Laminar flow clean bench/cabinet (standard: "biological hazard")
3) Water bath: 37ºC ± 1ºC
4) Inverse phase contrast microscope
5) Sterile glass tubes with caps (e.g., 5 mL)
6) Centrifuge (optionally: equipped with microtiter plate rotor)
7) Laboratory balance
8) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
9) Shaker for microtiter plates
10) Cell counter or hemocytometer
11) Pipetting aid
12) Pipettes, pipettors (multi-channel and single channel), dilution block
13) Cryotubes
14) Tissue culture flasks (e.g., 75 - 80 cm^2, 25 cm^2)
15) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Falcon tissue culture-treated)
16) pH paper (wide and narrow range)

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of 3T3 cells.]

3. Chemicals, Media, and Sera

- Dulbecco's Modification of Eagle's Medium (DMEM) without L-Glutamine; should have high glucose [4.5gm/l] (e.g., ICN-Flow Cat. No. 12-332-54)
- L-Glutamine 200 mM (e.g., ICN-Flow # 16-801-49)
- New Born Calf Serum (NBCS or NCS) (e.g., Biochrom # SO 125)
- 0.05 % Trypsin/0.02 % EDTA solution (e.g., SIGMA T 3924, ICN-Flow, # 16891-49)
- Phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}(for trypsinization)

- Hanks' Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+}(CMF-HBSS)
- Dulbecco's Phosphate Buffered Saline (D-PBS) [formulation containing calcium and magnesium cations; glucose optional] (for rinsing)
- Penicillin/streptomycin solution (e.g. ICN-Flow # 16-700-49)
- Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
- Dimethyl sulfoxide (DMSO), U.S.P. analytical grade (Store under nitrogen @ -20ºC)
- Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
- Glacial acetic acid, analytical grade
- Distilled H_2O or any purified water suitable for cell culture (sterile)
- Sterile paper towels (for blotting 96-well plates)

[Note: Due to lot variability of NBCS/NCS, first check a lot for growth stimulating properties with 3T3 cells (approximately 20-24 h doubling time) and then reserve a sufficient amount of NBCS/NCS. May use pre-tested serum lot from Phase Ia of the validation study if the serum has been stored under appropriate conditions and shelf-life has not expired.]

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard). All methods and procedures will be adequately documented.]

1. Media

DMEM (buffered with sodium bicarbonate) supplemented with (final concentrations in DMEM are quoted):

a) for freezing (Freeze Medium); contains 2X concentration of NBCS/NCS and DMSO of final freezing solution

40 %	NBCS/NCS
20 %	DMSO

b) for routine culture (Routine Culture Medium)

10 %	NBCS/NCS
4 mM	Glutamine

c) for solubility testing and test chemical dilution (Chemical Dilution Medium)

4 mM	Glutamine
200 IU/mL	Penicillin
200 µg/mL	Streptomycin

d) for dilution of NR stock solution (NR Dilution Medium)

5 %	NBCS/NCS
4 mM	Glutamine
100 IU/mL	Penicillin
100 µg/mL	Streptomycin

[Note: The Chemical Dilution Medium with test chemical will dilute the serum concentration of the Routine Culture Medium in the test plate to 5 %. Serum proteins may mask the toxicity of the test substance, but serum cannot be totally excluded because cell growth is markedly reduced in its absence.]

Completed media formulations should be kept at approximately 2-8° C and stored for no longer than two weeks.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay (e.g., SIGMA #N2889, 3.3 mg/mL). Store liquid tissue culture-grade NR Stock Solution at the storage conditions and shelf-life period recommended by the manufacturer.

If the liquid form is not available, the following formulation can be prepared.

EXAMPLE: 0.33 g NR Dye powder in 100 mL H_2O

The NR Stock Solution (powder in water) should be stored in the dark at room temperature for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

1 mL (3.3 mg NR dye/mL)	NR Stock Solution
99 mL	NR Dilution Medium (pre-warmed to 37° C)

The final concentration of the NR Medium is 33 μg NR dye/mL.
[Note: The NR medium may be centrifuged at approximately 600 x g for 10 min (to remove NR crystals). The NR Medium shall be filtered (e.g., Millipore filtering, 0.2 – 0.45 μm pore size) to reduce NR crystals. The temperature of the NR Medium should be maintained at 37° C (e.g., in a waterbath) before adding tothe cells and will be used within 15 minutes after removing from 37° C storage. Aliquots of NR Medium can be made on the day of testing and maintained at 37° C for later use.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

BALB/c 3T3 cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 75 - 80 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells

should be examined on a daily (i.e., on workdays) basis under a phase contrast microscope, and any changes in morphology or their adhesive properties noted in a Study Workbook.

2. Receipt of Cryopreserved BALB/c 3T3 Cells

Upon receipt of cryopreserved BALB/c 3T3 cells, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells

Thaw cells by putting ampules into a water bath at 37°C ± 1ºC. Leave for as brief a time as possible.

a) Resuspend the cells in pre-warmed Routine Culture Medium and transfer into pre-warmed Routine Culture Medium in a tissue-culture flask.
b) Incubate at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air.
c) When the cells have attached to the bottom of the flask (within 4 to 24 h), decant the supernatant and replace with fresh pre-warmed (37ºC) medium. Culture as described above.
d) Passage at least two times before using the cells in a cytotoxicity test.

A fresh batch of frozen cells from the stock lot of cells should be thawed out and cultured approximately every two months. This period resembles a sequence of about 18 passages.

4. Routine Culture of BALB/C 3T3 Cells

When cells exceed 50 % confluence (but less than 80 % confluent) they should be removed from the flask by trypsinization:

a) Decant medium, rinse cultures with 5 mL PBS or Hanks' BSS (without Ca^{2+}, Mg^{2+}) per 25 cm^2 flask (15 mL per 75 cm^2 flask). Wash cells by gentle agitation to remove any remaining serum that might inhibit the action of the trypsin.
b) Discard the washing solution.
c) Add 1-2 mL trypsin-EDTA solution per 25 cm^2 to the monolayer for a few seconds (e.g., 15-30 seconds).
d) Remove excess trypsin-EDTA solution and incubate the cells at room temperature.
e) After 2-3 minutes (min), lightly tap the flask to detach the cells into a single cell suspension.

5. Cell Counting

After detaching the cells, add 0.1-0.2 mL of pre-warmed (37ºC) Routine Culture Medium/cm^2 to the flask (e.g., 2.5 mL for a 25 cm^2 flask). Disperse the monolayer by gentle trituration. It is important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension obtained using a hemocytometer or cell counter (e.g., Coulter counter).

6. Subculture of Cells

After determination of cell number, the culture can be sub-cultured into other flasks or seeded into 96-well microtiter plates. BALB/c 3T3 cells are routinely passaged at suggested cell densities as listed in the table (approximate doubling time is 20-24 h). The individual laboratories will need to determine and adjust the final density to achieve appropriate growth.

Table 1. Cell Densities for Subculturing

Days in Culture	Seeding Density (cells/cm^2)	Total Cells per 25 cm^2 flask	Total Cells per 75 cm^2 flask
2	16800	4.2×10^5	1.26×10^6
3	8400	2.1×10^5	6.3×10^5
4	4200	1.05×10^5	3.15×10^5

[Note: It is important that cells have overcome the lag growth phase when they are used for the test.]

7. Freezing Cells (procedure required only if current stock of cells is depleted)

Stocks of BALB/c 3T3 cells can be stored in sterile, freezing tubes in a liquid nitrogen freezer. DMSO is used as a cryoprotective agent.

a) Centrifuge trypsinized cells at approximately 200 x g.
b) Suspend the cells in cold Routine Culture Medium (half the final freezing volume) so a final concentration of $1\text{-}5 \times 10^6$ cells/mL can be attained.
c) Slowly add cold Freeze Medium to the cells so that the solvent will equilibrate across the cell membranes. Bring the cell suspension to the final freezing volume. The final cell suspension will be 10 % DMSO. Aliquot the cell suspension into freezing tubes and fill to 1.8 mL.
d) Place the tubes into an insulated container (e.g., styrofoam trays) and place in a freezer (-70 to -80°C) for 24 h. This gives a freezing rate of approximately 1°C/min. The laboratory needs to ensure that the freezing protocol is applicable to the 3T3 cells and that the cells are viable when removed from cryopreservation.
e) Place the frozen tubes into liquid nitrogen for storage.

8. Preparation of Cells for Assays

e) Cultured cells that are going to be used in seeding the 96-well plates should be fed fresh medium the day before subculturing to the plates. On the day of plate seeding, prepare a cell suspension of 2.5×10^4 cells/mL in Routine Culture Medium. Using a multi-channel pipette, dispense 100 µl Routine Culture Medium only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate (See **Section VII.F.1**). In the remaining wells, dispense 100 µl of a cell suspension of 2.5×10^4 cells/mL (= 2.5×10^3 cells/well). The seeding density should be noted to ensure that the cells in the control wells are not overgrown after three days (i.e., 24 h incubation

in step **b** and 48 h exposure to test chemicals). Prepare one plate per chemical to be tested.

e) Incubate cells for 24 ± 1 h (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air) so that cells form a less than half confluent monolayer. This incubation period assures cell recovery and adherence and progression to exponential growth phase.

e) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

9. Determination of Doubling Time

a) A cell doubling time procedure was performed on the initial lot of cells that was used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined in Phase Ib if there is a change in the lot of cells used. Establish cells in culture and trypsinize cells as per **Section VII.C.4** for subculture. Resuspend cells in NR Dilution Medium (5 % NBCS/NCS). Seed cells at 4200 cells/cm^2.

b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).

c) After 4 - 6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue) if Study Director sees a need. Use appropriate size exclusion limits if using a Coulter counter. Determine the total number of cells and document. Repeat sampling at 24 h, 48 h, 72 h, and 96 h post inoculation. Change culture medium at 72 h or sooner in remaining dishes if indicated by pH drop.

d) Plot cell concentration (per mL of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Solubility Test

The preference of solvent for dissolving test chemicals is medium, DMSO, and then ethanol. Solubility shall be determined in a step-wise procedure that involves attempting to dissolve a test chemical at a relatively high concentration with the sequence of mechanical procedures specified in **Section VII.D.2.a.** If the chemical does not dissolve, the volume of solvent is increased so as to decrease the concentration by a factor of 10, and then the sequence of mechanical procedures in **Section VII.D.2.a** are repeated in an attempt to solubilize the chemical at the lower concentrations. For testing solubility in medium, the starting concentration is 20,000 μg/ml (i.e., 20 mg/mL) in Tier 1, but for DMSO and ethanol the starting concentration is 200,000 μg/ml (i.e., 200 mg/mL) in Tier 2. Weighing out chemical for each solvent (i.e., medium, DMSO, ethanol) can be done all at once, if convenient, but solubility testing (at each tier that calls for more than one solvent) is designed to be sequential - medium, then DMSO, then ethanol – in accordance with the solvent hierarchy (see **Figure 1**). This allows for testing to stop, rather than continue testing with less preferred solvents, if the test chemical dissolves in a more preferred solvent. For example, if a chemical is soluble in medium at a particular tier, testing may stop. Likewise, if a chemical is soluble in DMSO

at any tier, testing need not continue with ethanol. However, since the issue of primary importance is testing the solvents and concentrations of test chemical required by any one tier, sequential testing of solvents may be abandoned if the lab can test more efficiently in another way.

1. Determination of Solubility

a) Tier 1 begins with testing 20 mg/mL in Chemical Dilution Medium (see **Table 2**). Approximately 10 mg (10,000 μg) of the test chemical will be weighed into a glass tube and the weight will be documented. A volume of Chemical Dilution Medium, approximately 0.5 mL, will be added to the vessel so that the concentration is 20,000 μg/ml (20 mg/mL). The solution is mixed as specified in **Section VII.D.2.a.** If complete solubility is achieved in medium, then additional solubility procedures are not needed.

b) If the test chemical is insoluble in Chemical Dilution Medium, proceed to Tier 2 by adding enough medium, approximately 4.5 mL, to attempt to dissolve the chemical at 2 mg/mL by using the sequence of mixing procedures specified in **Section VII.D.2.a**. If the test chemical dissolves in Chemical Dilution Medium at 2 mg/mL, no further procedures are necessary. If the test chemical does NOT dissolve, weigh out approximately 100 mg test chemical in a second glass tube and add enough DMSO to make the total volume approximately 0.5 mL (for 200 mg/mL). In another glass tube, also add approximately 100 mg test chemical to enough ethanol to make the total volume approximately 0.5 mL (for 200 mg/mL). Mix both solutions as specified in **Section VII.D.2.a** in an attempt to solubilize the test chemical. If the chemical is soluble in either solvent, no additional solubility procedures are needed.

c) If the chemical is NOT soluble in Chemical Dilution Medium, DMSO, or ethanol at Tier 2, then continue to Tier 3 in Table 2 by adding enough solvent to increase the volume of the three Tier 2 solutions by 10 and attempt to solubilize again using the sequence of mixing procedures in **Section VII.D.2.a.** If the test chemical dissolves, no additional solubility procedures are necessary. If the test chemical does NOT dissolve, continue with Tier 4 and, if necessary, Tier 5 using DMSO and ethanol. Tier 4 begins by diluting the Tier 3 samples with DMSO or ethanol to bring the total volume to 50 mL. The mixing procedures in **Section VII.D.2.a** are again followed to attempt to solubilize the chemical. Tier 5 is performed, if necessary, by weighing out another two more samples of test chemical at ~10 mg each and adding ~50 mL DMSO or ethanol for a 200 μg/mL solution, and following the mixing procedures in **Section VII.D.2.a**.

Example: If complete solubility is not achieved at 20,000 μg/mL in Chemical Dilution Medium at Tier 1 using the mixing procedures specified in **Section VII.D.2.a,** then the procedure continues to Tier 2 by diluting the solution to 5 mL and mixing again as specified in **Section VII.D.2.a.** If the chemical is not soluble in Chemical Dilution Medium, two samples of ~ 100 mg test chemical are weighed to attempt to solubilize in DMSO and ethanol at 200,000 μg/mL (i.e., 200 mg/mL). Solutions are mixed following the sequence of procedures prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved at Tier 2, then the solutions (Chemical Dilution Medium, DMSO, and ethanol) prepared in Tier 2 are diluted by 10 so as to test 200 μg/mL in media, and 20,000 μg/mL in DMSO and

ethanol. This advances the procedure to Tier 3. Solutions are again mixed as prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved in Tier 3, the procedure continues to Tier 4, and to 5 if necessary (see **Figure 1** and **Table 2**).

Table 2 Determination of Solubility in Chemical Dilution Medium, DMSO, or Ethanol

TIER	1	2	3	4	5
Total Volume Chemical Dilution Medium	0.5 mL	5 mL	50 mL		
Concentration of Test Chemical (Add ~10 mg to a tube. Add enough medium to equal the first volume. Dilute to subsequent volumes if necessary.)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	200 µg/mL (0.20 mg/mL)		
Total Volume DMSO/Ethanol		0.5 mL	5 mL	50 mL	
Concentration of Test Chemical (Add ~100 mg to a large tube. Add enough DMSO or ethanol to equal the first volume. Dilute to subsequent volumes if necessary.)		200,000 µg/mL (200 mg/mL)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	
Total Volume DMSO/Ethanol					50 mL
Concentration of Test Chemical (Add ~10 mg to a large tube. Add enough DMSO or ethanol to equal 50 mL.)					200 µg/mL (0.2 mg/mL)
Equivalent Concentration on Cells	10,000 µg/mL (10 mg/mL)	1000 µg/mL (1 mg/mL)	100 µg/mL (0.1 mg/mL)	10 µg/mL (0.01 mg/mL)	1 µg/mL (0.001 mg/mL)

NOTE: The amounts of test chemical weighed and Chemical Dilution Medium added may be modified from the amounts given above, provided that the targeted concentrations specified for each tier are tested.

Figure 1. Solubility Flow Chart

TIER 1

STEP 1:	20 mg/mL test chemical (TC) in 0.5 mL Chemical Dilution Medium: • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 2.

TIER 2

STEP 2:	2 mg/mL TC in medium – increase volume from STEP 1 by 10 (i.e., to 5 mL) • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 3.

STEP 3:	200 mg/mL TC in DMSO
	• if TC soluble, then **STOP**.
	• if TC insoluble, test at 200 mg/mL in ETOH.
	  • if TC soluble, then **STOP.**
	  • If TC insoluble, go to STEP 4.

TIER 3

STEP 4:	0.2 mg/mL TC in medium – increase volume from STEP 2 by 10 (i.e., to 50 mL)
	• if TC soluble, then **STOP**.
	• if TC insoluble, test at 20 mg/mL in DMSO – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, test at 20 mg/mL in ETOH – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 5.

TIER 4

STEP 5:	2 mg/mL TC in DMSO – increase volume from STEP 4 by 10 (i.e., to 50 mL)
	• if TC soluble, then **STOP.**
	• if TC insoluble, test at 2 mg/mL in ETOH – increase volume from STEP 4 by 10 (i.e., to 50 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 6.

TIER 5

STEP 6:	0.2 mg/mL TC in 50 mL DMSO • if TC soluble, then **STOP.** • if TC insoluble, test at 0.2 mg/mL in 50 mL ETOH • **STOP**

2. Mechanical Procedures

a) The following hierarchy of mixing procedures will be followed to dissolve the test chemical:

1) Add test chemical to solvent as in Tier 1 of **Table 2**.
2) Gently mix. Vortex the tube (1 –2 minutes).
3) If test chemical hasn't dissolved, use sonication for up to 5 minutes.
4) If sonication doesn't work, then warm solution to 37°C. This can be performed by warming 5 mL tubes in a 37°C water bath for at least 5-10 minutes before evaluating solubility. Warm larger vessels for at least 15-20 minutes in a 37°C water bath before evaluating solubility.
5) Proceed to Tier 2 (and Tiers 3-5, if necessary of Table 2 and repeat procedures 2-4).

b) The preference of solvent for dissolving test chemicals is Chemical Dilution Medium, DMSO, and then ethanol. Thus, if (all solvents for a particular tier are tested simultaneously and) a test chemical dissolves in more than one solvent, then the choice of solvent follows this hierarchy. For example, if, at any tier, a chemical is soluble in Chemical Dilution Medium and DMSO, but not ethanol, the choice of solvent would be medium. If the chemical were insoluble in medium, but soluble in DMSO and ethanol, the choice of solvent would be DMSO.

After the lab has determined the preferred solvent for the test chemical and before proceeding to the cytotoxicity testing, the Study Director will discuss the solvent selection with the Study Management Team (SMT) of the validation study. The SMT will relate what solvent should be used in the assay for each chemical.

E. Preparation of Test Chemicals

[Note: Preparation under red or yellow light is recommended to preserve chemicals that degrade upon exposure to light.]

1. Test Chemicals in Solution

a) Allow test chemicals to equilibrate to room temperature before dissolving and diluting.

b) Prepare test chemical immediately prior to use. The solutions must not be cloudy nor have noticeable precipitate. Each stock dilution should have at least 1-2 mL total volume to ensure adequate solution for the test wells in a single 96-well plate.

c) For chemicals dissolved in DMSO or ethanol, the final DMSO or ethanol concentration for application to the cells must be 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations.

d) The stock solution for each test chemical should be prepared at the highest concentration found to be soluble in the solubility test. Thus, the highest test concentration applied to the cells in each range finding experiment is:

- 0.5 times the highest concentration found to be soluble in the solubility test, if the chemical was soluble in Chemical Dilution Medium, or
- 1/200 the highest concentration found to be soluble in the solubility test if the chemical was soluble in ethanol or DMSO.

e) The seven lower concentrations in the range finding experiment would then be prepared by successive dilutions that decrease by one log unit each. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in Chemical Dilution Medium before application to 3T3 cells.

Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme

If DMSO was determined to be the preferred solvent at Tier 2 of the solubility test (i.e., 200,000 μg/mL), dissolve the chemical in DMSO at 200,000 μg/mL for the chemical stock solution.

1) Label eight tubes 1 – 8. Add 0.9 mL solvent (e.g., DMSO) to tubes 2 -- 8.
2) Prepare stock solution of 200,000 μg test chemical/mL solvent in tube # 1.
3) Add 0.1 mL of 200,000 μg/mL dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 20,000 μg/mL).
4) Add 0.1 mL of 20,000 μg/mL dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 2,000 μg/mL)
5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.
6) Since each concentration is 200 fold greater than the concentration to be tested, make a 1:100 dilution by diluting 1 part dissolved chemical in each tube with 99 parts of Chemical Dilution Medium (e.g., 0.1 mL test chemical in DMSO + 9.9 mL Chemical Dilution Medium) to derive the eight 2X concentrations for application to 3T3 cells. Each 2X test chemical concentration will then contain 1 % v/v solvent. The 3T3 cells will have 0.05 mL Routine Culture Medium in the wells prior to application of the test chemical. By adding 0.05 mL of the appropriate 2X test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 1,000 μg/mL) in a total of 0.1 mL and the solvent concentration in the wells will be 0.5% v/v.
7) A test article prepared in Chemical Dilution Medium, DMSO, or ethanol may precipitate upon transfer into the Routine Culture Medium. The 2X dosing solutions should be evaluated for precipitates and the results will be recorded in the workbook. It will be permissible to test all of the dosing solutions in the dose range finding assay only. Doses containing test article precipitates should be avoided, and will not be used in the ICx determinations for either the range finding experiments or the definitive tests.

Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Measure the pH of the highest concentration of the test chemical in culture medium using pH paper. Document the pH and note the color of the medium for all dilutions. Do not adjust the pH.

3. Concentrations of Test Chemical

a) Range Finder Experiment

Test eight concentrations of the test chemical by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

The data from any well that has precipitate will be excluded from any calculations.

b) Main Experiment

[Note: After the range finding assay is completed, the concentration-response experiment shall be performed three times on three different days for each chemical (i.e., one plate per day per chemical.]

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller ($\sqrt[6]{10} = 1.47$). Cover the relevant concentration range ($\geq$10 % and $\leq$ 90 % effect) preferably with three points of a graded effect, but with a minimum of two points, one on each side of the IC_{50} value, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than one cytotoxic concentration on each side of the IC_{50} value shall be repeated, where possible, with a smaller dilution factor. In addition, the dilution scheme shall be adjusted in subsequent replicate assays (i.e., definitive assays), if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. (Taking into account pipetting errors, a progression factor of 1.21 is regarded the smallest factor achievable.)

Determine which test chemical concentration is closest to the IC_{50} value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

Maximum Doses to be Tested in the Main Experiments

If minimal or no cytotoxicity was measured in the dose range finding assay, a maximum dose for the main experiments will be established as follows:

- For test chemicals prepared in Chemical Dilution Medium, the highest test article concentration that may be applied to the cells in the main experiments will be either 100 mg/mL, or the maximum soluble dose. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of Chemical Dilution Medium will be added to the vessel so that the concentration is 200,000 µg/mL (200 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in medium, then 7 additional serial stock dosing solutions may be prepared from the 200 mg/mL 2X stock. If the test chemical is insoluble in medium at 200 mg/ml, proceed by adding medium, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- For test chemicals prepared in either DMSO or ethanol, the highest test article concentration that may be applied to the cells in the main experiments will be either 2.5 mg/mL, or less, depending upon the maximum solubility in solvent. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of the appropriate solvent (determined from the original solubility test) will be added to the vessel so that the concentration is 500,000 µg/mL (500 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in the solvent, then 7 additional serial stock dosing solutions may be prepared from the 500 mg/mL 200X stock. If the test chemical is insoluble in solvent at 500 mg/ml, proceed by adding solvent, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

c) Test Chemical Dilutions

The dosing factor of 3.16 (= $\sqrt[2]{10}$) divides a log into two equidistant steps, a factor of 2.15 (= $\sqrt[3]{10}$) divides a decade into three steps. The factor of 1.47 (= $\sqrt[6]{10}$) divides a log into six equidistant steps, and the factor of 1.21 (= $\sqrt[12]{10}$) divides the log into 12 steps.

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

F. Test Procedure

1. 96-Well Plate Configuration

The 3T3 NRU assay for test chemicals will use the 96-well plate configuration shown in **Figure 2**.

Figure 2. 96-Well Plate Configuration for Positive Control and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	b	b	b	b	b	b	b	b	b	b	b	b
B	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
C	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
D	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
E	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
F	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
G	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
H	b	b	b	b	b	b	b	b	b	b	b	b

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 – C_8$ = Test Chemicals or Positive Control (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)

2. Application of Test Chemical

a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized. The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs and/or Corning/Transtar model 4878 disposable reservoir liners, 8-channel). The second method utilizes a "dummy" plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate (with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the plate containing cells. More volume than needed for the test plate (i.e. greater than 50 µl/well) should be in the wells of the dummy plate. At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing.

b) After 24 h ± 1 h incubation of the cells, remove Routine Culture Medium from the cells by careful inversion of the plate (i.e., "dump") over an appropriate receptacle. Gently blot the plate on a sterile paper towel so that the monolayer is minimally disrupted. Do not use automatic plate washers for this procedure nor vacuum aspiration.

c) Immediately add 50 µL of fresh pre-warmed Routine Culture Medium to all of the wells, including the blanks. Add 50 µL of Chemical Dilution Medium to the blank wells. Then add 50 µL Chemical Dilution Medium containing either the appropriate

concentration of test chemical, the PC, or the VC (see **Figure 2** for the plate configuration). The solutions will be transferred from the dummy plate to the test plate by adding the vehicle control first then lowest to highest dose so that the same pipette tips on the eight channel pipettor can be used for the whole plate.

d) Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).

e) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in the development of the positive control database in Phase Ia of the Validation Study. The mean IC_{50} and ± two standard deviations (SD) of the IC_{50} of SLS (mutually agreed upon by the Testing Facility and the SMT) are the values that will be used as an acceptance criterion for test sensitivity for the 3T3 NRU assay. This plate will follow the same schedule and procedures as used for the test chemical plates.

3. Microscopic Evaluation

After at least 46 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

a) Carefully remove (i.e., "dump") the medium with test chemical and rinse the cells very carefully with 250 µL pre-warmed D-PBS. Remove the rinsing solution by dumping and remove excess by gently blotting on sterile paper towels. Add 250 µL NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3±0.1 h. Observe the cells briefly during the NR incubation (e.g., at 1, 2, and 3 h – Study Director's discretion) for NR crystal formation. Record observations in the Study Workbook. Study Director can decide to reject the experiment if excessive NR crystallization has occurred.

b) After incubation, remove the NR medium, and carefully rinse cells with 250 µl pre-warmed D-PBS.

c) Decant and blot D-PBS from the plate.
d) Add exactly 100 µl NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.
e) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution.
f) Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader (spectrophotometer), using the blanks as a reference. [Phase Ia data show the mean OD value for the plate blanks to be 0.051 ± 0.022 for 3T3 cells (± two standard deviations; data from 3 labs; N = 59). Use this value as a **guide** for assessment of the blank values.] Save raw data in the Excel format as provided by the SMT.

5. Quality Check of 3T3 NRU Assay

a) Test Acceptance Criteria

1) A test meets acceptance criteria, if the IC_{50} for SLS (PC) is within ± two (2) standard deviations of the historical mean established by the Test Facility (as per **VII.F.2.e**).
2) A test meets acceptance criteria if the corrected mean OD_{540} of VCs is ≥ 0.30 and ≤ 0.80.
3) A test meets acceptance criteria if the left and the right mean of the VCs do not differ by more than 15 % from the mean of all VCs.
4) A test meets acceptance criteria if a minimum of two points, one on each side of the IC_{50} value, are determined and fall within the range ≥10 % and ≤ 90 % effect.

[Note: All acceptance criteria must be met for an assay to be considered acceptable.]

b) Checks for Systematic Cell Seeding Errors

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

c) Quality Check of Concentration-Response

The IC_{50} derived from the concentration-response of the test chemicals will be backed by preferably three responses ≥ 10 % and ≤ 90 % inhibition of NRU and at least two responses, one on either side of the IC_{50} value (see **VII.E.3.b**). If this is not the case, and the concentration progression factor can be easily reduced, reject the experiment and repeat it with a smaller progression factor. In addition, the dilution scheme shall be adjusted in subsequent replicate assays, if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. Numerical scoring of the cells (see **VII.F.3**) should be determined and documented in the Study Workbook.

G. Data Analysis

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicate well) per test concentration (blanks will be subtracted). This value is compared with the mean NRU of all VC values (provided VC values have met the VC acceptance criteria). Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet provided by the SMT for determining cell viability and performing statistical analyses.

The concentration of a test chemical reflecting a 20 %, 50 %, and 80 % inhibition of cell viability (i.e., the IC_{20}, IC_{50}, and IC_{80}) is determined from the concentration-response by applying a Hill function to the concentration-response data. Statistical software (e.g., GraphPad PRISM® 3.0) specified by the SMT shall be used to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical. In addition, the SMT shall provide guidelines for calculating ICx values and confidence limits. The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the SMT/biostatistician through the designated contacts in electronic format and hard copy upon completion of testing. The SMT will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

Hackenberg, U. and H. Bartling. 1959. Messen und Rechnen im pharmakologischen Laboratorium mit einem speziellen Zahlensystem (WL24-System). Arch. Exp. Pathol. Pharmakol. 235: 437-463.

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IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**
(Print or type name)

_____ _____

Test Facility STUDY DIRECTOR **DATE**
(Print or type name)

Appendix C4

Test Method Protocol for the Normal Human Epidermal Keratinocyte (NHK) Neutral Red Uptake Cytotoxicity Test (Phase Ib)

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TEST METHOD PROTOCOL
for the NHK Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an In Vitro Validation Study
Phase Ib

November 15, 2002
Revised November 22, 2002
Revised by IIVS Nov. 26, 2002

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The Normal Human Keratinocyte (NHK) Neutral Red Uptake Cytotoxicity Test
A Test for Basal Cytotoxicity
Phase Ib

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the Normal Human Keratinocyte (NHK) Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and is in support of the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. NHK Neutral Red Uptake Cytotoxicity Test

The NHK NRU test will be performed to analyze the *in vitro* toxicity of three (3) blinded/coded test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for the predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals: *Blinded chemicals (3)*

B. Controls:
Positive: Sodium Lauryl Sulfate
Vehicle (Negative): Assay medium
Solvent (as needed): Assay medium with appropriate solvent used to prepare the test chemicals (**Section VII.E**)

IV. TESTING FACILITY AND KEY PERSONNEL

- Name:
- Address:

- Study Director:
- Laboratory Technician(s):
- Scientific Advisor:
- Quality Assurance Director:
- Safety Manager:
- Facility Management:

A. Test Schedule

1. Proposed Experimental Initiation Date:
2. Proposed Experimental Completion Date:
3. Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A.. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{logIC50} - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test chemical preparation, incubator function); all optical density data obtained from the spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used unless otherwise noted.]

1. Cell Lines

Normal Human Epidermal Keratinocytes (NHK)

Non-transformed cells; from cryopreserved primary or secondary cells (**Clonetics #CC-2507 or equivalent).** Cells will be Clonetics NHK cells.

Cambrex [Cambrex Bio Science, 8830 Biggs Ford Road, Walkersville, MD 21793-0127

Cambrex Europe [Cambrex Bio Science Verviers, S.P.R.L. Parc Industriel de Petit Rechain, B-4800 Verviers, BELGIUM]

2. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

a) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
b) Laminar flow clean bench (standard: "biological hazard")
c) Water bath: 37ºC ± 1ºC
d) Inverse phase contrast microscope
e) Sterile glass tubes with caps (e.g., 5mL)
f) Centrifuge (optionally: equipped with microtiter plate rotor)
g) Laboratory balance
h) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
i) Shaker for microtiter plates
j) Cell counter or hemocytometer
k) Pipetting aid
l) Pipettes, pipettors (multi-channel and single channel), dilution block
m) Cryotubes
n) Tissue culture flasks (75 - 80 cm^2, 25 cm^2)
o) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Corning/COSTAR tissue culture-treated)
p) pH paper (wide and narrow range)

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of NHK.]

3. Chemicals, Media, and Sera

a) Keratinocyte Basal Medium without Ca^{++} (KBM®, Clonetics CC-3104) that is completed by adding the KBM® SingleQuots® (Clonetics CC-4131) to achieve the proper concentrations of epidermal growth factor, insulin, hydrocortisone,

antimicrobial agents, bovine pituitary extract, and calcium (e.g., Clonetics Calcium SingleQuots®, 300 mM $CaCl_2$, Clonetics # CC-4202).

b) HEPES Buffered Saline Solution (HEPES-BSS) (e.g., Clonetics # CC-5022)
c) 0.025 % Trypsin/EDTA solution (e.g., Clonetics # CC-5012)
d) Trypsin Neutralizing Solution (TNS) (e.g., Clonetics # CC-5002)
e) Phosphate Buffered Saline (PBS)
f) Dulbecco's Phosphate Buffered Saline (D-PBS) [formulation containing calcium and magnesium cations; glucose optional] (for rinsing)
g) Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
h) Dimethyl sulfoxide (DMSO), U.S.P analytical grade (Store under nitrogen @ -20ºC)
i) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
j) Glacial acetic acid, analytical grade
k) Hanks' Balanced Salt Solution without Ca^{2+} or Mg^{2+} (CMF-HBSS) (e.g., Invitrogen # 14170)
l) Distilled H_2O or any purified water suitable for cell culture (sterile)
m) Sterile paper towels (for blotting 96-well plates)

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard).). All methods and procedures will be adequately documented.]

1. Media

a) Routine Culture Medium/Treatment Medium

KBM® (Clonetics CC-3104) supplemented with KBM® SingleQuots® (Clonetics CC-4131) and Clonetics Calcium SingleQuots® (CC-4202) to make 500 mL medium. Final concentration of supplements in medium are:

0.0001 ng/mL	Human recombinant epidermal growth factor
5 µg/mL	Insulin
0.5 µg/mL	Hydrocortisone
30 µg/mL	Gentamicin
15 ng/mL	Amphotericin B
0.10 mM	Calcium
30 µg/mL	Bovine pituitary extract

Complete media should be kept at 2-8°C and stored for no longer than two weeks.

NOTE:
KBM® SingleQuots® contain the following stock concentrations and volumes:

0.1 ng/mL	hEGF	0.5 mL
5.0 mg/mL	Insulin	0.5 mL
0.5 mg/mL	Hydrocortisone	0.5 mL

30 mg/mL	Gentamicin, 15 ug/mL Amphotericin-B	0.5 mL
7.5 mg/mL	Bovine Pituitary Extract (BPE)	2.0 mL

Clonetics Calcium SingleQuots® are 2 mL of 300mM calcium.

165 ul of solution per 500 mL calcium-free medium equals 0.10 mM calcium in the medium.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay (e.g., SIGMA #N2889, 3.3 mg/mL). Store liquid tissue culture-grade NR Stock Solution at the storage conditions and shelf-life period recommended by the manufacturer.

If the liquid form is not available, the following formulation can be prepared.

EXAMPLE: 0.33 g NR Dye powder in 100 mL H_2O

The NR Stock Solution (powder in water) should be stored in the dark at room temperature for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

1 mL (3.3 mg NR dye/mL)	NR Stock Solution
99 mL	Routine Culture Medium (pre-warmed to 37° C.)

The final concentration of the NR Medium is 33 μg NR dye/mL.
[Note: The NR medium may be centrifuged at approximately 600 x g for 10 min (to remove NR crystals). The NR Medium shall be filtered (e.g., Millipore filtering, 0.2 – 0.45 μm pore size) used to reduce NR crystals. The temperature of the NR Medium should be maintained at 37° C (e.g., in a waterbath) before adding to the cells and will be used within 15 minutes after removing from 37° C storage. Aliquots of NR Medium can be made on the day of testing and maintained at 37° C. for later use.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

NHK cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 25 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells should be

examined on a daily (i.e., on workdays) basis under a phase contrast microscope, and any changes in morphology or their adhesive properties must be noted in a Study Workbook.

2. Receipt of Cryopreserved Keratinocytes

Upon receipt of cryopreserved keratinocytes, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells and Establishing Cell Cultures

a) Thaw cells by putting ampules into a water bath at 37°C for as brief a time as possible. Do not thaw cells at room temperature or by hand. Seed the thawed cells into culture flasks as quickly as possible and with minimal handling.
b) Slowly (taking approximately 1-2 min) add 9 mL of pre-warmed Routine Culture Medium to the cells suspended in the cryoprotective solution and transfer cells into flasks containing pre-warmed Routine Culture Medium (See Table 1).
c) Incubate the cultures at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air until the cells attach to the flask (within 4 to 24 h), at which time the Routine Culture Medium should be removed and replaced with fresh Routine Culture Medium.
d) Unless otherwise specified, the cells should be incubated at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air and fed every 2-3 days until they exceed 50 % confluence (but less than 80 % confluent).

Table 1. Establishing Cell Cultures

Cells/25 cm^2 flask (in approximately 5 mL) 1 flask each cell concentration	6.25 x 10^4 (2500/cm^2)	1.25 x 10^5 (5000/cm^2)	2.25 x 10^5 (9000/cm^2)
Approximate Time to Subculture	96+ hours	72 - 96 hours	48 - 72 hours
Cells to 96-Well Plates	6 – 8 plates	6 – 8 plates	6 – 8 plates

Cell growth guidelines – actual growth of individual cell lots may vary.

4. Subculture of NHK Cells to 96-Well Plates

[Note: It is important that cells have overcome the lag growth phase when they are used for the test. Keratinocytes will be passaged only into the 96-well plates and will not be subcultured into flasks for use in later assays]

a) When the keratinocyte culture in a 25 cm^2 flask exceeds 50 % confluence (but less than 80 % confluent), remove the medium and rinse the culture twice with 5 mL HEPES-BSS. The second rinse should be left on the cells for approximately 5 minutes. Discard the washing solution.
b) Add 2 mL trypsin/EDTA solution to each flask and remove after 15 to 30 seconds. Incubate the flask at room temperature for 3 to 7 min. When more than 50 % of the cells become dislodged, rap the flask sharply against the palm of the hand.
c) When most of the cells have become detached from the surface, rinse the flask with 5 mL of room temperature TNS. If more than one flask is subcultured, the same 5 mL of TNS may be used to rinse a total of up to 2 flasks.

d) Then rinse the flask with 5 mL CMF-HBSS and transfer the cell suspension to a centrifuge tube.
e) Pellet the cells by centrifugation for 5 min at approximately 220 x g. Remove the supernatant by aspiration.
f) Resuspend the keratinocyte pellet by gentle trituration (to have single cells) in Routine Culture Medium. It is important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension using a hemocytometer or cell counter.
g) Prepare a cell suspension –1.6 – 2.0 x10^4 cells/mL in Routine Culture Medium. Using a multi-channel pipette, dispense 250 µl Routine Culture Medium only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate. In the remaining wells, dispense 125 µl of the cell suspension (2x10^3 – 2.5x10^3 cells/well). Prepare one plate per chemical to be tested (see **Figure 2**, **Section VII.F.1**).
h) Incubate cells (37ºC ± 1ºC, 90 % ± 5.0 % humidity, and 5 % ± 1 % CO_2/air) so that cells form a 20+ % monolayer (~48-72 h). This incubation period assures cell recovery and adherence and progression to exponential growth phase.
i) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

5. Determination of Doubling Time

a) A cell doubling time procedure was performed on the initial lot of cells that was used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined in Phase Ib if there is a change in the lot of cells used. Establish cells in culture and trypsinize cells as per **Section VII.C.4** for subculture. Resuspend cells in appropriate culture medium. Use **Table 1** to determine seeding densities.
b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).
c) After 4-6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue). Determine the total number of cells and document. Repeat sampling at 24 hr, 48 hr, 72 hr, and 96 hr post inoculation. Change culture medium at 72 hr or sooner in remaining dishes if indicated by pH drop.
d) Plot cell concentration (per mL of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. The doubling time will be in the log (exponential) phase of the growth curve. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Solubility Test

The preference of solvent for dissolving test chemicals is medium, DMSO, and then ethanol. Solubility shall be determined in a step-wise procedure that involves attempting to dissolve a test chemical at a relatively high concentration with the sequence of mechanical procedures specified in **Section VII.D.2.a.** If the chemical does not dissolve, the volume of solvent is

increased so as to decrease the concentration by a factor of 10, and then the sequence of mechanical procedures in **Section VII.D.2.a** are repeated in an attempt to solubilize the chemical at the lower concentrations. For testing solubility in media, the starting concentration is 20,000 μg/ml (i.e., 20 mg/mL) in Tier 1, but for DMSO and ethanol the starting concentration is 200,000 μg/ml (i.e., 200 mg/mL) in Tier 2. Weighing out chemical for each solvent (i.e., media, DMSO, ethanol) can be done all at once, if convenient, but solubility testing (at each tier that calls for more than one solvent) is designed to be sequential - media, then DMSO, then ethanol – in accordance with the solvent hierarchy (see **Figure 1**). This allows for testing to stop, rather than continue testing with less preferred solvents, if the test chemical dissolves in a more preferred solvent. For example, if a chemical is soluble in medium at a particular tier, testing may stop. Likewise, if a chemical is soluble in DMSO at any tier, testing need not continue with ethanol. However, since the issue of primary importance is testing the solvents and concentrations of test chemical required by any one tier, sequential testing of solvents may be abandoned if the lab can test more efficiently in another way.

1. Determination of Solubility

a) Tier 1 begins with testing 20 mg/mL in Routine Culture Medium (see **Table 2**). Approximately 10 mg (10,000 μg) of the test chemical will be weighed into a glass tube and the weight will be documented. A volume of Routine Culture Medium, approximately 0.5 mL, will be added to the vessel so that the concentration is 20,000 μg/ml (20 mg/mL). The solution is mixed as specified in **Section VII.D.2.a.** If complete solubility is achieved in media, then additional solubility procedures are not needed.

b) If the test chemical is insoluble in medium, proceed to Tier 2 by adding enough medium, approximately 4.5 mL, to attempt to dissolve the chemical at 2 mg/mL by using the sequence of mixing procedures specified in **Section VII.D.2.a**. If the test chemical dissolves in medium at 2 mg/mL, no further procedures are necessary. If the test chemical does NOT dissolve, weigh out approximately 100 mg test chemical in a second glass tube and add enough DMSO to make the total volume approximately 0.5 mL (for 200 mg/mL). In another glass tube, also add approximately 100 mg test chemical to enough ethanol to make the total volume approximately 0.5 mL (for 200 mg/mL). Mix both solutions as specified in **Section VII.D.2.a** in an attempt to solubilize the test chemical. If the chemical is soluble in either solvent, no additional solubility procedures are needed.

Table 2 Determination of Solubility in Routine Culture Medium, DMSO, or Ethanol

TIER	1	2	3	4	5
Total Volume Medium	0.5 mL	5 mL	50 mL		
Concentration of Test Chemical (Add ~10 mg to a tube. Add enough medium to equal the first volume. Dilute to subsequent volumes if necessary.)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	200 µg/mL (0.20 mg/mL)		
Total Volume DMSO/Ethanol		0.5 mL	5 mL	50 mL	
Concentration of Test Chemical (Add ~100 mg to a large tube. Add enough DMSO or ethanol to equal the first volume. Dilute to subsequent volumes if necessary.)		200,000 µg/mL (200 mg/mL)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	
Total Volume DMSO/Ethanol					50 mL
Concentration of Test Chemical (Add ~10 mg to a large tube. Add enough DMSO or ethanol to equal 50 mL.)					200 µg/mL (0.2 mg/mL)
Equivalent Concentration on Cells	10,000 µg/mL (10 mg/mL)	1000 µg/mL (1 mg/mL)	100 µg/mL (0.1 mg/mL)	10 µg/mL (0.01 mg/mL)	1 µg/mL (0.001 mg/mL)

c) If the chemical is NOT soluble in media, DMSO, or ethanol at Tier 2, then continue to Tier 3 in **Table 2** by adding enough solvent to increase the volume of the three Tier 2 solutions by 10 and attempt to solubilize again using the sequence of mixing procedures in **Section VII.D.2.a.** If the test chemical dissolves, no additional solubility procedures are necessary. If the test chemical does NOT dissolve, continue with Tier 4 and, if necessary, Tier 5 using DMSO and ethanol. Tier 4 begins by diluting the Tier 3 samples with DMSO or ethanol to bring the total volume to 50 mL. The mixing procedures in **Section VII.D.2.a** are again followed to attempt to solubilize the chemical. Tier 5 is performed, if necessary, by weighing out another two more samples of test chemical at ~10 mg each and adding ~50 mL DMSO or ethanol for a 200 µg/mL solution, and following the mixing procedures in **Section VII.D.2.a**.

Example: If complete solubility is not achieved at 20,000 µg/mL in Routine Culture Medium at Tier 1 using the mixing procedures specified in **Section VII.D.2.a,** then the procedure continues to Tier 2 by diluting the solution to 5 mL and mixing again

as specified in **Section VII.D.2.a.** If the chemical is not soluble in medium, two samples of ~ 100 mg test chemical are weighed to attempt to solubilize in DMSO and ethanol at 200,000 μg/mL (i.e., 200 mg/mL). Solutions are mixed following the sequence of procedures prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved at Tier 2, then the solutions (media, DMSO, and ethanol) prepared in Tier 2 are diluted by 10 so as to test 200 μg/mL in media, and 20,000 μg/mL in DMSO and ethanol. This advances the procedure to Tier 3. Solutions are again mixed as prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved in Tier 3, the procedure continues to Tier 4, and to 5 if necessary (see **Figure 1** and **Table 2**).

NOTE: The amounts of test chemical weighed and Routine Culture Medium added may be modified from the amounts given above, provided that the targeted concentrations specified for each tier are tested.

Figure 1. Solubility Flow Chart

TIER 1

STEP 1:	20 mg/mL test chemical (TC) in 0.5 mL medium: • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 2.

TIER 2

STEP 2:	2 mg/mL TC in medium – increase volume from STEP 1 by 10 (i.e., to 5 mL) • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 3.
STEP 3:	200 mg/mL TC in DMSO j) if TC soluble, then **STOP**. k) if TC insoluble, test at 200 mg/mL in ETOH. l) if TC soluble, then **STOP.** m) If TC insoluble, go to STEP 4.

TIER 3

STEP 4:	0.2 mg/mL TC in medium – increase volume from STEP 2 by 10 (i.e., to 50 mL) • if TC soluble, then **STOP**. • if TC insoluble, test at 20 mg/mL in DMSO – increase volume from STEP 3 by 10 (i.e., to 5 mL). • if TC soluble, then **STOP.** • if TC insoluble, test at 20 mg/mL in ETOH – increase volume from STEP 3 by 10 (i.e., to 5 mL). • if TC soluble, then **STOP.** • if TC insoluble, then go to STEP 5.

TIER 4

STEP 5:	2 mg/mL TC in DMSO – increase volume from STEP 4 by 10 (i.e., to 50 mL) • if TC soluble, then **STOP.** • if TC insoluble, test at 2 mg/mL in ETOH – increase volume from STEP 4 by 10 (i.e., to 50 mL). • if TC soluble, then **STOP.** • if TC insoluble, then go to STEP 6.

TIER 5

STEP 6:	0.2 mg/mL TC in 50 mL DMSO • if TC soluble, then **STOP.** • if TC insoluble, test at 0.2 mg/mL in 50 mL ETOH • **STOP**

2. Mechanical Procedures

a) The following hierarchy of mixing procedures will be followed to dissolve the test chemical:

1) Add test chemical to solvent as in Tier 1 of Table 2.
2) Gently mix. Vortex the tube (1 –2 minutes).
3) If test chemical hasn't dissolved, use sonication for up to 5 minutes.
4) If sonication doesn't work, then warm solution to 37°C. This can be performed by warming 5 mL tubes in a 37°C water bath for at least 5-10 minutes before evaluating solubility. Warm larger vessels for at least 15-20 minutes in a 37°C water bath before evaluating solubility.
5) Proceed to Tier 2 (and Tiers 3-5, if necessary of Table 2 and repeat procedures 2-4).

b) The preference of solvent for dissolving test chemicals is medium, DMSO, and then ethanol. Thus, if a test chemical dissolves in more than one solvent at any one solubility-testing tier, then the choice of solvent follows this hierarchy. For example, if, at any tier, a chemical is soluble in medium and DMSO, but not ethanol, the choice of solvent would be medium. If the chemical were insoluble in medium, but soluble in DMSO and ethanol, the choice of solvent would be DMSO.

After the lab has determined the preferred solvent for the test chemical and before proceeding to the cytotoxicity testing, the Study Director will discuss the solvent selection with the Study Management Team (SMT) of the validation study. The SMT will relate what solvent should be used in the assay for each chemical.

E. Preparation of Test Chemicals

[Note: Preparation under red or yellow light is recommended to preserve chemicals that degrade upon exposure to light.]

1. Test Chemical in Solution

a) Allow test chemicals to equilibrate to room temperature before dissolving and diluting.

b) Prepare test chemical immediately prior to use. The solutions must not be cloudy nor have noticeable precipitate. Each stock dilution should have at least 1-2 mL total volume to ensure adequate solution for the test wells in a single 96-well plate.

c) For chemicals dissolved in DMSO or ethanol, the final DMSO or ethanol concentration for application to the cells must be 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations.

d) The stock solution for each test chemical should be prepared at the highest concentration found to be soluble in the solubility test. Thus, the highest test concentration applied to the cells in each range finding experiment is:
- 0.5 times the highest concentration found to be soluble in the solubility test, if the chemical was soluble in medium, or

- 1/200 the highest concentration found to be soluble in the solubility test if the chemical was soluble in ethanol or DMSO.

e) The seven lower concentrations in the range finding experiment would then be prepared by successive dilutions that decrease by one log unit each. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in medium before application to NHK cells.

Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme

If DMSO was determined to be the preferred solvent at Tier 2 of the solubility test (i.e., 200,000 μg/mL), dissolve the chemical in DMSO at 200,000 μg/mL for the chemical stock solution.

1) Label eight tubes 1 – 8. Add 0.9 mL solvent (e.g., DMSO) to tubes 2 -- 8.
2) Prepare stock solution of 200,000 μg test chemical/mL solvent in tube # 1.
3) Add 0.1 mL of 200,000 μg/mL dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 20,000 μg/mL).
4) Add 0.1 mL of 20,000 μg/mL dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 2,000 μg/mL)
5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.
6) Since each concentration is 200 fold greater than the concentration to be tested, make a 1:100 dilution by diluting 1 part dissolved chemical in each tube with 99 parts of culture medium (e.g., 0.1 mL of test chemical in DMSO + 9.9 mL culture medium) to derive the eight 2X concentrations for application to NHK cells. Each 2X test chemical concentration will then contain 1 % v/v solvent. The NHK cells will have 0.125 mL of culture medium in the wells prior to application of the test chemical. By adding 0.125 mL of the appropriate 2X test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 1,000 μg/mL) in a total of 0.250 mL and the solvent concentration in the wells will be 0.5% v/v.
7) A test article prepared in DMSO or ethanol may precipitate upon transfer into the Routine Culture Medium. The 2X dosing solutions should be evaluated for precipitates and the results will be recorded in the workbook. It will be permissible to test all of the dosing solutions in the dose range finding assay only. Doses containing test article precipitates should be avoided, and will not be used in the ICx determinations for either the range finding experiments or the definitive tests.

Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Measure the pH of the highest concentration of the test chemical in culture medium using pH paper. Document the pH and note the color of the medium for all dilutions. Do not adjust the pH.

3. Concentrations of Test Chemical

a) Range Finder Experiment

Test eight concentrations of the test chemical by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

The data from any well that has precipitate will be excluded from any calculations.

b) Main Experiment

[Note: After the range finding assay is completed, the concentration-response experiment shall be performed three times on three different days for each chemical (i.e., one plate per day per chemical).]

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller ($\sqrt[6]{10} = 1.47$). Cover the relevant concentration range ($\geq$10 % and $\leq$ 90 % effect) preferably with three points of a graded effect, but with a minimum of two points, one on each side of the IC_{50} value, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than one cytotoxic concentration on each side of the IC_{50} value shall be repeated, where possible, with a smaller dilution factor. In addition, the dilution scheme shall be adjusted in subsequent replicate assays (i.e., definitive assays), if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. (Taking into account pipetting errors, a progression factor of 1.21 is regarded the smallest factor achievable.)

Determine which test chemical concentration is closest to the IC_{50} value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

Maximum Doses to be Tested in the Main Experiments

If minimal or no cytotoxicity was measured in the dose range finding assay, a maximum dose for the main experiments will be established as follows:

- For test chemicals prepared in Routine Culture Medium, the highest test article concentration that may be applied to the cells in the main experiments will be either 100 mg/mL, or the maximum soluble dose. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of Routine Culture Medium will be added to the vessel so that the concentration is 200,000 µg/mL (200 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in medium, then 7 additional serial stock dosing solutions may be prepared from the 200 mg/mL 2X stock. If the test chemical is insoluble in medium at 200 mg/ml, proceed by adding medium, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- For test chemicals prepared in either DMSO or ethanol, the highest test article concentration that may be applied to the cells in the main experiments will be either 2.5 mg/mL, or less, depending upon the maximum solubility in solvent. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of the appropriate solvent (determined from the original solubility test) will be added to the vessel so that the concentration is 500,000 μg/mL (500 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in the solvent, then 7 additional serial stock dosing solutions may be prepared from the 500 mg/mL 200X stock. If the test chemical is insoluble in solvent at 500 mg/ml, proceed by adding solvent, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

c) Test Chemical Dilutions

The dosing factor of 3.16 (= $^{2}\sqrt{10}$) divides a log into two equidistant steps, a factor of 2.15 (= $^{3}\sqrt{10}$) divides a decade into three steps. The factor of 1.47 (= $^{6}\sqrt{10}$) divides a log into six equidistant steps, and the factor of 1.21 (= $^{12}\sqrt{10}$) divides the log into 12 steps.

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

F. Test Procedure

1. 96-Well Plate Configuration

The NHK NRU assay for test chemicals will use the 96-well plate configuration shown in Figure 2.

Figure 2. 96-Well Plate Configuration for Positive Control and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	b	b	b	b	b	b	b	b	b	b	b	b
B	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
C	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
D	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
E	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
F	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
G	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
H	b	b	b	b	b	b	b	b	b	b	b	b

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 - C_8$ = Test Chemicals or Positive Control (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)

2. Application of Test Chemical

a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized. The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs and/or Corning/Transtar model 4878 disposable reservoir liners, 8-channel). The second method utilizes a "dummy" plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate (with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the plate containing cells. More volume than needed for the test plate (i.e. greater than 125 µl/well) should be in the wells of the dummy plate. At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing.

b) After 48 - 72 h (i.e., after cells attain 20-30+ % confluency [see Section VII.C.4(h)[) incubation of the cells, add 125 µl of the appropriate concentration of test chemical, the PC, or the VC (see Figure 2 for the plate configuration) directly to the test wells. Do not remove Routine Culture Medium for re-feeding the cells.Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).

c) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in the development of the positive control database in Phase Ia of the Validation Study. The mean IC_{50} and two standard deviations (SD) of the IC_{50} of SLS are the values that will be used as an acceptance criterion for test sensitivity for the NHK NRU assay. This plate will follow the same schedule and procedures as used for the test chemical plates.

3. Microscopic Evaluation

After at least 46 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

b) Carefully remove (i.e., "dump") the Routine Culture Medium (with test chemical) and rinse the cells very carefully with 250 µL pre-warmed D-PBS. Remove the rinsing solution by dumping and remove excess by gently blotting on sterile paper towels. Add 250 µL NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3±0.1 h. Observe the cells briefly during the NR incubation (e.g., at 1, 2, and 3 h – Study Director 's discretion) for NR crystal formation. Record observations in the Study Workbook. Study Director can decide to reject the experiment if excessive NR crystallization has occurred.
c) After incubation, remove the NR medium, and carefully rinse cells with 250 µL pre-warmed D-PBS.
d) Decant and blot D-PBS from the plate. (Optionally: centrifuge the reversed plate.)
e) Add exactly 100 µL NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.
f) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution.

g) Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader (spectrophotometer), using the blanks as a reference. [Phase Ia data show the mean OD value for the plate blanks to be 0.058 ± 0.032 for NHK cells (mutually agreed upon by Testing Facility and SMT; data from 3 labs; N = 75). Use this value as a **guide** for assessment of the blank values.] Save raw data in the Excel format as provided by the Study Management Team.

5. Quality Check of Assay

a) Test Acceptance Criteria

1) A test meets acceptance criteria, if the IC_{50} for SLS is within two standard deviations of the historical mean established by the Test Facility (as per **VII.F.2.c**).
2) A test meets acceptance criteria if the corrected mean OD_{540} of VCs is ≥ 0.60 and ≤ 1.70
3) A test meets acceptance criteria if the left and the right mean of the VCs do not differ by more than 15 % from the mean of all VCs.
4) A test meets acceptance criteria if a minimum of two points, one on each side of the IC_{50} value, are determined and fall within the range ≥10 % and ≤ 90 % effect.

[Note: All acceptance criteria must be met for an assay to be considered acceptable.]

b) Checks for Systematic Cell Seeding Errors

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

c) Quality Check of Concentration-Response

The IC_{50} derived from the concentration-response of the test chemicals should be backed by preferably three responses ≥ 10 and ≤ 90 % inhibition of NRU and at least two responses, one on either side of the IC_{50} value (see **VII.E.3.b**). If this is not the case, and the concentration progression factor can be easily reduced, reject the experiment and repeat it with a smaller progression factor. In addition, the dilution scheme shall be adjusted in subsequent replicate assays, if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. Numerical scoring of the cells (see **VII.F.3**) should be determined and documented in the Study Workbook.

G. Data Analysis

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicates wells) per test concentration. This value is compared with the mean NRU of all VC values (provided VC values have met the VC acceptance criteria). Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet provided by the Study Management Team for determining cell viability and performing statistical analyses.

The concentration of a test chemical reflecting a 20 %, 50 %, and 80 % inhibition of cell viability (i.e., the IC_{20}, IC_{50}, and IC_{80}) is determined from the concentration-response by applying a Hill function to the concentration-response data. Statistical software (e.g., GraphPad PRISM® 3.0) specified by the Study Management Team shall be used to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical. In addition, the Study Management Team shall provide guidelines for calculating ICx values and confidence limits. The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the Study Management Team/biostatistician through the designated contacts in electronic format and hard copy upon completion of testing. The Study Management Team will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

Clonetics Normal Human Keratinocyte Systems Instructions for Use, AA-1000-4-Rev.03/00. (http://www.clonetics.com).

Hackenberg, U. and H. Bartling. 1959. Messen und Rechnen im pharmakologischen Laboratorium mit einem speziellen Zahlensystem (WL24-System). Arch. Exp. Pathol. Pharmakol. 235: 437-463.

Triglia, D., P.T. Wegener, J. Harbell, K. Wallace, D. Matheson, and C. Shopsis. 1989. Interlaboratory validation study of the keratinocyte neutral red bioassay from Clonetics Corporation. In *Alternative Methods in Toxicology*, Volume 7. A.M. Goldberg, ed., pp. 357-365. Mary Ann Liebert, Inc., New York.

IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**

(Print or type name)

_____ _____

Testing Facility STUDY DIRECTOR **DATE**
(Print or type name)

Appendix C5

Test Method Protocol for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test (Phase II)

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TEST METHOD PROTOCOL
for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an *In Vitro* Validation Study
Phase II

May 15, 2003

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test
A Test for Basal Cytotoxicity
Phase II

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the BALB/c 3T3 Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and supports the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test

The 3T3 NRU test will be performed to analyze the *in vitro* toxicity of nine (9) blinded/coded test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for the predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name:	National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)	
B. Address:	P.O. Box 12233 Research Triangle Park, NC 27709	
C. Representative:	*Named Representative*	

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals:	*Blinded Chemicals (9)*	
B. Controls:	Positive:	Sodium Lauryl Sulfate
	Vehicle (Negative):	Assay medium (DMEM containing 5% NBCS, 4 mM L-Glutamine, 100 IU/mL Penicillin, 100 µg/mL Streptomycin)
	Solvent (as needed):	Assay medium with appropriate solvent used to prepare the test chemicals (**Section VII.E**)

IV. TESTING FACILITY AND KEY PERSONNEL

A. Facility Information

1) Name:
2) Address:
3) Study Director:
4) Laboratory Technician(s):
5) Scientific Advisor:
6) Quality Assurance Director:
7) Safety Manager:
8) Facility Management:

B. Test Schedule

1) Proposed Experimental Initiation Date:
2) Proposed Experimental Completion Date:
3) Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\log IC50 - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test

chemical preparation, incubator function); all optical density data obtained from the spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

1. Cell Lines

BALB/c 3T3 cells, clone 31
CCL-163, LGC Reference Materials, Customer Service, Queens Road, Teddington, Middlesex, TW110LY, UK
CCL-163, American Type Culture Collection [ATCC], Manassas, VA, USA)

2. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

a) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
b) Laminar flow clean bench/cabinet (standard: "biological hazard")
c) Water bath: 37ºC ± 1ºC
d) Inverse phase contrast microscope
e) Sterile glass tubes with caps (e.g., 5 mL)
f) Centrifuge (optionally: equipped with microtiter plate rotor)
g) Laboratory balance
h) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
i) Shaker for microtiter plates
j) Cell counter or hemocytometer
k) Pipetting aid
l) Pipettes, pipettors (multi-channel and single channel; multichannel repeater pipette), dilution block
m) Cryotubes
n) Tissue culture flasks (e.g., 75 - 80 cm^2, 25 cm^2)
o) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Falcon tissue culture-treated)
p) pH paper (wide and narrow range)
q) Multichannel reagent reservoir
r) Waterbath sonicator
s) Magnetic stirrer
t) Antistatic bar ionizer/antistatic gun (optional for neutralizing static on 96-well plates)
u) Dry heat block (optional)

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of 3T3 cells. Multi-channel repeater pipettes may be used for plating cells in the 96-well plates, dispensing plate rinse solutions, NR medium, and desorb solution. Do not use the repeater pipette for dispensing test chemicals to the cells.]

3. Chemicals, Media, and Sera

a) Dulbecco's Modification of Eagle's Medium (DMEM) without L-Glutamine; should have high glucose [4.5gm/l] (e.g., ICN-Flow Cat. No. 12-332-54)
b) L-Glutamine 200 mM (e.g., ICN-Flow # 16-801-49)
c) New Born Calf Serum (NBCS or NCS) (e.g., Biochrom # SO 125)
d) 0.05 % Trypsin/0.02 % EDTA solution (e.g., SIGMA T 3924, ICN-Flow, # 16891-49)
e) Phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}(for trypsinization)
f) Hanks' Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+}(CMF-HBSS)
g) Dulbecco's Phosphate Buffered Saline (D-PBS) [formulation containing calcium and magnesium cations; glucose optional] (for rinsing)
h) Penicillin/streptomycin solution (e.g. ICN-Flow # 16-700-49)
i) Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
j) Dimethyl sulfoxide (DMSO), U.S.P. analytical grade (Store under nitrogen @ -20ºC)
k) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
l) Glacial acetic acid, analytical grade
m) Distilled H_2O or any purified water suitable for cell culture (sterile)
n) Sterile paper towels (for blotting 96-well plates)

[Note: Due to lot variability of NBCS/NCS, first check a lot for growth stimulating properties with 3T3 cells (approximately 20-24 h doubling time) and then reserve a sufficient amount of NBCS/NCS. May use pre-tested serum lot from Phases Ia and Ib of the validation study if the serum has been stored under appropriate conditions and shelf-life has not expired.]

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard). All methods and procedures will be adequately documented.]

1. Media

DMEM (buffered with sodium bicarbonate) supplemented with (final concentrations in DMEM are quoted):

a) for freezing (Freeze Medium); contains 2X concentration of NBCS/NCS and DMSO of final freezing solution
   40 % NBCS/NCS
   20 % DMSO

b) for routine culture (Routine Culture Medium)
   10 % NBCS/NCS
   4 mM Glutamine

c) for solubility testing and test chemical dilution (Chemical Dilution Medium)

4 mM	Glutamine
200 IU/mL	Penicillin
200 µg/mL	Streptomycin

d) for dilution of NR stock solution (NR Dilution Medium)

5 %	NBCS/NCS
4 mM	Glutamine
100 IU/mL	Penicillin
100 µg/mL	Streptomycin

[Note: The Chemical Dilution Medium with test chemical will dilute the serum concentration of the Routine Culture Medium in the test plate to 5 %. Serum proteins may mask the toxicity of the test substance, but serum cannot be totally excluded because cell growth is markedly reduced in its absence.]

Completed media formulations should be kept at approximately 2-8° C and stored for no longer than two weeks.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay (e.g., SIGMA #N2889, 3.3 mg/mL). Store liquid tissue culture-grade NR Stock Solution at the storage conditions and shelf-life period recommended by the manufacturer.

If the liquid form is not available, the following formulation can be prepared.

EXAMPLE: 0.25 g NR Dye powder in 100 mL H_2O

The NR Stock Solution (powder in water) should be stored in the dark at room temperature for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

0.758 mL (3.3 mg NR dye/mL solution)	NR Stock Solution
99.242 mL	NR Dilution Medium (pre-warmed to 37° C)

The final concentration of the NR Medium is **25 µg NR dye/mL** and aliquots will be prepared on the day of application.

[Note: The NR Medium shall be filtered (e.g., Millipore filtering, 0.2 – 0.45 µm pore size) to reduce NR crystals. Aliquots of the NR Medium should be maintained at 37° C (e.g., in a waterbath) before adding to the cells and used within 30 min of preparation but also used within 15 min after removing from 37° C storage.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

BALB/c 3T3 cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 75 - 80 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells should be examined on a daily (i.e., on workdays) basis under a phase contrast microscope, and any changes in morphology or their adhesive properties noted in a Study Workbook.

2. Receipt of Cryopreserved BALB/c 3T3 Cells

Upon receipt of cryopreserved BALB/c 3T3 cells, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells

Thaw cells by putting ampules into a water bath at 37°C ± 1ºC. Leave for as brief a time as possible.

a) Resuspend the cells in pre-warmed Routine Culture Medium and transfer into pre-warmed Routine Culture Medium in a tissue-culture flask.

b) Incubate at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air.

c) When the cells have attached to the bottom of the flask (within 4 to 24 h), decant the supernatant and replace with fresh pre-warmed (37ºC) medium. Culture as described above.

d) Passage at least two times before using the cells in a cytotoxicity test.

A fresh batch of frozen cells from the stock lot of cells should be thawed out and cultured approximately every two months. This period resembles a sequence of about 18 passages.

4. Routine Culture of BALB/C 3T3 Cells

When cells exceed 50 % confluence (but less than 80 % confluent) they should be removed from the flask by trypsinization:

a) Decant medium, briefly rinse cultures with 5 mL PBS or Hanks' BSS (without Ca^{2+}, Mg^{2+}) per 25 cm^2 flask (15 mL per 75 cm^2 flask). Wash cells by gentle agitation to remove any remaining serum that might inhibit the action of the trypsin.

b) Discard the washing solution. Repeat the rinsing procedure and discard the washing solution.

c) Add 1-2 mL trypsin-EDTA solution per 25 cm^2 to the monolayer for a few seconds (e.g., 15-30 seconds).

d) Remove excess trypsin-EDTA solution and incubate the cells at room temperature.

e) After 2-3 minutes (min), lightly tap the flask to detach the cells into a single cell suspension.

5. Cell Counting

After detaching the cells, add 0.1-0.2 mL of pre-warmed (37ºC) Routine Culture Medium/cm^2 to the flask (e.g., 2.5 mL for a 25 cm^2 flask). Disperse the monolayer by gentle trituration. It is important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension obtained using a hemocytometer or cell counter (e.g., Coulter counter).

6. Subculture of Cells

After determination of cell number, the culture can be sub-cultured into other flasks or seeded into 96-well microtiter plates. BALB/c 3T3 cells are routinely passaged at suggested cell densities as listed in the table (approximate doubling time is 20-24 h). The individual laboratories will need to determine and adjust the final density to achieve appropriate growth.

Table 1. Cell Densities for Subculturing

Days in Culture	Seeding Density (cells/cm^2)	Total Cells per 25 cm^2 flask	Total Cells per 75 cm^2 flask
2	16800	4.2×10^5	1.26×10^6
3	8400	2.1×10^5	6.3×10^5
4	4200	1.05×10^5	3.15×10^5

[Note: It is important that cells have overcome the lag growth phase when they are used for the test.]

7. Freezing Cells (procedure required only if current stock of cells is depleted)

Stocks of BALB/c 3T3 cells can be stored in sterile, freezing tubes in a liquid nitrogen freezer. DMSO is used as a cryoprotective agent.

a) Centrifuge trypsinized cells at approximately 200 x g.

b) Suspend the cells in cold Routine Culture Medium (half the final freezing volume) so a final concentration of $1\text{-}5 \times 10^6$ cells/mL can be attained.

c) Slowly add cold Freeze Medium to the cells so that the solvent will equilibrate across the cell membranes. Bring the cell suspension to the final freezing

volume. The final cell suspension will be 10 % DMSO. Aliquot the cell suspension into freezing tubes and fill to 1.8 mL.

d) Place the tubes into an insulated container (e.g., styrofoam trays) and place in a freezer (-70 to -80°C) for 24 h. This gives a freezing rate of approximately 1°C/min. The laboratory needs to ensure that the freezing protocol is applicable to the 3T3 cells and that the cells are viable when removed from cryopreservation.

e) Place the frozen tubes into liquid nitrogen for storage.

8. Preparation of Cells for Assays

a) Cultured cells that are going to be used in seeding the 96-well plates should be fed fresh medium the day before subculturing to the plates. On the day of plate seeding, prepare a cell suspension of 2.0 – 3.0x10^4 cells/mL in Routine Culture Medium. Using a multi-channel pipette, dispense 100 µl Routine Culture Medium only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate (See **Section VII.F.1**). In the remaining wells, dispense 100 µl of a cell suspension of 2.0 – 3.0x10^4 cells/mL (= 2.0 – 3.0x10^3 cells/well). The seeding density should be noted to ensure that the cells in the control wells are not overgrown after three days (i.e., 24 h incubation in step **b** and 48 h exposure to test chemicals). Prepare one plate per chemical to be tested.

b) Incubate cells for 24 ± 2 h (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air) so that cells form a less than half (< 50%) confluent monolayer. This incubation period assures cell recovery and adherence and progression to exponential growth phase.

c) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

9. Determination of Doubling Time

a) A cell doubling time procedure was performed on the initial lot of cells that was used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined in Phase II if there is a change in the lot of cells used. Establish cells in culture and trypsinize cells as per **Section VII.C.4** for subculture. Resuspend cells in NR Dilution Medium (5 % NBCS/NCS). Seed cells at 4200 cells/cm^2.

b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).

c) After 4 - 6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count

cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue; Nigrosin) if Study Director sees a need. Use appropriate size exclusion limits if using a Coulter counter. Determine the total number of cells and document. Repeat sampling at 24 h, 48 h, 72 h, and 96 h post inoculation. Change culture medium at 72 h or sooner in remaining dishes if indicated by pH drop.

d) Plot cell concentration (per mL of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Solubility Test

The preference of solvent for dissolving test chemicals is medium, DMSO, and then ethanol. Solubility shall be determined in a step-wise procedure that involves attempting to dissolve a test chemical at a relatively high concentration with the sequence of mechanical procedures specified in **Section VII.D.2.a.** If the chemical does not dissolve, the volume of solvent is increased so as to decrease the concentration by a factor of 10, and then the sequence of mechanical procedures in **Section VII.D.2.a** are repeated in an attempt to solubilize the chemical at the lower concentrations. For testing solubility in medium, the starting concentration is 20,000 μg/ml (i.e., 20 mg/mL) in Tier 1, but for DMSO and ethanol the starting concentration is 200,000 μg/ml (i.e., 200 mg/mL) in Tier 2. Weighing out chemical for each solvent (i.e., medium, DMSO, ethanol) can be done all at once, if convenient, but solubility testing (at each tier that calls for more than one solvent) is designed to be sequential - medium, then DMSO, then ethanol – in accordance with the solvent hierarchy (see **Figure 1**). This allows for testing to stop, rather than continue testing with less preferred solvents, if the test chemical dissolves in a more preferred solvent. For example, if a chemical is soluble in medium at a particular tier, testing may stop. Likewise, if a chemical is soluble in DMSO at any tier, testing need not continue with ethanol. However, since the issue of primary importance is testing the solvents and concentrations of test chemical required by any one tier, sequential testing of solvents may be abandoned if the lab can test more efficiently in another way.

1. Determination of Solubility

a) Tier 1 begins with testing 20 mg/mL in Chemical Dilution Medium (see **Table 2**). Approximately 10 mg (10,000 μg) of the test chemical will be weighed into a glass tube and the weight will be documented. A volume of Chemical Dilution Medium, approximately 0.5 mL, will be added to the vessel so that the concentration is 20,000 μg/ml (20 mg/mL). The solution is mixed as specified in **Section VII.D.2.a.** If complete solubility is achieved in medium, then additional solubility procedures are not needed.

b) If the test chemical is insoluble in Chemical Dilution Medium, proceed to Tier 2 by adding enough medium, approximately 4.5 mL, to attempt to dissolve the chemical at 2 mg/mL by using the sequence of mixing procedures specified in **Section VII.D.2.a**. If the test chemical dissolves in Chemical Dilution Medium at 2 mg/mL, no further procedures are necessary. If the test chemical does NOT dissolve, weigh out approximately 100 mg test chemical in a second glass tube and add enough DMSO to

make the total volume approximately 0.5 mL (for 200 mg/mL) and attempt to dissolve the chemical as specified in **Section VII.D.2.a**. If the test chemical does not dissolve in DMSO, weigh out approximately 100 mg test chemical in another glass tube and add enough ethanol to make the total volume approximately 0.5 mL (for 200 mg/mL) and attempt to dissolve the chemical as specified in **Section VII.D.2.a**. If the chemical is soluble in either solvent, no additional solubility procedures are needed.

c) If the chemical is NOT soluble in Chemical Dilution Medium, DMSO, or ethanol at Tier 2, then continue to Tier 3 in Table 2 by adding enough solvent to increase the volume of the three Tier 2 solutions by 10 and attempt to solubilize again using the sequence of mixing procedures in **Section VII.D.2.a.** If the test chemical dissolves, no additional solubility procedures are necessary. If the test chemical does NOT dissolve, continue with Tier 4 and, if necessary, Tier 5 using DMSO and ethanol. Tier 4 begins by diluting the Tier 3 samples with DMSO or ethanol to bring the total volume to 50 mL. The mixing procedures in **Section VII.D.2.a** are again followed to attempt to solubilize the chemical. Tier 5 is performed, if necessary, by weighing out another two more samples of test chemical at ~10 mg each and adding ~50 mL DMSO or ethanol for a 200 µg/mL solution, and following the mixing procedures in **Section VII.D.2.a**.

Example: If complete solubility is not achieved at 20,000 µg/mL in Chemical Dilution Medium at Tier 1 using the mixing procedures specified in **Section VII.D.2.a,** then the procedure continues to Tier 2 by diluting the solution to 5 mL and mixing again as specified in **Section VII.D.2.a.** If the chemical is not soluble in Chemical Dilution Medium, two samples of ~ 100 mg test chemical are weighed to attempt to solubilize in DMSO and ethanol at 200,000 µg/mL (i.e., 200 mg/mL). Solutions are mixed following the sequence of procedures prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved at Tier 2, then the solutions (Chemical Dilution Medium, DMSO, and ethanol) prepared in Tier 2 are diluted by 10 so as to test 200 µg/mL in media, and 20,000 µg/mL in DMSO and ethanol. This advances the procedure to Tier 3. Solutions are again mixed as prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved in Tier 3, the procedure continues to Tier 4, and to 5 if necessary (see **Figure 1** and **Table 2**).

Table 2 Determination of Solubility in Chemical Dilution Medium, DMSO, or Ethanol

TIER	1	2	3	4	5
Total Volume Chemical Dilution Medium	0.5 mL	5 mL	50 mL		
Concentration of Test Chemical (Add ~10 mg to a tube. Add enough medium to equal the first volume. Dilute to subsequent volumes if necessary.)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	200 µg/mL (0.20 mg/mL)		
Total Volume DMSO/Ethanol		0.5 mL	5 mL	50 mL	
Concentration of Test Chemical (Add ~100 mg to a large tube. Add enough DMSO or ethanol to equal the first volume. Dilute to subsequent volumes if necessary.)		200,000 µg/mL (200 mg/mL)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	
Total Volume DMSO/Ethanol					50 mL
Concentration of Test Chemical (Add ~10 mg to a large tube. Add enough DMSO or ethanol to equal 50 mL.)					200 µg/mL (0.2 mg/mL)
Equivalent Concentration on Cells	10,000 µg/mL (10 mg/mL)	1000 µg/mL (1 mg/mL)	100 µg/mL (0.1 mg/mL)	10 µg/mL (0.01 mg/mL)	1 µg/mL (0.001 mg/mL)

[NOTE: The amounts of test chemical weighed and Chemical Dilution Medium added may be modified from the amounts given above, provided that the targeted concentrations specified for each tier are tested.]

Figure 1. Solubility Flow Chart

TIER 1

STEP 1:	20 mg/mL test chemical (TC) in 0.5 mL Chemical Dilution Medium: • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 2.

TIER 2

STEP 2:	2 mg/mL TC in medium – increase volume from STEP 1 by 10 (i.e., to 5 mL) • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 3.

STEP 3:	200 mg/mL TC in DMSO
	• if TC soluble, then **STOP**.
	• if TC insoluble, test at 200 mg/mL in ETOH.
	  • if TC soluble, then **STOP.**
	  • If TC insoluble, go to STEP 4.

TIER 3

STEP 4:	0.2 mg/mL TC in medium – increase volume from STEP 2 by 10 (i.e., to 50 mL)
	• if TC soluble, then **STOP**.
	• if TC insoluble, test at 20 mg/mL in DMSO – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, test at 20 mg/mL in ETOH – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 5.

TIER 4

STEP 5:	2 mg/mL TC in DMSO – increase volume from STEP 4 by 10 (i.e., to 50 mL)
	• if TC soluble, then **STOP.**
	• if TC insoluble, test at 2 mg/mL in ETOH – increase volume from STEP 4 by 10 (i.e., to 50 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 6.

TIER 5

STEP 6:	0.2 mg/mL TC in 50 mL DMSO • if TC soluble, then **STOP.** • if TC insoluble, test at 0.2 mg/mL in 50 mL ETOH • **STOP**

2. Mechanical Procedures

a) The following hierarchy of mixing procedures will be followed to dissolve the test chemical:

1) Add test chemical to solvent as in Tier 1 of **Table 2**.

2) Gently mix. Vortex the tube (1 –2 minutes).

3) If test chemical hasn't dissolved, use sonication for up to 5 minutes.

4) If sonication doesn't work, then warm solution to 37°C for 5 - 60 min. This can be performed by warming tubes in a 37°C water bath or in a CO_2 incubator at 37°C. The solution may be stirred during warming (stirring in a CO_2 incubator will help maintain proper pH).

5) Proceed to Tier 2 (and Tiers 3-5, if necessary of Table 2 and repeat procedures 2-4).

b) The preference of solvent for dissolving test chemicals is Chemical Dilution Medium, DMSO, and then ethanol. Thus, if (all solvents for a particular tier are tested simultaneously and) a test chemical dissolves in more than one solvent, then the choice of solvent follows this hierarchy. For example, if, at any tier, a chemical is soluble in Chemical Dilution Medium and DMSO, but not ethanol, the choice of solvent would be medium. If the chemical were insoluble in medium, but soluble in DMSO and ethanol, the choice of solvent would be DMSO.

After the lab has determined the preferred solvent for the test chemical and before proceeding to the cytotoxicity testing, the Study Director will discuss the solvent selection with the Study Management Team (SMT) of the validation study. The SMT will relate what solvent should be used in the assay for each chemical. If the laboratory has attempted all solubility testing without success, then the SMT will provide additional guidance for achieving test chemical solubility. The SMT anticipates that all validation study test chemicals will be tested in the NRU assays.

E. Preparation of Test Chemicals

[Note: Preparation under red or yellow light is recommended to preserve chemicals that degrade upon exposure to light.]

1. Test Chemicals in Solution

a) Allow test chemicals to equilibrate to room temperature before dissolving and diluting.

b) Prepare test chemical immediately prior to use. Test chemical solutions should not be prepared in bulk for use in subsequent tests. The solutions must not be cloudy nor have noticeable precipitate. Each stock dilution should have at least 1-2 mL total volume to ensure adequate solution for the test wells in a single 96-well plate. The SMT may direct the Study Director to store an aliquot (e.g., 1 mL) of the highest 2X

stock solution (e.g., low solubility chemicals) in a freezer (e.g., -70°C) for use in future chemical analyses.

c) For chemicals dissolved in DMSO or ethanol, the final DMSO or ethanol concentration for application to the cells must be 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations.

d) The stock solution for each test chemical should be prepared at the highest concentration found to be soluble in the solubility test. Thus, the highest test concentration applied to the cells in each range finding experiment is:
   - 0.5 times the highest concentration found to be soluble in the solubility test, if the chemical was soluble in Chemical Dilution Medium, or
   - 1/200 the highest concentration found to be soluble in the solubility test if the chemical was soluble in ethanol or DMSO.

e) The seven lower concentrations in the range finding experiment would then be prepared by successive dilutions that decrease by one log unit each. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in Chemical Dilution Medium before application to 3T3 cells.

Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme

If DMSO was determined to be the preferred solvent at Tier 2 of the solubility test (i.e., 200,000 μg/mL), dissolve the chemical in DMSO at 200,000 μg/mL for the chemical stock solution.

1) Label eight tubes 1 – 8. Add 0.9 mL solvent (e.g., DMSO) to tubes 2 -- 8.

2) Prepare stock solution of 200,000 μg test chemical/mL solvent in tube # 1.

3) Add 0.1 mL of 200,000 μg/mL dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 20,000 μg/mL).

4) Add 0.1 mL of 20,000 μg/mL dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 2,000 μg/mL)

5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.

6) Since each concentration is 200 fold greater than the concentration to be tested, make a 1:100 dilution by diluting 1 part dissolved chemical in each tube with 99 parts of Chemical Dilution Medium (e.g., 0.1 mL test chemical in DMSO + 9.9 mL Chemical Dilution Medium) to derive the eight 2X concentrations for application to 3T3 cells. Each 2X test chemical concentration will then contain 1 % v/v solvent. The 3T3 cells will have 0.05 mL Routine Culture Medium in the wells prior to application of the test chemical. By adding 0.05 mL of the appropriate 2X test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 1,000 μg/mL) in a total of 0.1 mL and the solvent concentration in the wells will be 0.5% v/v.

7) A test article prepared in Chemical Dilution Medium, DMSO, or ethanol may precipitate upon transfer into the Routine Culture Medium. The 2X dosing solutions

should be evaluated for precipitates and the results will be recorded in the workbook. It will be permissible to test all of the dosing solutions in the dose range finding assay only. Doses containing test article precipitates should be avoided, and will not be used in the ICx determinations for either the range finding experiments or the definitive tests.

Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Measure the pH of the highest concentration of the test chemical in culture medium using pH paper (e.g., pH 0 - 14 to estimate and pH 5 – 10 to determine more precise value). The pH paper should be in contact with the solution for approximately one minute. Document the final pH (i.e., in the EXCEL template) and note the color of the medium for all dilutions. Do not adjust the pH.

3. Concentrations of Test Chemical

a) Range Finder Experiment

Test eight concentrations of the test chemical by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

The data from any well that has precipitate will be excluded from any calculations.

b) Main Experiment

[Note: After the range finding assay is completed, the concentration-response experiment shall be performed three times on three different days for each chemical (i.e., one plate per day per chemical.]

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller ($\sqrt[6]{10} = 1.47$). Cover the relevant concentration range (≥10 % and ≤ 90 % effect) preferably with three points of a graded effect, but with a minimum of two points, one on each side of the estimated IC_{50} value, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than one cytotoxic concentration on each side of the IC_{50} value shall be repeated, where possible, with a smaller dilution factor. Each experiment should have at least one cytotoxicity value ≥ 10.0 % and ≤ 50.0 % viability and at least one cytotoxicity value > 50.0 % and ≤ 90.0 % viability. In addition, the dilution scheme shall be adjusted in subsequent replicate assays (i.e., definitive assays), if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. (Taking into account pipetting errors, a progression factor of 1.21 is regarded the smallest factor achievable.)

Determine which test chemical concentration is closest to the IC_{50} value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

Maximum Doses to be Tested in the Main Experiments

If minimal or no cytotoxicity was measured in the dose range finding assay, a maximum dose for the main experiments will be established as follows:

- For test chemicals prepared in Chemical Dilution Medium, the highest test article concentration that may be applied to the cells in the main experiments will be either 100 mg/mL, or the maximum soluble dose. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of Chemical Dilution Medium will be added to the vessel so that the concentration is 200,000 µg/mL (200 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in medium, then 7 additional serial stock dosing solutions may be prepared from the 200 mg/mL 2X stock. If the test chemical is insoluble in medium at 200 mg/ml, proceed by adding medium, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- For test chemicals prepared in either DMSO or ethanol, the highest test article concentration that may be applied to the cells in the main experiments will be either 2.5 mg/mL, or less, depending upon the maximum solubility in solvent. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of the appropriate solvent (determined from the original solubility test) will be added to the vessel so that the concentration is 500,000 µg/mL (500 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in the solvent, then 7 additional serial stock dosing solutions may be prepared from the 500 mg/mL 200X stock. If the test chemical is insoluble in solvent at 500 mg/ml, proceed by adding solvent, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

c) Test Chemical Dilutions

The dosing factor of 3.16 (= $\sqrt[2]{10}$) divides a log into two equidistant steps, a factor of 2.15 (= $\sqrt[3]{10}$) divides a decade into three steps. The factor of 1.47 (= $\sqrt[6]{10}$) divides a log into six equidistant steps, and the factor of 1.21 (= $\sqrt[12]{10}$) divides the log into 12 steps.

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

F. Test Procedure

1. 96-Well Plate Configuration

The 3T3 NRU assay for test chemicals will use the 96-well plate configuration as shown in **Figure 2**.

Figure 2. 96-Well Plate Configuration for Positive Control and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb
B	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
C	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
D	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
E	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
F	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
G	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
H	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 – C_8$ = Test Chemicals or Positive Control (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)
VCb = VEHICLE CONTROL BLANK

2. Application of Test Chemical

a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized.

1) The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs; or Corning/Transtar model 4878 disposable reservoir liners, 8-channel; or other multichannel reservoirs).

2) The second method utilizes a “dummy” plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate (with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the

plate containing cells. More volume than needed for the test plate (i.e. greater than 50 µl/well) should be in the wells of the dummy plate.

At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing. Do not use a multichannel repeater pipette for dispensing test chemical to the plates.

b) After 24 h ± 2 h incubation of the cells, remove Routine Culture Medium from the cells by careful inversion of the plate (i.e., "dump") over an appropriate receptacle. Gently blot the plate on a sterile paper towel so that the monolayer is minimally disrupted. Do not use automatic plate washers for this procedure nor vacuum aspiration.

c) Immediately add 50 µL of fresh pre-warmed Routine Culture Medium to all of the wells, including the blanks. Fifty microliters (50 µL) of dosing solution will be rapidly transferred from the 8-channel reservoir (or dummy plate) to the appropriate wells of the test plate using a single delivery multi-channel pipettor. For example, the VC may be transferred first (into columns 1, 2, 11, and 12), followed by the test article dosing solutions from lowest to highest dose, so that the same pipette tips on the multi-channel pipettor can be used for the whole plate. [The Vehicle Control blank (VCb) wells (column 1, column 12, wells A2, A11, H2, H11) will receive the Vehicle Control dosing solutions (which should include any solvents used). Blanks for wells A3 – A10 and H3 – H10 shall receive the appropriate test chemical solutions for each concentration (e.g., wells A3 and H3 receive C_1 solution). [The test chemical blanks in rows A and H will be used for their respective test chemical concentrations.]

d) Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).

e) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in the development of the positive control database in Phase I of the Validation Study. If multiple sets of test chemical plates are set up, then clearly designate the positive control plates for each set; each set will be an individual entity. The mean IC_{50} ± two and a half standard deviations (SD) for the SLS acceptable tests from Phases Ia and Ib (after the removal of outliers) are the values that will be used as an acceptance criterion for test sensitivity for the 3T3 NRU assay. This plate will follow the same schedule and procedures as used for the test chemical plates (including appropriate chemical concentrations in the appropriate wells – see sections VII.F.1 and F.2).

3. Microscopic Evaluation

After at least 46 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the

test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

a) Carefully remove (i.e., "dump") the medium with test chemical and rinse the cells very carefully with 250 µL pre-warmed D-PBS. Remove the rinsing solution by dumping and remove excess by gently blotting on sterile paper towels. Add 250 µL NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3±0.1 h. Observe the cells briefly during the NR incubation (e.g., between 2 and 3 h – Study Director's discretion) for NR crystal formation. Record observations in the Study Workbook. Study Director can decide to reject the experiment if excessive NR crystallization has occurred.

b) After incubation, remove the NR medium, and carefully rinse cells with 250 µl pre-warmed D-PBS.

c) Decant and blot D-PBS from the plate.

d) Add exactly 100 µl NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.

e) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution. Plates should be protected from light by using a cover during shaking.

f) Plates should be still for at least five minutes after removal from the plate shaker (or orbital mixer). Observe the wells for bubbles. Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader (spectrophotometer), using the blanks as a reference. [Note: Phases Ia and Ib data show the mean OD value for the plate blanks to be 0.057 ± 0.043 for 3T3 cells (± 2.5 standard deviations; data from 3 labs; N = 189). Use this range as a **guide** for assessment of the blank values.] Save raw data in the Excel format as provided by the SMT.

5. Quality Check of 3T3 NRU Assay

a) Test Acceptance Criteria

1) A test meets acceptance criteria, if the IC_{50} for SLS (PC) is within ± two and a half (2.5) standard deviations of the historical mean established by the Test Facility (as per **VII.F.2.e**).

2) A test meets acceptance criteria if the left and the right mean of the VCs do not differ by more than 15.0 % from the mean of all VCs.

3) A test meets acceptance criteria if:
   - at least one calculated cytotoxicity value ≥ 10.0 % and ≤ 50.0 % viability and
   - at least one calculated cytotoxicity value > 50.0 % and ≤ 90.0 % viability.

4) A test meets acceptance criteria if the r^2 (coefficient of determination) value calculated for the Hill model fit (i.e., from PRISM® software) is ≥ 0.90. A test does not meet acceptance criteria if the r^2 value is < 0.80. If the r^2 value is ≥ 0.80 and < 0.90 ("gray zone"), then the SMT will evaluate the model fit and make the determination of whether or not the test meets the acceptance criteria and relate the information to the Study Director.

   [Note: All acceptance criteria must be met for an assay to be considered acceptable.]

   [A corrected mean $OD_{540 \pm 10nm}$ of 0.103 - 0.813 for the VCs is a target range but will not be a test acceptance criterion. Range determined from Phase Ib VC OD values from 3 laboratories (mean ± 2.5 standard deviations, N = 98).]

b) Checks for Systematic Cell Seeding Errors

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

c) Quality Check of Concentration-Response

The IC_{50} derived from the concentration-response of the test chemicals will be backed by preferably three responses ≥ 10 % and ≤ 90 % inhibition of NRU and at least two responses, one on either side of the IC_{50} value (see sections **VII.E.3.b** and **VII.F.5.a.3**). If this is not the case, and the concentration progression factor can be easily reduced, reject the experiment and repeat it with a smaller progression factor. In addition, the dilution scheme shall be adjusted in subsequent replicate assays, if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. Numerical scoring of the cells (see **VII.F.3**) should be determined and documented in the Study Workbook.

G. Data Analysis

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicate well) per test concentration (blanks will be subtracted). The Study Director will use good biological/scientific judgment for determining "unusable" wells that will be excluded from the statistical analysis. This value is compared with the mean NRU of all VC values (provided VC values have met the VC acceptance criteria). Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet (template with macros provided by the SMT) that will automatically determine cell viability and perform statistical analyses (including determination of outliers).

The concentration of a test chemical reflecting a 20 %, 50 %, and 80 % inhibition of cell viability (i.e., the IC_{20}, IC_{50}, and IC_{80}) is determined from the concentration-response by applying a Hill function to the concentration-response data. Statistical software (e.g., GraphPad PRISM® 3.0) specified by the SMT shall be used to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical. In addition, the SMT shall provide guidelines for calculating ICx values and confidence limits. The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the SMT/biostatistician through the designated contacts in electronic format and hard copy upon completion of testing. The SMT will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

Hackenberg, U. and H. Bartling. 1959. Messen und Rechnen im pharmakologischen Laboratorium mit einem speziellen Zahlensystem (WL24-System). Arch. Exp. Pathol. Pharmakol. 235: 437-463.

Spielmann, H., S. Gerner, S. Kalweit, R. Moog, T. Wirnserberger, K. Krauser, R. Kreiling, H. Kreuzer, N.P. Luepke, H.G. Miltenburger, N. Müller, P. Murmann, W. Pape, B. Siegmund, J. Spengler, W. Steiling, and F.J. Wiebel. 1991. Interlaboratory assessment of alternatives to the Draize eye irritation test in Germany. Toxicol. *In Vitro* 5: 539-542.

IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**
(Print or type name)

_____ _____

Test Facility STUDY DIRECTOR **DATE**
(Print or type name)

Appendix C6

Test Method Protocol for the Normal Human Epidermal Keratinocyte (NHK) Neutral Red Uptake Cytotoxicity Test (Phase II)

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TEST METHOD PROTOCOL
for the NHK Neutral Red Uptake Cytotoxicity Test

A Test for Basal Cytotoxicity for an In Vitro Validation Study
Phase II

May 15, 2003

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

TEST METHOD PROTOCOL

The Normal Human Keratinocyte (NHK) Neutral Red Uptake Cytotoxicity Test A Test for Basal Cytotoxicity Phase II

I. PURPOSE

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the Normal Human Keratinocyte (NHK) Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter-laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and is in support of the *in vitro* validation study organized by NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay.

A. NHK Neutral Red Uptake Cytotoxicity Test

The NHK NRU test will be performed to analyze the *in vitro* toxicity of nine (9) blinded/coded test chemicals. This test will be used to determine IC_{20}, IC_{50}, and IC_{80} values for the predetermined set of test chemicals of varying toxicities.

II. SPONSOR

A. Name: National Institute of Environmental Health Sciences (NIEHS); The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

B. Address: P.O. Box 12233
Research Triangle Park, NC 27709

C. Representative: *Named Representative*

III. IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

A. Test Chemicals: *Blinded chemicals (9)*

B. Controls:

Positive:	Sodium Lauryl Sulfate
Vehicle (Negative):	Assay medium
Solvent (as needed):	Assay medium with appropriate solvent used to prepare the test chemicals (**Section VII.E**)

IV. TESTING FACILITY AND KEY PERSONNEL

A. Facility Information

1) Name:
2) Address:
3) Study Director:
4) Laboratory Technician(s):
5) Scientific Advisor:
6) Quality Assurance Director:
7) Safety Manager:
8) Facility Management:

B. Test Schedule

1) Proposed Experimental Initiation Date:
2) Proposed Experimental Completion Date:
3) Proposed Report Date:

V. TEST SYSTEM

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

VI. DEFINITIONS

A.. *Hill function*: a four parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{logIC50} - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

B. *Documentation*: all methods and procedures will be noted in a Study Workbook; logs will be maintained for general laboratory procedures and equipment (e.g., media preparation, test chemical preparation, incubator function); all optical density data obtained from the

spectrophotometer plate reader will be saved in electronic and paper formats; all calculations of ICx values and other derived data will be in electronic and paper format; all data will be archived.

VII. PROCEDURES

A. Materials

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used unless otherwise noted.]

1. Cell Lines

Normal Human Epidermal Keratinocytes (NHK)

Non-transformed cells; from cryopreserved primary or secondary cells (**Clonetics #CC-2507 or equivalent).** Cells will be Clonetics NHK cells.

Cambrex [Cambrex Bio Science, 8830 Biggs Ford Road, Walkersville, MD 21793-0127

Cambrex Europe [Cambrex Bio Science Verviers, S.P.R.L. Parc Industriel de Petit Rechain, B-4800 Verviers, BELGIUM]

2. Technical Equipment

[Note: Suggested brand names/vendors are listed in parentheses. Equivalents may be used.]

a) Incubator: 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air
b) Laminar flow clean bench (standard: "biological hazard")
c) Water bath: 37ºC ± 1ºC
d) Inverse phase contrast microscope
e) Sterile glass tubes with caps (e.g., 5mL)
f) Centrifuge (optionally: equipped with microtiter plate rotor)
g) Laboratory balance
h) 96-well plate spectrophotometer (i.e., plate reader) equipped with 540 nm ± 10 nm filter
i) Shaker for microtiter plates
j) Cell counter or hemocytometer
k) Pipetting aid
l) Pipettes, pipettors (multi-channel and single channel; multichannel repeater pipette), dilution block
m) Cryotubes
n) Tissue culture flasks (75 - 80 cm^2, 25 cm^2)
o) 96-well flat bottom tissue culture microtiter plates (e.g., Nunc # 167 008; Corning/COSTAR tissue culture-treated)
p) pH paper (wide and narrow range)
q) Multichannel reagent reservoir
r) Waterbath sonicator
s) Magnetic stirrer

t) Antistatic bar ionizer/antistatic gun (optional for neutralizing static on 96-well plates)
u) Dry heat block (optional)

[Note: Tissue culture flasks and microtiter plates should be prescreened to ensure that they adequately support the growth of NHK. Multi-channel repeater pipettes may be used for plating cells in the 96-well plates, dispensing plate rinse solutions, NR medium, and desorb solution. Do not use the repeater pipette for dispensing test chemicals to the cells.]

3. Chemicals, Media, and Sera

a) Keratinocyte Basal Medium without Ca^{++} (KBM®, Clonetics CC-3104) that is completed by adding the KBM® SingleQuots® (Clonetics CC-4131) to achieve the proper concentrations of epidermal growth factor, insulin, hydrocortisone, antimicrobial agents, bovine pituitary extract, and calcium (e.g., Clonetics Calcium SingleQuots®, 300 mM $CaCl_2$, Clonetics # CC-4202).
b) HEPES Buffered Saline Solution (HEPES-BSS) (e.g., Clonetics # CC-5022)
c) 0.025 % Trypsin/EDTA solution (e.g., Clonetics # CC-5012)
d) Trypsin Neutralizing Solution (TNS) (e.g., Clonetics # CC-5002)
e) Phosphate Buffered Saline (PBS)
f) Dulbecco's Phosphate Buffered Saline (D-PBS) [formulation containing calcium and magnesium cations; glucose optional] (for rinsing)
g) Neutral Red (NR) Dye – tissue culture-grade; liquid form (e.g., SIGMA N 2889); powder form (e.g., SIGMA N 4638)
h) Dimethyl sulfoxide (DMSO), U.S.P analytical grade (Store under nitrogen @ -20ºC)
i) Ethanol (ETOH), U.S.P. analytical grade (100 %, non-denatured for test chemical preparation; 95 % can be used for the desorb solution)
j) Glacial acetic acid, analytical grade
k) Hanks' Balanced Salt Solution without Ca^{2+} or Mg^{2+} (CMF-HBSS) (e.g., Invitrogen # 14170)
l) Distilled H_2O or any purified water suitable for cell culture (sterile)
m) Sterile paper towels (for blotting 96-well plates)

B. Preparations of Media and Solutions

[Note: All solutions (except NR stock solution, NR medium and NR desorb), glassware, pipettes, etc., shall be sterile and all procedures should be carried out under aseptic conditions and in the sterile environment of a laminar flow cabinet (biological hazard standard). All methods and procedures will be adequately documented.]

1. Media

a) Routine Culture Medium/Treatment Medium

KBM® (Clonetics CC-3104) supplemented with KBM® SingleQuots® (Clonetics CC-4131) and Clonetics Calcium SingleQuots® (CC-4202) to make 500 mL medium. Final concentration of supplements in medium are:

0.0001 ng/mL	Human recombinant epidermal growth factor
5 μg/mL	Insulin
0.5 μg/mL	Hydrocortisone

30 μg/mL	Gentamicin
15 ng/mL	Amphotericin B
0.10 mM	Calcium
30 μg/mL	Bovine pituitary extract

Complete media should be kept at 2-8°C and stored for no longer than two weeks.

NOTE:
KBM® SingleQuots® contain the following stock concentrations and volumes:

0.1 ng/mL	hEGF	0.5 mL
5.0 mg/mL	Insulin	0.5 mL
0.5 mg/mL	Hydrocortisone	0.5 mL
30 mg/mL	Gentamicin, 15 ug/mL Amphotericin-B	0.5 mL
7.5 mg/mL	Bovine Pituitary Extract (BPE)	2.0 mL

Clonetics Calcium SingleQuots® are 2 mL of 300mM calcium.

165 μl of solution per 500 mL calcium-free medium equals 0.10 mM calcium in the medium.

2. Neutral Red (NR) Stock Solution

The liquid tissue culture-grade stock NR Solution will be the first choice for performing the assay (e.g., SIGMA #N2889, 3.3 mg/mL). Store liquid tissue culture-grade NR Stock Solution at the storage conditions and shelf-life period recommended by the manufacturer.

If the liquid form is not available, the following formulation can be prepared.

EXAMPLE: 0.33 g NR Dye powder in 100 mL H_2O

The NR Stock Solution (powder in water) should be stored in the dark at room temperature for up to two months.

3. Neutral Red (NR) Medium

EXAMPLE:

1.0 mL (3.3 mg NR dye/mL)	NR Stock Solution
99.0 mL	Routine Culture Medium (pre-warmed to 37° C.)

The final concentration of the NR Medium is **33 μg NR dye/mL** and aliquots will be prepared on the day of application.

[Note: The NR Medium shall be filtered (e.g., Millipore filtering, 0.2 – 0.45 μm pore size) used to reduce NR crystals. Aliquots of the NR Medium should be maintained at 37° C (e.g., in a waterbath) before adding to the cells and used within 30 min of preparation but also used within 15 min after removing from 37° C storage.]

4. Ethanol/Acetic Acid Solution (NR Desorb)

1 %	Glacial acetic acid solution
50 %	Ethanol
49 %	H_2O

C. Methods

1. Cell Maintenance and Culture Procedures

NHK cells are routinely grown as a monolayer in tissue culture grade flasks (e.g., 25 cm^2) at 37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air. The cells should be examined on a daily (i.e., on workdays) basis under a phase contrast microscope, and any changes in morphology or their adhesive properties must be noted in a Study Workbook.

2. Receipt of Cryopreserved Keratinocytes

Upon receipt of cryopreserved keratinocytes, the vial(s) of cells shall be stored in a liquid nitrogen freezer until needed.

3. Thawing Cells and Establishing Cell Cultures

a) Thaw cells by putting ampules into a water bath at 37°C for as brief a time as possible. Do not thaw cells at room temperature or by hand. Seed the thawed cells into culture flasks as quickly as possible and with minimal handling.

b) Slowly (taking approximately 1-2 min) add 9 mL of pre-warmed Routine Culture Medium to the cells suspended in the cryoprotective solution and transfer cells into flasks containing pre-warmed Routine Culture Medium (See Table 1).

c) Incubate the cultures at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air until the cells attach to the flask (within 4 to 24 h), at which time the Routine Culture Medium should be removed and replaced with fresh Routine Culture Medium.

d) Unless otherwise specified, the cells should be incubated at 37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air and fed every 2-3 days until they exceed 50 % confluence (but less than 80 % confluent).

Table 1. Establishing Cell Cultures

Cells/25 cm^2 flask (in approximately 5 mL) 1 flask each cell concentration	6.25 x 10^4 (2500/cm^2)	1.25 x 10^5 (5000/cm^2)	2.25 x 10^5 (9000/cm^2)
Approximate Time to Subculture	96+ hours	72 - 96 hours	48 - 72 hours
Cells to 96-Well Plates	6 – 8 plates	6 – 8 plates	6 – 8 plates

Cell growth guidelines – actual growth of individual cell lots may vary.

4. Subculture of NHK Cells to 96-Well Plates

[Note: It is important that cells have overcome the lag growth phase when they are used for the test. Keratinocytes will be passaged only into the 96-well plates and will not be subcultured into flasks for use in later assays]

a) When the keratinocyte culture in a 25 cm^2 flask exceeds 50 % confluence (but less than 80 % confluent), remove the medium and rinse the culture twice with 5 mL HEPES-BSS. The first rinse may be left on the cells for up to 5 minutes and the second rinse should remain on the cells for approximately 5 minutes. Discard the washing solutions.

b) Add 2 mL trypsin/EDTA solution to each flask and remove after 15 to 30 seconds. Incubate the flask at room temperature for 3 to 7 min. When more than 50 % of the cells become dislodged, rap the flask sharply against the palm of the hand.

c) When most of the cells have become detached from the surface, rinse the flask with 5 mL of room temperature TNS. If more than one flask is subcultured, the same 5 mL of TNS may be used to rinse a total of up to two flasks.

d) Then rinse the flask with 5 mL CMF-HBSS and transfer the cell suspension to a centrifuge tube.

e) Pellet the cells by centrifugation for 5 min at approximately 220 x g. Remove the supernatant by aspiration.

f) Resuspend the keratinocyte pellet by gentle trituration (to have single cells) in Routine Culture Medium. It is important to obtain a single cell suspension for exact counting. Count a sample of the cell suspension using a hemocytometer or cell counter.

g) Prepare a cell suspension –1.6 – 2.0 $x10^4$ cells/mL in Routine Culture Medium. Using a multi-channel pipette, dispense 125 µl Routine Culture Medium only into the peripheral wells (blanks) of a 96-well tissue culture microtiter plate. In the remaining wells, dispense 125 µl of the cell suspension ($2x10^3$ – $2.5x10^3$ cells/well). Prepare one plate per chemical to be tested (see **Figure 2**, **Section VII.F.1**).

h) Incubate cells (37ºC ± 1ºC, 90 % ± 5.0 % humidity, and 5 % ± 1 % CO_2/air) so that cells form a 20+ % monolayer (~48-72 h). This incubation period assures cell recovery and adherence and progression to exponential growth phase.

i) Examine each plate under a phase contrast microscope to assure that cell growth is relatively even across the microtiter plate. This check is performed to identify experimental and systemic cell seeding errors. Record observations in the Study Workbook.

5. Determination of Doubling Time

a) A cell doubling time procedure was performed on the initial lot of cells that was used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined in Phase II if there is a change in the lot of cells used. Establish cells in culture and trypsinize cells as per **Section VII.C.4** for subculture. Resuspend cells in appropriate culture medium. Use **Table 1** to determine seeding densities.

b) Seed five sets of cell culture vessels in triplicate for each cell type (e.g., 15 tissue culture dishes [60mm x 15mm]). Use appropriate volume of culture medium for the culture vessels. Note number of cells placed into each culture dish. Place dishes into the incubators (37ºC ± 1ºC, 90 % ± 5 % humidity, 5.0 % ± 1 % CO_2/air).

c) After 4-6 hours (use the same initial measurement time for each subsequent doubling time experiment), remove three culture dishes and trypsinize cells. Count cells using a cell counter or hemocytometer. Cell viability may be determined by dye exclusion (e.g., Trypan Blue; Nigrosin). Determine the total number of cells and document. Repeat sampling at 24 hr, 48 hr, 72 hr, and 96 hr post inoculation. Change culture medium at 72 hr or sooner in remaining dishes if indicated by pH drop.

d) Plot cell concentration (per mL of medium) on a log scale against time on a linear scale. Determine lag time and population doubling time. The doubling time will be in the log (exponential) phase of the growth curve. Additional dishes and time are needed if the entire growth curve is to be determined (lag phase, log phase, plateau phase).

D. Solubility Test

The preference of solvent for dissolving test chemicals is medium, DMSO, and then ethanol. Solubility shall be determined in a step-wise procedure that involves attempting to dissolve a test chemical at a relatively high concentration with the sequence of mechanical procedures specified in **Section VII.D.2.a.** If the chemical does not dissolve, the volume of solvent is increased so as to decrease the concentration by a factor of 10, and then the sequence of mechanical procedures in **Section VII.D.2.a** are repeated in an attempt to solubilize the chemical at the lower concentrations. For testing solubility in media, the starting concentration is 20,000 μg/ml (i.e., 20 mg/mL) in Tier 1, but for DMSO and ethanol the starting concentration is 200,000 μg/ml (i.e., 200 mg/mL) in Tier 2. Weighing out chemical for each solvent (i.e., media, DMSO, ethanol) can be done all at once, if convenient, but solubility testing (at each tier that calls for more than one solvent) is designed to be sequential - media, then DMSO, then ethanol – in accordance with the solvent hierarchy (see **Figure 1**). This allows for testing to stop, rather than continue testing with less preferred solvents, if the test chemical dissolves in a more preferred solvent. For example, if a chemical is soluble in medium at a particular tier, testing may stop. Likewise, if a chemical is soluble in DMSO at any tier, testing need not continue with ethanol. However, since the issue of primary importance is testing the solvents and concentrations of test chemical required by any one tier, sequential testing of solvents may be abandoned if the lab can test more efficiently in another way.

1. Determination of Solubility

a) Tier 1 begins with testing 20 mg/mL in Routine Culture Medium (see **Table 2**). Approximately 10 mg (10,000 μg) of the test chemical will be weighed into a glass tube and the weight will be documented. A volume of Routine Culture Medium, approximately 0.5 mL, will be added to the vessel so that the concentration is 20,000 μg/ml (20 mg/mL). The solution is mixed as specified in **Section VII.D.2.a.** If complete solubility is achieved in media, then additional solubility procedures are not needed.

b) If the test chemical is insoluble in medium, proceed to Tier 2 by adding enough medium, approximately 4.5 mL, to attempt to dissolve the chemical at 2 mg/mL by using the sequence of mixing procedures specified in **Section VII.D.2.a**. If the test chemical dissolves in medium at 2 mg/mL, no further procedures are necessary. If the test chemical does NOT dissolve, weigh out approximately 100 mg test chemical in a second glass tube and add enough DMSO to make the total volume approximately 0.5 mL (for 200 mg/mL), and attempt to dissolve the chemical as specified in **Section VII.D.2.a**. If the chemical does not dissolve in DMSO, weigh out approximately 100 mg test chemical in another glass tube and add enough ethanol to make the total volume approximately 0.5 mL (for 200 mg/mL) and attempt to dissolve the chemical as specified in **Section VII.D.2.a**. If the chemical is soluble in either solvent, no additional solubility procedures are needed.

c) If the chemical is NOT soluble in media, DMSO, or ethanol at Tier 2, then continue to Tier 3 in **Table 2** by adding enough solvent to increase the volume of the three Tier 2 solutions by 10 and attempt to solubilize again using the sequence of mixing procedures in **Section VII.D.2.a.** If the test chemical dissolves, no additional solubility procedures are necessary. If the test chemical does NOT dissolve, continue with Tier 4 and, if necessary, Tier 5 using DMSO and ethanol. Tier 4 begins by diluting the Tier 3 samples with DMSO or ethanol to bring the total volume to 50 mL. The mixing procedures in **Section VII.D.2.a** are again followed to attempt to solubilize the chemical. Tier 5 is performed, if necessary, by weighing out another two more samples of test chemical at ~10 mg each and adding ~50 mL DMSO or ethanol for a 200 μg/mL solution, and following the mixing procedures in **Section VII.D.2.a**.

Example: If complete solubility is not achieved at 20,000 μg/mL in Routine Culture Medium at Tier 1 using the mixing procedures specified in **Section VII.D.2.a,** then the procedure continues to Tier 2 by diluting the solution to 5 mL and mixing again as specified in **Section VII.D.2.a.** If the chemical is not soluble in medium, two samples of ~ 100 mg test chemical are weighed to attempt to solubilize in DMSO and ethanol at 200,000 μg/mL (i.e., 200 mg/mL). Solutions are mixed following the sequence of procedures prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved at Tier 2, then the solutions (media, DMSO, and ethanol) prepared in Tier 2 are diluted by 10 to test 200 μg/mL in media, and 20,000 μg/mL in DMSO and ethanol. This advances the procedure to Tier 3. Solutions are again mixed as prescribed in **Section VII.D.2.a** in an attempt to dissolve. If solubility is not achieved in Tier 3, the procedure continues to Tier 4, and to 5 if necessary (see **Figure 1** and **Table 2**).

Table 2 Determination of Solubility in Routine Culture Medium, DMSO, or Ethanol

TIER	1	2	3	4	5
Total Volume Medium	0.5 mL	5 mL	50 mL		
Concentration of Test Chemical (Add ~10 mg to a tube. Add enough medium to equal the first volume. Dilute to subsequent volumes if necessary.)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	200 µg/mL (0.20 mg/mL)		
Total Volume DMSO/Ethanol		0.5 mL	5 mL	50 mL	
Concentration of Test Chemical (Add ~100 mg to a large tube. Add enough DMSO or ethanol to equal the first volume. Dilute to subsequent volumes if necessary.)		200,000 µg/mL (200 mg/mL)	20,000 µg/mL (20 mg/mL)	2,000 µg/mL (2 mg/mL)	
Total Volume DMSO/Ethanol					50 mL
Concentration of Test Chemical (Add ~10 mg to a large tube. Add enough DMSO or ethanol to equal 50 mL.)					200 µg/mL (0.2 mg/mL)
Equivalent Concentration on Cells	10,000 µg/mL (10 mg/mL)	1000 µg/mL (1 mg/mL)	100 µg/mL (0.1 mg/mL)	10 µg/mL (0.01 mg/mL)	1 µg/mL (0.001 mg/mL)

NOTE: The amounts of test chemical weighed and Routine Culture Medium added may be modified from the amounts given above, provided that the targeted concentrations specified for each tier are tested.

Figure 1. Solubility Flow Chart

TIER 1

STEP 1:	20 mg/mL test chemical (TC) in 0.5 mL medium: • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 2.

TIER 2

STEP 2:	2 mg/mL TC in medium – increase volume from STEP 1 by 10 (i.e., to 5 mL) • if TC soluble, then **STOP**. • if TC insoluble, then go to STEP 3.

STEP 3:	200 mg/mL TC in DMSO
	• if TC soluble, then **STOP**.
	• if TC insoluble, test at 200 mg/mL in ETOH.
	  • if TC soluble, then **STOP.**
	  • If TC insoluble, go to STEP 4.

TIER 3

STEP 4:	0.2 mg/mL TC in medium – increase volume from STEP 2 by 10 (i.e., to 50 mL)
	• if TC soluble, then **STOP**.
	• if TC insoluble, test at 20 mg/mL in DMSO – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, test at 20 mg/mL in ETOH – increase volume from STEP 3 by 10 (i.e., to 5 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 5.

TIER 4

STEP 5:	2 mg/mL TC in DMSO – increase volume from STEP 4 by 10 (i.e., to 50 mL)
	• if TC soluble, then **STOP.**
	• if TC insoluble, test at 2 mg/mL in ETOH – increase volume from STEP 4 by 10 (i.e., to 50 mL).
	  • if TC soluble, then **STOP.**
	  • if TC insoluble, then go to STEP 6.

TIER 5

STEP 6:	0.2 mg/mL TC in 50 mL DMSO • if TC soluble, then **STOP.** • if TC insoluble, test at 0.2 mg/mL in 50 mL ETOH • **STOP**

2. Mechanical Procedures

a) The following hierarchy of mixing procedures will be followed to dissolve the test chemical:

1) Add test chemical to solvent as in Tier 1 of **Table 2**.

2) Gently mix. Vortex the tube (1 –2 minutes).

3) If test chemical hasn't dissolved, use sonication for up to 5 minutes.

4) If sonication doesn't work, then warm solution to 37°C for 5 – 60 minutes. This can be performed by warming tubes in a 37°C water bath or in a CO_2 incubator at 37°C. The solution may be stirred during warming (stirring in a CO_2 incubator will help maintain proper pH).

5) Proceed to Tier 2 (and Tiers 3-5, if necessary of Table 2 and repeat procedures 2-4).

b) The preference of solvent for dissolving test chemicals is medium, DMSO, and then ethanol. Thus, if a test chemical dissolves in more than one solvent at any one solubility-testing tier, then the choice of solvent follows this hierarchy. For example, if, at any tier, a chemical is soluble in medium and DMSO, but not ethanol, the choice of solvent would be medium. If the chemical were insoluble in medium, but soluble in DMSO and ethanol, the choice of solvent would be DMSO.

After the lab has determined the preferred solvent for the test chemical and before proceeding to the cytotoxicity testing, the Study Director will discuss the solvent selection with the Study Management Team (SMT) of the validation study. The SMT will relate what solvent should be used in the assay for each chemical. If the laboratory has attempted all solubility testing without success, then the SMT will provide additional guidance for achieving test chemical solubility. The SMT anticipates that all validation study test chemicals will be tested in the NRU assays.

E. Preparation of Test Chemicals

[Note: Preparation under red or yellow light is recommended to preserve chemicals that degrade upon exposure to light.]

1. Test Chemical in Solution

a) Allow test chemicals to equilibrate to room temperature before dissolving and diluting.

b) Prepare test chemical immediately prior to use. Test chemical solutions should not be prepared in bulk for use in subsequent tests. The solutions must not be cloudy nor have noticeable precipitate. Each stock dilution should have at least 1-2 mL total volume to ensure adequate solution for the test wells in a single 96-well plate. The SMT may direct the Study Director to store an aliquot (e.g., 1 mL) of the highest 2X

stock solution (e.g., low solubility chemicals) in a freezer (e.g., -70°C) for use in future chemical analyses.

c) For chemicals dissolved in DMSO or ethanol, the final DMSO or ethanol concentration for application to the cells must be 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations.

d) The stock solution for each test chemical should be prepared at the highest concentration found to be soluble in the solubility test. Thus, the highest test concentration applied to the cells in each range finding experiment is:
   - 0.5 times the highest concentration found to be soluble in the solubility test, if the chemical was soluble in medium, or
   - 1/200 the highest concentration found to be soluble in the solubility test if the chemical was soluble in ethanol or DMSO.

e) The seven lower concentrations in the range finding experiment would then be prepared by successive dilutions that decrease by one log unit each. The following example illustrates the preparation of test chemical in solvent and the dilution of dissolved test chemical in medium before application to NHK cells.

   Example: Preparation of Test Chemical in Solvent Using a Log Dilution Scheme

   If DMSO was determined to be the preferred solvent at Tier 2 of the solubility test (i.e., 200,000 μg/mL), dissolve the chemical in DMSO at 200,000 μg/mL for the chemical stock solution.

   1) Label eight tubes 1 – 8. Add 0.9 mL solvent (e.g., DMSO) to tubes 2 -- 8.

   2) Prepare stock solution of 200,000 μg test chemical/mL solvent in tube # 1.

   3) Add 0.1 mL of 200,000 μg/mL dilution from tube #1 to tube #2 to make a 1:10 dilution in solvent (i.e., 20,000 μg/mL).

   4) Add 0.1 mL of 20,000 μg/mL dilution from tube #2 to tube #3 to make another 1:10 dilution (i.e., 1:100 dilution from stock solution) in solvent (i.e., 2,000 μg/mL)

   5) Continuing making serial 1:10 dilutions in the prepared solvent tubes.

   6) Since each concentration is 200 fold greater than the concentration to be tested, make a 1:100 dilution by diluting 1 part dissolved chemical in each tube with 99 parts of culture medium (e.g., 0.1 mL of test chemical in DMSO + 9.9 mL culture medium) to derive the eight 2X concentrations for application to NHK cells. Each 2X test chemical concentration will then contain 1 % v/v solvent. The NHK cells will have 0.125 mL of culture medium in the wells prior to application of the test chemical. By adding 0.125 mL of the appropriate 2X test chemical concentration to the appropriate wells, the test chemical will be diluted appropriately (e.g., highest concentration in well will be 1,000 μg/mL) in a total of 0.250 mL and the solvent concentration in the wells will be 0.5% v/v.

7) A test article prepared in DMSO or ethanol may precipitate upon transfer into the Routine Culture Medium. The 2X dosing solutions should be evaluated for precipitates and the results will be recorded in the workbook. It will be permissible to test all of the dosing solutions in the dose range finding assay only. Doses containing test article precipitates should be avoided, and will not be used in the ICx determinations for either the range finding experiments or the definitive tests.

Document all test chemical preparations in the Study Workbook.

2. pH of Test Chemical Solutions

Measure the pH of the highest concentration of the test chemical in culture medium using pH paper (e.g., pH 0 – 14 to estimate and pH 5 – 10 to determine more precise value). The pH paper should be in contact with the solution for approximately one minute. Document the pH and note the color of the medium for all dilutions. Do not adjust the pH.

3. Concentrations of Test Chemical

a) Range Finder Experiment

Test eight concentrations of the test chemical by diluting the stock solution with a constant factor covering a large range. The initial dilution series shall be log dilutions (e.g., 1:10, 1:100, 1:1000, etc.).

The data from any well that has precipitate will be excluded from any calculations.

b) Main Experiment

[Note: After the range finding assay is completed, the concentration-response experiment shall be performed three times on three different days for each chemical (i.e., one plate per day per chemical)]

Depending on the slope of the concentration-response curve estimated from the range finder, the dilution/progression factor in the concentration series of the main experiment should be smaller ($\sqrt[6]{10} = 1.47$). Cover the relevant concentration range ($\geq$10 % and $\leq$ 90 % effect) preferably with three points of a graded effect, but with a minimum of two points, one on each side of the estimated IC_{50} value, avoiding too many non-cytotoxic and/or 100 %-cytotoxic concentrations. Experiments revealing less than one cytotoxic concentration on each side of the IC_{50} value shall be repeated, where possible, with a smaller dilution factor. Each experiment should have at least one cytotoxicity value $\geq$ 10.0 % and $\leq$ 50.0 % viability and at least one cytotoxicity value > 50.0 % and $\leq$ 90.0 % viability. In addition, the dilution scheme shall be adjusted in subsequent replicate assays (i.e., definitive assays), if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. (Taking into account pipetting errors, a progression factor of 1.21 is regarded the smallest factor achievable.)

Determine which test chemical concentration is closest to the IC_{50} value (e.g., 50 % cytotoxicity). Use that value as a central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

Maximum Doses to be Tested in the Main Experiments

If minimal or no cytotoxicity was measured in the dose range finding assay, a maximum dose for the main experiments will be established as follows:

- For test chemicals prepared in Routine Culture Medium, the highest test article concentration that may be applied to the cells in the main experiments will be either 100 mg/mL, or the maximum soluble dose. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of Routine Culture Medium will be added to the vessel so that the concentration is 200,000 µg/mL (200 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in medium, then 7 additional serial stock dosing solutions may be prepared from the 200 mg/mL 2X stock. If the test chemical is insoluble in medium at 200 mg/ml, proceed by adding medium, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

- For test chemicals prepared in either DMSO or ethanol, the highest test article concentration that may be applied to the cells in the main experiments will be either 2.5 mg/mL, or less, depending upon the maximum solubility in solvent. Test chemical will be weighed into a glass tube and the weight will be documented. A volume of the appropriate solvent (determined from the original solubility test) will be added to the vessel so that the concentration is 500,000 µg/mL (500 mg/mL). The solution is mixed as specified in **Section VII.D.2.a**. If complete solubility is achieved in the solvent, then 7 additional serial stock dosing solutions may be prepared from the 500 mg/mL 200X stock. If the test chemical is insoluble in solvent at 500 mg/ml, proceed by adding solvent, in small incremental amounts, to attempt to dissolve the chemical by using the sequence of mixing procedures specified in **Section VII.D.2.a**. The highest soluble stock solution will be used to prepare the 7 additional serial stock dosing solutions.

c) Test Chemical Dilutions

The dosing factor of 3.16 ($= \sqrt[2]{10}$) divides a log into two equidistant steps, a factor of 2.15 ($= \sqrt[3]{10}$) divides a decade into three steps. The factor of 1.47 ($= \sqrt[6]{10}$) divides a log into six equidistant steps, and the factor of 1.21 ($= \sqrt[12]{10}$) divides the log into 12 steps.

EXAMPLE:

10						31.6						100
10				21.5				46.4				100
10		14.7		21.5		31.6		46.4		68.1		100
10	12.1	14.7	17.8	21.5	26.1	31.6	38.3	46.4	56.2	68.1	82.5	100

The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration, dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

F. Test Procedure

1. 96-Well Plate Configuration

The NHK NRU assay for test chemicals will use the 96-well plate configuration shown in Figure 2.

Figure 2. 96-Well Plate Configuration for Positive Control and Test Chemical Assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb
B	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
C	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
D	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
E	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
F	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
G	VCb	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	VCb
H	VCb	VCb	C_1b	C_2b	C_3b	C_4b	C_5b	C_6b	C_7b	C_8b	VCb	VCb

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 – C_8$ = Test Chemicals or Positive Control (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)
VCb = VEHICLE CONTROL BLANK

2. Application of Test Chemical

a) Two optional methods for rapidly applying the 2X dosing solutions onto the 96-well plates may be utilized.

1) The first method is to add each of the 2X dosing solutions into labeled, sterile reservoirs (e.g., Corning/Costar model 4870 sterile polystyrene 50 mL reagent reservoirs or Corning/Transtar model 4878 disposable reservoir liners, 8-channel; or other multichannel reservoirs).

2) The second method utilizes a "dummy" plate (i.e., an empty sterile 96-well plate) prepared to hold the dosing solutions immediately prior to treatment of the test plate (with cells). The test chemical and control dosing solutions should be dispensed into the dummy plate in the same pattern/order as will be applied to the plate containing cells. More volume than needed for the test plate (i.e. greater than 125 µl/well) should be in the wells of the dummy plate.

At the time of treatment initiation, a multi-channel micropipettor is used to transfer the 2X dosing solutions, from the reservoirs or dummy plate, to the appropriate wells on the treatment plate (as described in step c. below). These methods will ensure that the dosing solutions can be transferred rapidly to the appropriate wells of the test plate to initiate treatment times and to minimize the range of treatment initiation times across a large number of treatment plates, and to prevent "out of order" dosing. Do not use a multichannel repeater pipette for dispensing test chemical to the plates.

b) After 48 - 72 h (i.e., after cells attain 20+ % confluency [see Section VII.C.4(h)]) incubation of the cells, add 125 µl of the appropriate concentration of test chemical, the PC, or the VC (see Figure 2 for the plate configuration) directly to the test wells. Do not remove Routine Culture Medium for re-feeding the cells. The dosing solutions will be rapidly transferred from the 8-channel reservoir (or dummy plate) to the test plate using a single delivery multi-channel pipettor. For example, the VC may be transferred first (into columns 1, 2, 11, and 12), followed by the test article dosing solutions from lowest to highest dose, so that the same pipette tips on the multi-channel pipettor can be used for the whole plate. [The Vehicle Control blank (VCb) wells (column 1, column 12, wells A2, A11, H2, H11) will receive the Vehicle Control dosing solutions (which should include any solvents used). Blanks for wells A3 – A10 and H3 – H10 shall receive the appropriate test chemical solution for each concentration (e.g., wells A3 and H3 receive C_1 solution). The test chemical blanks in rows A and H will be used for their respective test chemical concentrations.] Incubate cells for 48 h ± 0.5 h (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air).

c) **Positive Control**: For each set of test chemical plates used in an assay, a separate plate of positive control concentrations will be set up following the concentration range established in the development of the positive control database in Phase I of the Validation Study. If multiple sets of test chemical plates are set up, then clearly designate the positive control plates for each set; each set will be an individual entity. The mean IC_{50} ± two and a half standard deviations (SD) for the SLS acceptable tests from Phases Ia and Ib (after the removal of outliers) are the values that will be used as an acceptance criterion for test sensitivity for the NHK NRU assay. This plate will follow the same schedule and procedures as used for the test chemical plates (including appropriate chemical concentrations in the appropriate wells – see sections VII.F.1 and F.2)..

3. Microscopic Evaluation

After at least 46 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record any changes in morphology of the cells due to the cytotoxic effects of the test chemical, but do not use these records for any quantitative measure of cytotoxicity. Undesirable growth characteristics of control cells may indicate experimental error and

may be cause for rejection of the assay. Use the following Visual Observations Codes in the description of cell culture conditions.

Visual Observations Codes

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

4. Measurement of NRU

a) Carefully remove (i.e., "dump") the Routine Culture Medium (with test chemical) and rinse the cells very carefully with 250 µL pre-warmed D-PBS. Remove the rinsing solution by dumping and remove excess by gently blotting on sterile paper towels. Add 250 µL NR medium (to all wells including the blanks) and incubate (37ºC ± 1ºC, 90 % ± 5 % humidity, and 5.0 % ± 1 % CO_2/air) for 3±0.1 h. Observe the cells briefly during the NR incubation (e.g., between 2 and 3 h – Study Director's discretion) for NR crystal formation. Record observations in the Study Workbook. Study Director can decide to reject the experiment if excessive NR crystallization has occurred.

b) After incubation, remove the NR medium, and carefully rinse cells with 250 µL pre-warmed D-PBS.

c) Decant and blot D-PBS from the plate. (Optionally: centrifuge the reversed plate.)

d) Add exactly 100 µL NR Desorb (ETOH/acetic acid) solution to all wells, including blanks.

e) Shake microtiter plate rapidly on a microtiter plate shaker for 20 – 45 min to extract NR from the cells and form a homogeneous solution. Plates should be protected from light by using a cover during shaking.

f) Plates should be still for at least five minutes after removal from the plate shaker (or orbital mixer). Observe the wells for bubbles. Measure the absorption (within 60 minutes of adding NR Desorb solution) of the resulting colored solution at 540 nm ± 10 nm in a microtiter plate reader (spectrophotometer), using the blanks as a reference. [Phases Ia and Ib data show the mean OD value for the plate blanks to be 0.055 ± 0.035 for NHK cells (± 2.5 standard deviations; data from 3 labs; N = 156). Use this range as a **guide** for assessment of the blank values.] Save raw data in the Excel format as provided by the SMT.

5. Quality Check of Assay

a) Test Acceptance Criteria

1) A test meets acceptance criteria, if the IC_{50} for SLS is within two and a half (2.5) standard deviations of the historical mean established by the Test Facility (as per **VII.F.2.c**).

2) A test meets acceptance criteria if the left and the right mean of the VCs do not differ by more than 15.0 % from the mean of all VCs.

3) A test meets acceptance criteria if:
   - at least one calculated cytotoxicity value ≥ 10.0 % and ≤ 50.0 % viability and
   - at least one calculated cytotoxicity value > 50.0 % and ≤ 90.0 % viability.

4) A test meets acceptance criteria if the r^2 (coefficient of determination) value calculated for the Hill model fit (i.e., from PRISM® software) is ≥ 0.90. A test does not meet acceptance criteria if the r^2 value is < 0.80. If the r^2 value is ≥ 0.80 and < 0.90 ("gray zone"), then the SMT will evaluate the model fit and make the determination of whether or not the test meets the acceptance criteria and relate the information to the Study Director.

[Note: All acceptance criteria must be met for an assay to be considered acceptable.]

[A corrected mean $OD_{540 \pm 10nm}$ of 0.205 - 1.645 for the VCs is a target range but will not be a test acceptance criterion. Range determined from Phase Ib VC OD values from 3 laboratories (mean ± 2.5 standard deviations, N = 69).]

b) Checks for Systematic Cell Seeding Errors

To check for systematic cell seeding errors, untreated VCs are placed both at the left side (row 2) and the right side (row 11 for the test plates) of the 96-well plate. Aberrations in the cell monolayer for the VCs may reflect a volatile and toxic test article present in the assay.

Checks for cell seeding errors may also be performed by examining each plate under a phase contrast microscope to assure that cell quantity is consistent.

c) Quality Check of Concentration-Response

The IC_{50} derived from the concentration-response of the test chemicals should be backed by preferably three responses ≥ 10 and ≤ 90 % inhibition of NRU and at least two responses, one on either side of the IC_{50} value (see sections **VII.E.3.b and VII.F.5.a.3**). If this is not the case, and the concentration progression factor can be easily reduced, reject the experiment and repeat it with a smaller progression factor. In addition, the dilution scheme shall be adjusted in subsequent replicate assays, if necessary, to increase the number of points on both sides of the IC_{50} in the 10-90% response range. Numerical scoring of the cells (see **VII.F.3**) should be determined and documented in the Study Workbook.

G. Data Analysis

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicates wells) per test concentration. The Study Director will use good biological/scientific judgment for determining "unusable" wells that will be excluded from the statistical analysis. This value is compared with the mean NRU of all VC values (provided VC values have met the VC acceptance criteria). Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability. Data from the microtiter plate reader shall be transferred to the Excel® spreadsheet (template with macros provided by the SMT) that will automatically determine cell viability and perform statistical analyses (including determination of outliers).

The concentration of a test chemical reflecting a 20 %, 50 %, and 80 % inhibition of cell viability (i.e., the IC_{20}, IC_{50}, and IC_{80}) is determined from the concentration-response by applying a Hill function to the concentration-response data. Statistical software (e.g., GraphPad PRISM® 3.0) specified by the SMT shall be used to calculate IC_{20}, IC_{50}, and IC_{80} values (and the associated confidence limits) for each test chemical. In addition, the SMT shall provide guidelines for calculating ICx values and confidence limits. The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the SMT/biostatistician through the designated contacts in electronic format and hard copy upon completion of testing. The SMT will be directly responsible for the statistical analyses of the Validation Study data.

VIII. REFERENCES

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IX. APPROVAL

_____ _____

SPONSOR REPRESENTATIVE **DATE**

(Print or type name)

_____ _____

Testing Facility STUDY DIRECTOR **DATE**
(Print or type name)

Appendix D

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Appendix D1

SAS Code for ANOVA and Contrasts

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```
 options nodate nonumber;
libname lib "S:\NIEHS\EXP Studies\BasicResearch\Haseman\Cytotoxicity
Validation\Post Phase III Analysis and Data\data sets";


proc sort data=lib.anovadata; by chemical cell lab;

ods trace on;
ods listing close;
ods output OverallANOVA=temp;
ods output Contrasts=temp1;
proc glm data=lib.anovadata;
class lab;
by chemical cell;
model log_ic50=lab;
contrast 'Comparing IIVS to FRAME and ECBC'
lab -.5 -.5 1;
contrast 'Comparing ECBC to FRAME and IIVS'
lab 1 -.5 -.5;
contrast 'Comparing FRAME to ECBC and IIVS'
lab -.5 1 -.5;

run;ods listing;
*proc print data=temp1;run;

data lib.contrast_results; set temp1;
keep chemical cell Source ProbF;
run;

*proc print data=lib.contrast_results;run;

data lib.anova_results; set temp;
if Source="Error" then delete;
if Source="Corrected Total" then delete;
keep chemical cell ProbF;
run;

proc sort data=lib.anova_results; by chemical cell;

/*proc print data=lib.anova_results;
var chemical cell ProbF;
run;*/

data temp;
set lib.anova_results;
keep chemical cell ProbF;

proc export data=temp
   outfile='S:\NIEHS\EXP Studies\BasicResearch\Haseman\Cytotoxicity
Validation\Post Phase III Analysis and Data\data sets\Anova Results.txt'
   dbms=TAB;

run;
```

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Appendix D2

SAS Code for Regression Comparisons

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```
dm 'output; clear';
dm 'log; clear';
*************************************************************************
*
*filename: task3.sas
*creation date: 08/02/06
*study:niceatm
*investigator:
*purpose: perform the individual lab regressions (tasks 1-4)
* note: 3 models are fit:
* (a) full model
* (b) reduced model with separate intercepts + common slope
* (c) separate intercepts + separate slopes model
*authors:mike riggs
*input data medium: sas data sets
*
*************************************************************************;
*
*
*compare rc to niceatm regressions, by cell type
*note: the input data set anal3 was created by taking the 47 3t3
*chemicals and the 51 nhk chemicals and computing their
*means by cell line
*
*
*
*************************************************************************;
proc mixed data=anal3 maxiter=200;
 by celline;
 class est_type;
 model log_ld50=est_type logic50_lab est_type*logic50_lab/outpredm=predat;
 title1 'ancova model (estimation type = trt) log-scale lab regressions, by
cell line';
 title2 '(test for slope differences)';
run;
quit;

****compute the full-model rsquare from the model residuals and predictions
***;
****note: proc mixed does not compute rsq, so you need to do it
yourself***;

data pred3t3 prednhk;
 set predat;
 if celline='3t3' then output pred3t3;
else output prednhk;
run;

proc summary data=pred3t3 nway;
 var log_ld50;
 output out=sumdat
mean=_mean_;
run;

data pred3t3;
 if _n_=1 then set sumdat;
 set pred3t3;
```

```
run;

data comp;
 set pred3t3 end=eof;
 sst+((log_ld50-_mean_)**2);
 sse+(resid**2);
 n=_n_ ;
 if eof then output;
run;

data comp;
 set comp;
 rsq=(sst-sse)/sst;
 label _mean_='response*mean'
sst='total sum*of squares'
sse='error sum*of squares'
rsq='r-squared';
run;

proc print data=comp split='*';
 var n _mean_ sst sse rsq;
 format rsq 5.3;
title1 'full ancova model r-square for 3t3 cell line (task 3)';
run;


proc summary data=prednhk nway;
 var log_ld50;
 output out=sumdat
mean=_mean_;
run;

data prednhk;
 if _n_=1 then set sumdat;
 set prednhk;
run;

data comp;
 set prednhk end=eof;
 sst+((log_ld50-_mean_)**2);
 sse+(resid**2);
 n=_n_ ;
 if eof then output;
run;

data comp;
 set comp;
 rsq=(sst-sse)/sst;
 label _mean_='response*mean'
sst='total sum*of squares'
sse='error sum*of squares'
rsq='r-squared';
run;

proc print data=comp split='*';
 var n _mean_ sst sse rsq;
 format rsq 5.3;
```

```
title1 'full ancova model r-square for nhk cell line (task 3)';
run;


proc mixed data=anal3 maxiter=200;
 by celline;
 class est_type;
 model log_ld50=est_type est_type*logic50_lab/noint solution cl alpha=0.05;
 * the following contrast is the simultaneous test of equal intercepts and
slopes ***;
 contrast 'lab vs. rc' est_type -1 1,
 est_type*logic50_lab -1 1;
 title1 'ancova model (trt=estimation type) log-scale lab regressions, by
cell line';
 title2 '(separate slope estimates)';
run;
quit;

proc mixed data=anal3 maxiter=200;
 by celline;
 model log_ld50=logic50_lab/solution cl alpha=0.05;
 title1 'ancova model (estimation type = trt) log-scale lab regressions, by
cell line';
 title2 '(estimate homogeneous slope with single intercept)';
run;
quit;
```

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Appendix E

Neutral Red Dye Experiments

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APPENDIX E
Neutral Red Dye Experiments

Appendix E1: Institute for *In Vitro* Sciences (IIVS) Assessment of Protocol Variables in the NICEATM/ECVAM Evaluation of Cytotoxicity Assays

IIVS performed experiments using the 3T3 cells and the NRU test methods before the NICEATM/ECVAM validation study was initiated. The laboratory examined: optimal solvent concentrations (DMSO and ETOH), cell seeding densities, doubling times, and exposure duration of a test chemical (24, 48, and 72-hour exposures). Data are presented in the appendix.

Appendix E2: Neutral Red (NR) Dye Experiments – 3T3 Cells

IIVS performed three sets of experiments to compare the optical density (OD) readings obtained in an NRU assay using various concentrations of NR dye and different incubation periods.

- Experiment 1: NR Stain Time Course in 3T3 Cells; NRU incubation times: 0.25, 0.50, 1.0, 2.0, and 3.0 hour.
- Experiment 2: Neutral Red Stain Prepared in DMEM/5%NCS; Test of NR Preparation 1 Day Prior to Use; Tested in 90-100% Confluent 3T3 Cultures
- Experiment 3: Neutral Red Stain Prepared in DMEM/5%NCS; Filtered Immediately before Use; Tested in 90-100% Confluent 3T3 Cultures

Appendix E3: Neutral Red (NR) Dye Experiments – NHK Cells

IIVS performed three sets of experiments to compare the optical density (OD) readings obtained in an NRU assay using various concentrations of NR dye and different incubation periods.

- Experiment 1: NR Stain Time Course in NHK Cells; NRU incubation times: 0.25, 0.50, 1.0, 2.0, and 3.0 hour.
- Experiment 2: Neutral Red Stain Prepared in KGM; Test of NR Preparation 1 Day Prior to Use; Tested in 90-100% Confluent NHK Cultures
- Experiment 3: Neutral Red Stain Prepared in KGM; Filtered Immediately before Use; Tested in 90-100% Confluent NHK Cultures

Appendix E4: Neutral Red (NR) Dye Experiments – Concentration vs Time – 3T3 Cells

ECBC performed experiments using the 3T3 cells and the NRU test methods.

- *in vitro* cytotoxicity NRU tests (3T3 cells) using SLS (range = 100 μg/mL to 6.7 μg/mL)
- NR dye mixed with DMEM culture medium with 10% NCS; final concentrations = 25 μg/mL and 50 μg/mL
- Tests performed with two NRU incubation times: 1 hour and 3 hours

μg NR dye/mL	NRU Incubation Time (hours)	Mean Vehicle Control OD_{540} Value
25	1	0.255
25	3	0.508
50	1	0.330
50	3	0.457

Appendix E1

Institute for *In Vitro* Sciences (IIVS) Assessment of Protocol Variables in the NICEATM/ECVAM Evaluation of Cytotoxicity Assays

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INSTITUTE FOR *IN VITRO* SCIENCES (IIVS)
ASSESSMENT OF PROTOCOL VARIABLES IN THE NTP EVALUATION OF CYTOTOXICITY ASSAYS
APRIL 2002

BALB/c 3T3 Cells

I. What is the acceptable solvent concentration?

Two solvents, DMSO and ETOH, were assayed in the 3T3 assay to determine acceptable concentrations. Multiple exposure times were assessed since the final assay exposure time was not yet established. Various cell seeding concentrations were tested since these experiments were run concurrently with others which used to determine optimal seeding density.

Table 1.

ETOH										
	Date	**2%**	**1%**	**0.50%**		**Seeding Density**				
48hour	2/26/02	58%	72%	100%		$9X10^3$ cells/ml				
	2/26/02	49%	73%	102%		$4.5X10^3$ cells/ml				
72hour	2/26/02	67%	75%	105%		$9X10^3$ cells/ml				
	2/26/02	68%	82%	108%		$4.5X10^3$ cells/ml				

DMSO										
	Date	**2%**	**1%**	**0.5%**	**0.4%**	**0.3%**	**0.2%**	**0.1%**	**Seeding Density**	
24hour	3/19/02		76%	91%	92%	99%	100%	101.6%	$2X10^4$ cells/ml	
48hour	2/26/02	25%	54%	83%					$9X10^3$ cells/ml	
	2/26/02	27%	56%	78%					$4.5X10^3$ cells/ml	
	3/19/02		116%	123%	122%	120%	117%	108.8%	$1X10^4$ cells/ml	
72hour	2/26/02	20%	52%	86%					$9X10^3$ cells/ml	
	2/26/02	19%	56%	93%					$4.5X10^3$ cells/ml	
	3/19/02		58%	89%	102%	102%	112%	110.1%	$5X10^3$ cells/ml	

We concluded from these experiments that 0.5% ETOH was the optimal ETOH concentration (little to no toxicity), and that 0.5% was probably acceptable for DMSO as a trade-off between slight toxicity and ability to test chemicals to higher does levels.

From about the middle of March 2002 on, we used 0.5% in all of our experiments where DMSO was called for as a solvent. This gave us a number of opportunities to further determine the toxicity of DMSO by comparing the solvent control wells with the media control wells in the same experiment.

Table 2.

DMSO			
Date & Exposure Time	OD Assay Medium Wells	OD Solvent Wells	% Survival in Solvent
24hour 3/19/02	0.502	0.474	94.5%
	0.441	0.394	89.4%
48hour 3/19/02	0.587	0.536	91.4%
	0.582	0.545	93.6%
72hour 3/19/02	0.687	0.601	87.6%
	0.666	0.588	88.3%

The average survival in 0.5% DMSO from **Table 2** was 90.8%.

II. Doubling Time Experiments

We ran a series of experiments designed primarily to determine the appropriate original seeding density for 24, 48, and 72 hour exposure times. We judged our results on visual observations of the cells at the conclusion of the experiment (control cells should be just confluent at 24, 48, or 72 hours), and on the shape of the growth curve.

Figure 1.

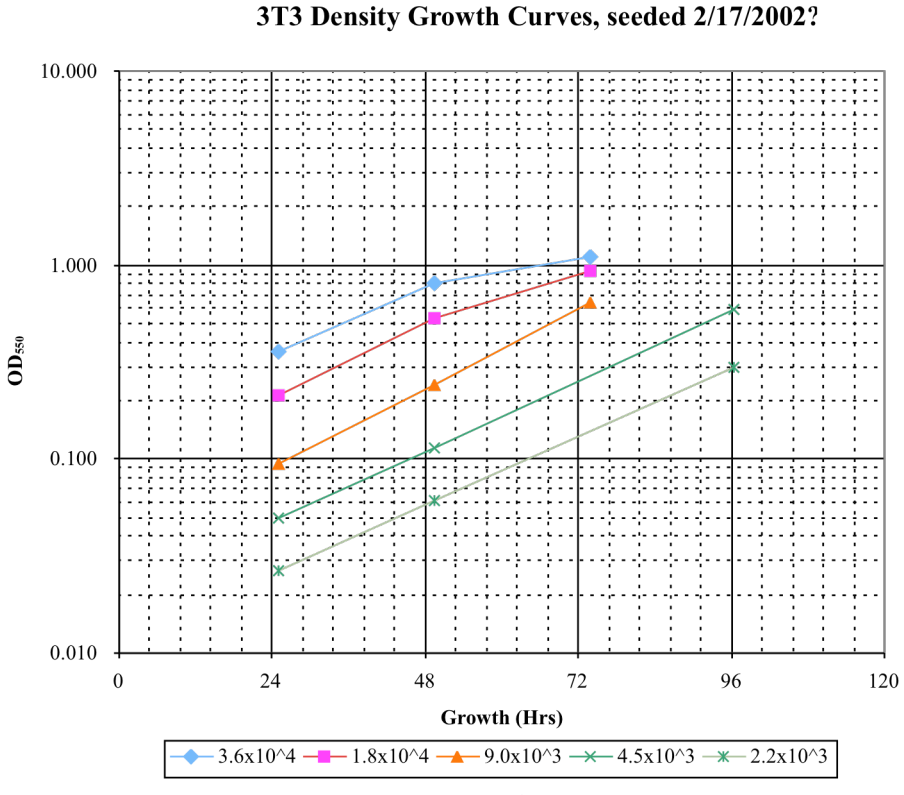
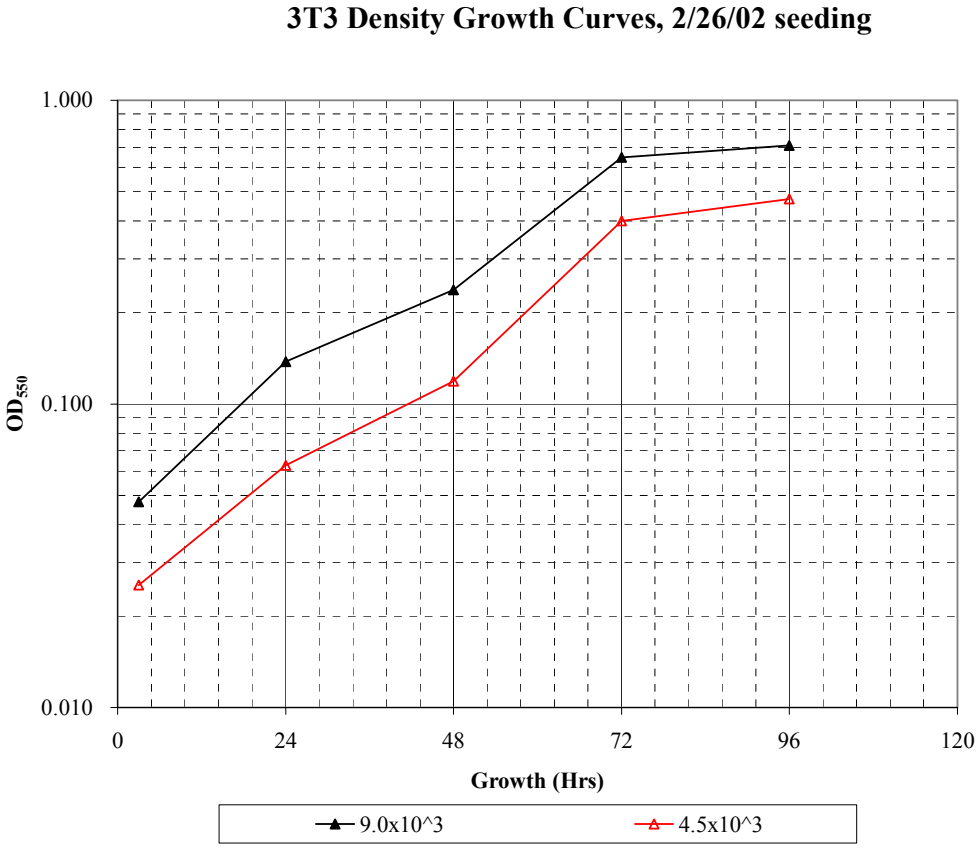


Figure 2.



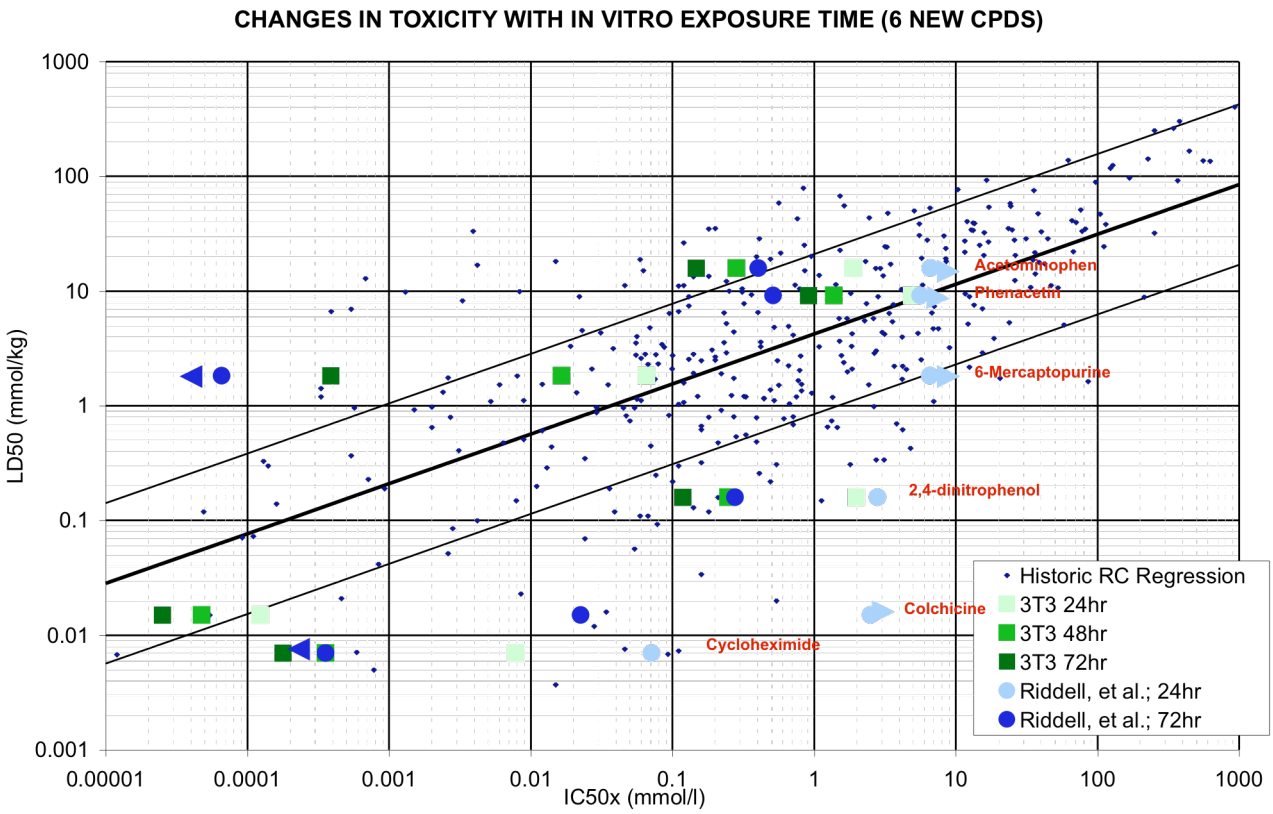
We have concluded from these growth curves that our 3T3 cells have a doubling time of about 19 hours and that cell concentration of: $1x10^4$ cells/ml (24hour); $5x10^3$ cells/ml (48hour); and $2.5x10^3$ (72hour) are acceptable.

III. Exposure Duration

The exposure question was first raised by Richard Clothier who indicated that a paper by Riddell, et al. (1986) showed a number of chemicals whose toxicity changed greatly between a 24 hour and a 72 hour exposure (for 25/50 materials there was little change and for 25/50 materials there was a change). We examined the paper and chose to investigate six chemicals that showed some of the largest differences between 24 hour and 72 hour.

Our initial studies gave similar results to those of Riddell et al. (1986). However we felt that the cell number for the longer exposures was not optimal, and we conducted additional studies to determine a standard seeding density for each exposure period. Using this methodology we looked at the 6 materials in a standardized fashion at 24, 48 and 72 hours. Our results are shown in **Fig. 3**.

Figure 3.

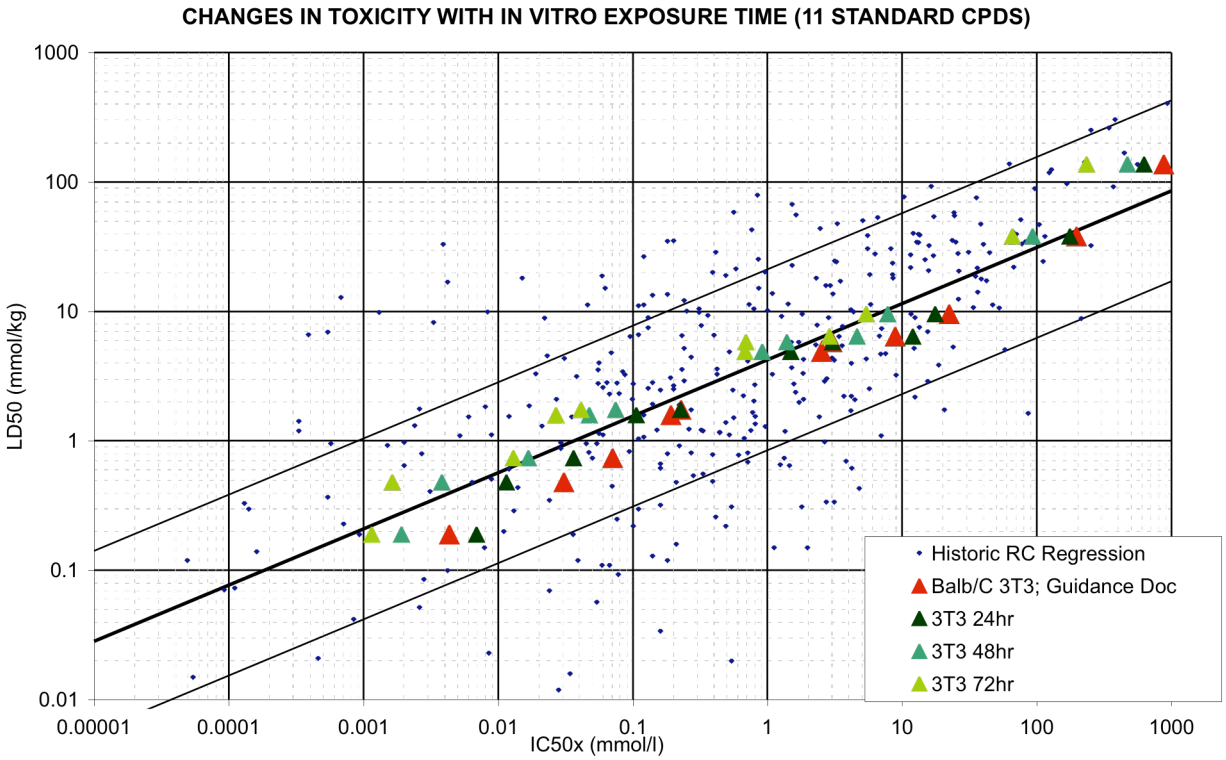


In this figure the historic Halle et al. (1992) data are shown as small blue dots and the regression line as a dark black line. To add perspective we have included the Riddell, et al. (1986) data as a light blue diamond (24hour) or a dark blue diamond (72hour). Arrows emerging from certain points indicate that the value is less than or greater than that point. Our values are graphed in increasing shades of green from light (24hour) to dark (72hour). All green values are averages of at least two separate experiments. It appears that our data are somewhat different than Riddell, et al. (1986), i.e., most differences are not as great as originally seen. Nonetheless the values, as expected, do become more toxic with increased exposure time. We feel that 48 hours is probably the optimal time for these data if the Halle regression is considered some type of a standard.

Next we asked whether a 48 hour exposure time would affect our earlier results with the 11 chemicals presented in the *Guidance Document* (ICCVAM 2001b). If these numbers were changed significantly, this might cause us to make significant modification to our guidance.

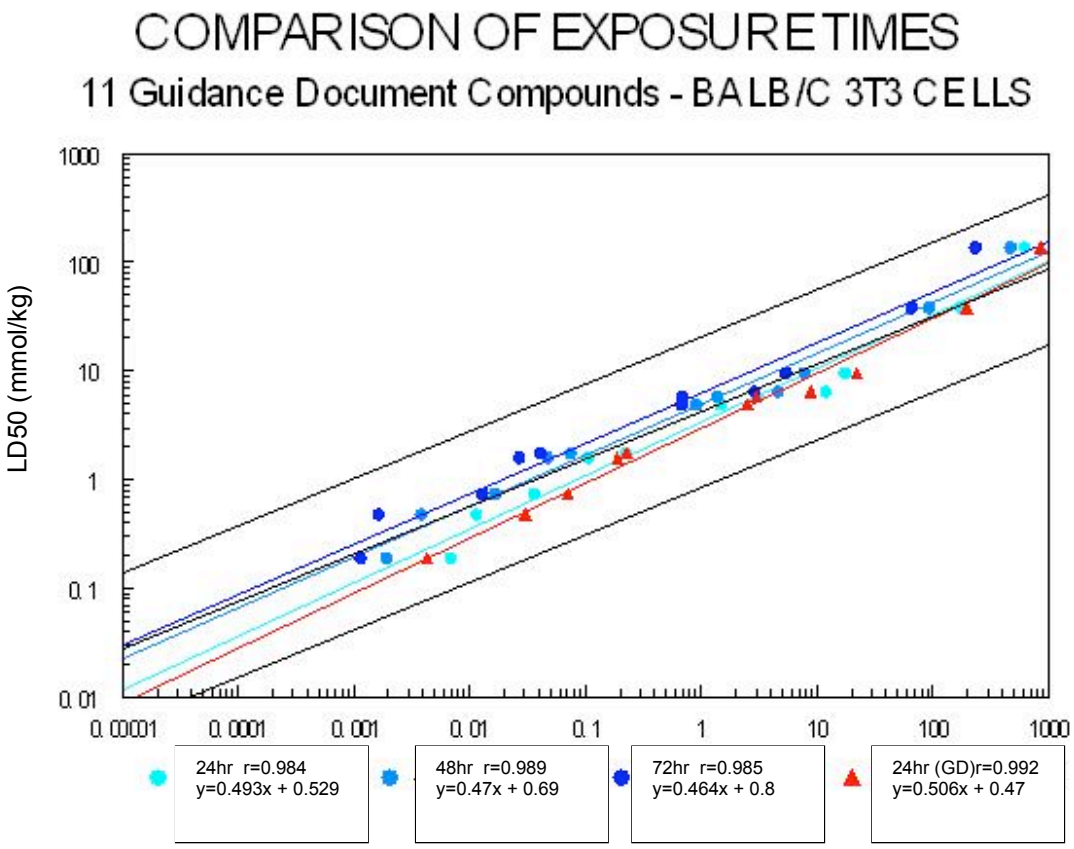
To assess the effect of increasing exposure time on the 11 chemicals, we tested them with exposure times of 24, 48 and 72 hours as shown in **Fig. 4**.

Figure 4.



The data shown on the graph are averages of duplicate experiments. It can be seen that although each of the chemicals becomes more toxic with increased exposure, all points are still within the 0.5 log range of the regression line. It again appears that 48 hour exposure fits the regression more closely, however we regraphed the data in **Fig. 5** to show the regression line and statistics for each of the new sets of data.

Figure 5.



In this figure it can be seen that all the regression lines for the 3 new time points plus the *Guidance Document* data (red triangles) fall with in the regression boundaries. It again appears that the 48 hour values best fit the original regression line.

We now feel that for the 3T3 cells an extended exposure period (>24hour) should be used, and that 48 hours seems to help identify the more toxic compounds while not over estimating the less toxic ones.

REFERENCES

Halle W, Spielmann H. 1992. Two procedures for the prediction of acute toxicity (LD50) from cytotoxicity data. Altern Lab Anim 20:40-49.

ICCVAM. 2001b. Guidance Document On Using *In Vitro* Data To Estimate In Vivo Starting Doses For Acute Toxicity. NIH Publication No. 01-4500. Research Triangle Park, NC:National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/ [accessed 01 November 2006].

Riddell RJ, Panacer DS, Wilde SM, Clothier RH, Balls M. 1986. The importance of exposure period and cell type in *in vitro* cytotoxicity tests. Altern Lab Anim 14:86-92.

Appendix E2

Neutral Red (NR) Dye Experiments – 3T3 Cells – IIVS

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Test Facility : IIVS
Chemical Code : N/A
2nd Chem. Code*: NRU

Study Number.: R&D - NR Stain Time Course in 3T3
96-Well Plate ID : 1
Experiment ID : RD96023T

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank											Blank
C	Blank											Blank
D	Blank	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min	Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.048	0.046	0.045	0.047	0.047	0.046	0.046	0.044	0.044	0.043	0.044	0.038
B	0.048	0.753	0.794	0.595	0.607	0.415	0.396	0.267	0.282	0.219	0.213	0.039
C	0.047	0.866	0.766	0.668	0.668	0.406	0.391	0.257	0.256	0.227	0.220	0.038
D	0.046	0.844	0.794	0.607	0.622	0.393	0.387	0.228	0.262	0.213	0.217	0.038
E	0.046	0.717	0.805	0.627	0.610	0.384	0.375	0.239	0.266	0.210	0.206	0.038
F	0.044	0.776	0.769	0.618	0.665	0.378	0.398	0.277	0.301	0.186	0.202	0.038
G	0.043	0.717	0.807	0.639	0.616	0.385	0.349	0.265	0.269	0.211	0.195	0.036
H	0.044	0.044	0.045	0.044	0.045	0.045	0.043	0.043	0.045	0.045	0.041	0.036

CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

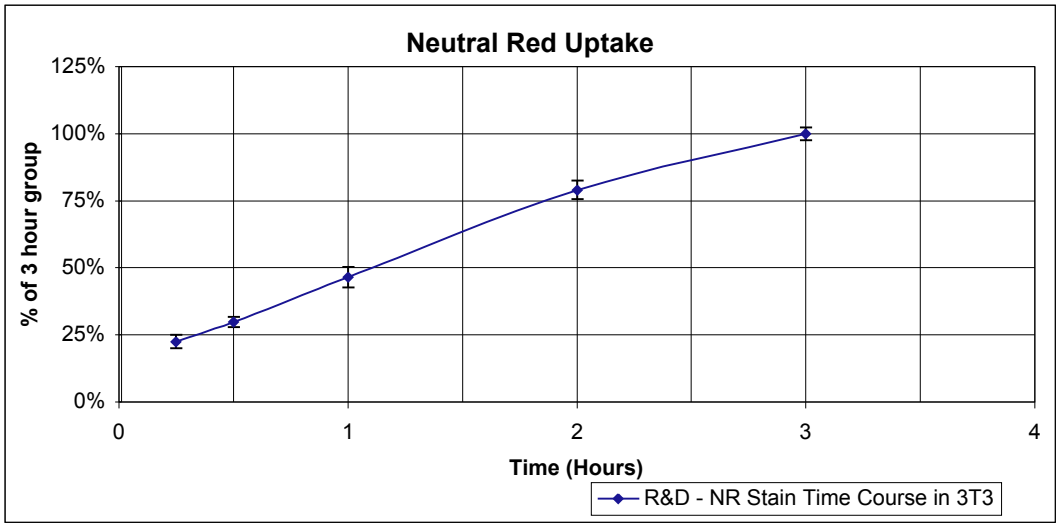
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.005	0.003	0.002	0.004	0.004	0.003	0.003	0.001	0.001	0.000	0.001	-0.005
B	0.005	0.710	0.751	0.552	0.564	0.372	0.353	0.224	0.239	0.176	0.170	-0.004
C	0.004	0.823	0.723	0.625	0.625	0.363	0.348	0.214	0.213	0.184	0.177	-0.005
D	0.003	0.801	0.751	0.564	0.579	0.350	0.344	0.185	0.219	0.170	0.174	-0.005
E	0.003	0.674	0.762	0.584	0.567	0.341	0.332	0.196	0.223	0.167	0.163	-0.005
F	0.001	0.733	0.726	0.575	0.622	0.335	0.355	0.234	0.258	0.143	0.159	-0.005
G	0.000	0.674	0.764	0.596	0.573	0.342	0.306	0.222	0.226	0.168	0.152	-0.007
H	0.001	0.000	0.002	0.001	0.002	0.002	0.000	0.000	0.002	0.002	-0.002	-0.007

Mean Blank =	0.043

RELATIVE VIABILITY (% OF VEHICLE CONTROL)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		95.8%	101.4%	74.5%	76.1%	50.2%	47.6%	30.2%	32.2%	23.7%	22.9%	
C		111.1%	97.6%	84.3%	84.3%	49.0%	46.9%	28.9%	28.7%	24.8%	23.9%	
D		108.1%	101.4%	76.1%	78.1%	47.2%	46.4%	24.9%	29.5%	22.9%	23.5%	
E		91.0%	102.8%	78.8%	76.5%	46.0%	44.8%	26.4%	30.1%	22.5%	22.0%	
F		98.9%	98.0%	77.6%	83.9%	45.2%	47.9%	31.6%	34.8%	19.3%	21.4%	
G		91.0%	103.1%	80.4%	77.3%	46.1%	41.3%	29.9%	30.5%	22.6%	20.5%	
H												

	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min
Conc. (µg/mL) :										
Mean Corr. OD :	0.736	0.746	0.582	0.588	0.350	0.339	0.212	0.229	0.168	0.166
SD :	0.064	0.018	0.026	0.028	0.014	0.018	0.019	0.016	0.014	0.010
Mean 3 hour :	0.741									
Mean Blank :	0.043									
% of 3 hour:	99.3%	100.7%	78.6%	79.4%	47.3%	45.8%	28.6%	31.0%	22.6%	22.3%
SD :	8.6%	2.4%	3.5%	3.7%	1.9%	2.5%	2.5%	2.2%	1.9%	1.3%
% CV :	8.63%	2.37%	4.42%	4.72%	4.08%	5.42%	8.73%	7.14%	8.22%	5.76%
hours			3	2	1	0.50	0.25			
% of 3 hour:			100.0%	79.0%	46.5%	29.8%	22.5%			


Neutral Red Uptake
125%
100%
75%
50%
25%
0%
% of 3 hour group
0
1
2
3
4
Time (Hours)
R&D - NR Stain Time Course in 3T3

Neutral Red Stain Prepared in DMEM5%NCS - TEST OF NR PREP 1 DAY PRIOR TO USE
Tested in 90-100% Confluent 3T3 Cultures

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank											Blank
C	Blank		50 ug/ml			50 ug/ml				33 ug/ml		Blank
D	Blank		Prepared and filtered			Filtered before use				Filtered before use		Blank
E	Blank		in evening before use									Blank
F	Blank		Filtered before use									Blank
G	Blank											Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.045	0.045	0.045	0.044	0.056	0.056	0.056	0.057	0.053	0.051	0.051	0.052
B	0.043	0.383	0.459	0.417	0.541	0.631	0.639	0.635	0.637	0.686	0.656	0.052
C	0.045	0.389	0.397	0.379	0.557	0.536	0.621	0.559	0.590	0.618	0.612	0.051
D	0.043	0.383	0.429	0.350	0.539	0.575	0.545	0.629	0.613	0.658	0.652	0.053
E	0.042	0.361	0.345	0.334	0.579	0.585	0.577	0.573	0.626	0.635	0.599	0.051
F	0.044	0.368	0.412	0.374	0.582	0.588	0.578	0.572	0.687	0.647	0.641	0.050
G	0.042	0.415	0.451	0.422	0.600	0.620	0.616	0.632	0.572	0.744	0.637	0.050
H	0.044	0.042	0.043	0.043	0.057	0.059	0.055	0.057	0.050	0.057	0.050	0.054

CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.002	0.002	0.002	0.001	0.013	0.013	0.013	0.014	0.010	0.008	0.008	0.009
B	0.000	0.340	0.416	0.374	0.498	0.588	0.596	0.592	0.594	0.643	0.613	0.009
C	0.002	0.346	0.354	0.336	0.514	0.493	0.578	0.516	0.547	0.575	0.569	0.008
D	0.000	0.340	0.386	0.307	0.496	0.532	0.502	0.586	0.570	0.615	0.609	0.010
E	-0.001	0.318	0.302	0.291	0.536	0.542	0.534	0.530	0.583	0.592	0.556	0.008
F	0.001	0.325	0.369	0.331	0.539	0.545	0.535	0.529	0.644	0.604	0.598	0.007
G	-0.001	0.372	0.408	0.379	0.557	0.577	0.573	0.589	0.529	0.701	0.594	0.007
H	0.001	0.000	0.000	0.000	0.014	0.016	0.012	0.014	0.007	0.014	0.007	0.011

Mean Blank =	0.052	(Only the 14 wells from the 33 ug/ml group)

	Neutral Red Stain Concentration									
Conc. (µg/mL) :		50.0			50.0				33.0	
Mean Corr. OD :	0.340	0.372	0.336	0.523	0.546	0.553	0.557	0.578	0.621	0.590
SD :	0.019	0.042	0.035	0.025	0.034	0.035	0.035	0.040	0.045	0.023
Group mean corr OD:		0.349			0.545				0.596	

Note: Significant crystal formation was observed in the DMEM5%NCS/NR prepared 1 day prior,
and the color was essentailly medium-colored. Much NR stain stripped out of solution.
No ppt or crystalization observed in the wells during the NR loading of cells.

Neutral Red Stain Prepared in DMEM5%NCS/Filtered immediately before use
Tested in 90-100% Confluent 3T3 Cultures

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	
B	Blank	50 ug/ml	50 ug/ml	28 ug/ml	28 ug/ml	16 ug/ml	16 ug/ml	9 ug/ml	9 ug/ml	5 ug/ml	5 ug/ml	
C	Blank											
D	Blank											Empty
E	Blank											
F	Blank											
G	Blank											
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076	0.051	0.05	0.045	0.044	0.041	0.041	0.041	0.039	0.038	0.037	0.037
B	0.058	0.553	0.535	0.58	0.587	0.421	0.353	0.225	0.221	0.149	0.145	0.037
C	0.053	0.561	0.503	0.517	0.549	0.338	0.345	0.213	0.203	0.144	0.155	0.035
D	0.048	0.493	0.527	0.489	0.495	0.351	0.331	0.196	0.196	0.143	0.161	0.038
E	0.047	0.491	0.497	0.528	0.571	0.312	0.321	0.188	0.195	0.132	0.172	0.038
F	0.073	0.606	0.697	0.53	0.6	0.36	0.373	0.239	0.218	0.143	0.163	0.036
G	0.072	0.63	0.497	0.563	0.592	0.399	0.39	0.235	0.21	0.145	0.157	0.037
H	0.056	0.089	0.055	0.043	0.045	0.041	0.04	0.039	0.039	0.042	0.04	0.036

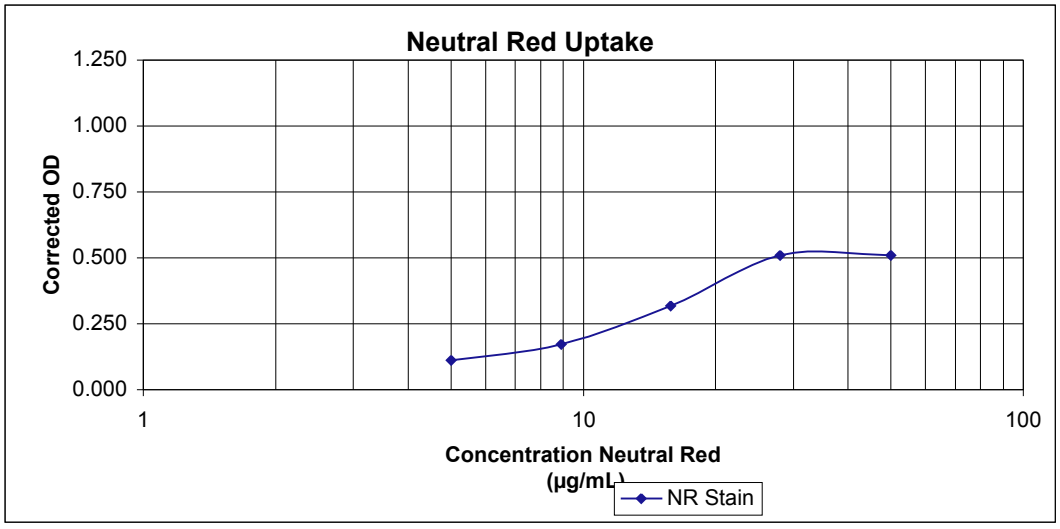
CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.033	0.008	0.007	0.002	0.001	-0.002	-0.002	-0.002	-0.004	-0.005	-0.006	-0.006
B	0.015	0.510	0.492	0.537	0.544	0.378	0.310	0.182	0.178	0.106	0.102	-0.006
C	0.010	0.518	0.460	0.474	0.506	0.295	0.302	0.170	0.160	0.101	0.112	-0.008
D	0.005	0.450	0.484	0.446	0.452	0.308	0.288	0.153	0.153	0.100	0.118	-0.005
E	0.004	0.448	0.454	0.485	0.528	0.269	0.278	0.145	0.152	0.089	0.129	-0.005
F	0.030	0.563	0.654	0.487	0.557	0.317	0.330	0.196	0.175	0.100	0.120	-0.007
G	0.029	0.587	0.454	0.520	0.549	0.356	0.347	0.192	0.167	0.102	0.114	-0.006
H	0.013	0.000	0.012	0.000	0.002	-0.002	-0.003	-0.004	-0.004	-0.001	-0.003	-0.007

Mean Blank =	0.039	(Only the 4 wells from the 5.0 ug/ml group)

	Neutral Red Stain Concentration									
Conc. (µg/mL) :	50.0	50.0	28.0	28.0	15.8	15.8	8.9	8.9	5.0	5.0
Mean Corr. OD :	0.512	0.499	0.491	0.522	0.320	0.309	0.173	0.164	0.099	0.116
SD :	0.057	0.077	0.033	0.039	0.040	0.026	0.021	0.011	0.006	0.009
Group mean corr OD:	0.506		0.507		0.315		0.168		0.107	

graph	x	50.0	28.0	15.8	8.9	5.0	
	y	0.506	0.507	0.315	0.168	0.107	



Appendix E3

Neutral Red (NR) Dye Experiments – NHK Cells – IIVS

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Test Facility : IIVS
Chemical Code : N/A
2nd Chem. Code*: NRU

Study Number.: R&D - NR Stain Time Course in NHK
96-Well Plate ID : 1
Experiment ID : RD9602NK

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank											Blank
C	Blank											Blank
D	Blank	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min	Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.057	0.053	0.059	0.058	0.054	0.055	0.053	0.052	0.051	0.048	0.049	0.035
B	0.068	1.501	1.564	1.311	1.327	0.998	1.052	0.671	0.649	0.438	0.474	0.037
C	0.057	1.549	1.482	1.376	1.372	1.082	1.076	0.714	0.697	0.494	0.474	0.034
D	0.058	1.540	1.503	1.415	1.422	1.026	0.995	0.724	0.698	0.482	0.474	0.036
E	0.057	1.553	1.532	1.388	1.453	1.060	1.010	0.675	0.634	0.459	0.462	0.034
F	0.057	1.632	1.600	1.396	1.380	1.066	1.074	0.656	0.628	0.470	0.429	0.033
G	0.054	1.462	1.514	1.357	1.439	1.069	1.010	0.708	0.606	0.474	0.437	0.035
H	0.057	0.054	0.053	0.052	0.051	0.055	0.051	0.049	0.047	0.050	0.046	0.034

CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

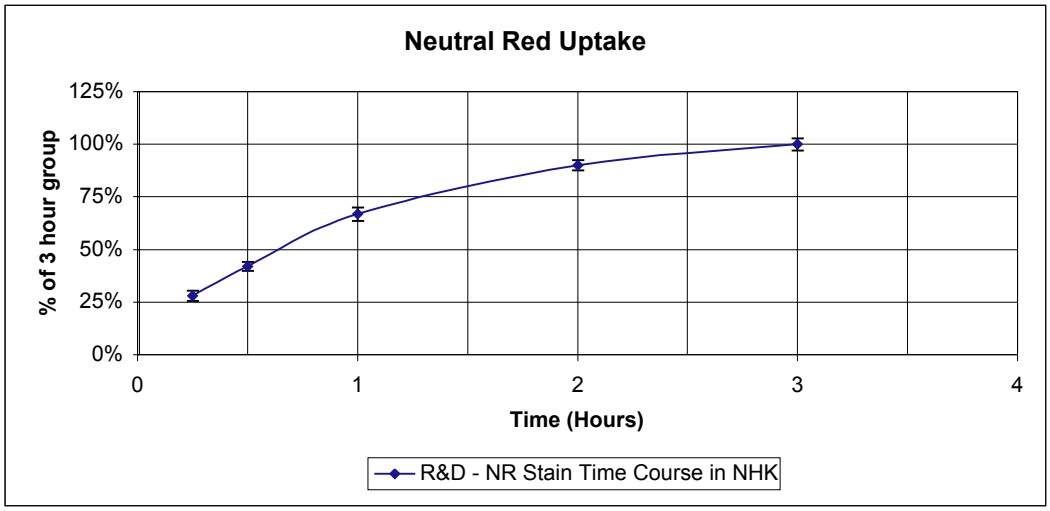
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.007	0.003	0.009	0.008	0.004	0.005	0.003	0.002	0.001	-0.002	-0.001	-0.015
B	0.018	1.451	1.514	1.261	1.277	0.948	1.002	0.621	0.599	0.388	0.424	-0.013
C	0.007	1.499	1.432	1.326	1.322	1.032	1.026	0.664	0.647	0.444	0.424	-0.016
D	0.008	1.490	1.453	1.365	1.372	0.976	0.945	0.674	0.648	0.432	0.424	-0.014
E	0.007	1.503	1.482	1.338	1.403	1.010	0.960	0.625	0.584	0.409	0.412	-0.016
F	0.007	1.582	1.550	1.346	1.330	1.016	1.024	0.606	0.578	0.420	0.379	-0.017
G	0.004	1.412	1.464	1.307	1.389	1.019	0.960	0.658	0.556	0.424	0.387	-0.015
H	0.007	0.000	0.003	0.002	0.001	0.005	0.001	-0.001	-0.003	0.000	-0.004	-0.016

Mean Blank =	0.050

RELATIVE VIABILITY (% OF VEHICLE CONTROL)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		97.6%	101.9%	84.9%	85.9%	63.8%	67.4%	41.8%	40.3%	26.1%	28.6%	
C		100.9%	96.4%	89.2%	89.0%	69.5%	69.1%	44.7%	43.6%	29.9%	28.6%	
D		100.3%	97.8%	91.9%	92.3%	65.7%	63.6%	45.4%	43.6%	29.1%	28.6%	
E		101.1%	99.7%	90.0%	94.4%	68.0%	64.6%	42.1%	39.3%	27.5%	27.7%	
F		106.5%	104.3%	90.6%	89.5%	68.4%	68.9%	40.8%	38.9%	28.3%	25.5%	
G		95.0%	98.5%	88.0%	93.5%	68.6%	64.6%	44.3%	37.4%	28.6%	26.1%	
H												

	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min
Conc. (µg/mL) :										
Mean Corr. OD :	1.490	1.483	1.324	1.349	1.001	0.987	0.642	0.602	0.420	0.409
SD :	0.057	0.043	0.036	0.048	0.032	0.036	0.028	0.038	0.019	0.020
Mean 3 hour :	1.486									
Mean Blank :	0.050									
% of 3 hour:	100.2%	99.8%	89.1%	90.8%	67.3%	66.4%	43.2%	40.5%	28.3%	27.5%
SD :	3.8%	2.9%	2.4%	3.2%	2.1%	2.4%	1.9%	2.5%	1.3%	1.4%
% CV :	3.83%	2.91%	2.75%	3.53%	3.17%	3.61%	4.29%	6.28%	4.62%	4.97%
hours			3	2	1	0.50	0.25			
% of 3 hour:			100.0%	89.9%	66.8%	41.9%	27.9%			


Neutral Red Uptake
125%
100%
75%
50%
25%
0%
% of 3 hour group
0
1
2
3
4
Time (Hours)
R&D - NR Stain Time Course in NHK

Neutral Red Stain Prepared in KGM - TEST OF NR PREP 1 DAY PRIOR TO USE
Tested in 90-100% Confluent NHK Cultures

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank										
C	Blank		50 ug/ml			50 ug/ml				33 ug/ml	
D	Blank		Prepared and filtered			Filtered before use				Filtered before use	
E	Blank		in evening before use								
F	Blank		Filtered before use								
G	Blank										
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11
A	0.062	0.061	0.063	0.064	0.063	0.062	0.060	0.060	0.052	0.053	0.051
B	0.055	1.306	1.545	1.530	1.514	1.403	1.421	1.297	1.249	1.136	1.134
C	0.060	1.530	1.520	1.554	1.471	1.536	1.416	1.415	1.308	1.160	1.189
D	0.062	1.454	1.527	1.513	1.511	1.472	1.491	1.438	1.217	1.192	1.173
E	0.067	1.423	1.433	1.505	1.577	1.469	1.448	1.474	1.199	1.249	1.158
F	0.057	1.423	1.591	1.577	1.577	1.403	1.431	1.347	1.250	1.235	1.102
G	0.065	1.430	1.468	1.393	1.319	1.432	1.304	1.416	1.243	1.117	1.110
H	0.064	0.059	0.060	0.064	0.064	0.065	0.061	0.064	0.060	0.055	0.060

CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

	1	2	3	4	5	6	7	8	9	10	11
A	0.012	0.011	0.013	0.014	0.013	0.012	0.010	0.010	0.002	0.003	0.001
B	0.005	1.256	1.495	1.480	1.464	1.353	1.371	1.247	1.199	1.086	1.084
C	0.010	1.480	1.470	1.504	1.421	1.486	1.366	1.365	1.258	1.110	1.139
D	0.012	1.404	1.477	1.463	1.461	1.422	1.441	1.388	1.167	1.142	1.123
E	0.017	1.373	1.383	1.455	1.527	1.419	1.398	1.424	1.149	1.199	1.108
F	0.007	1.373	1.541	1.527	1.527	1.353	1.381	1.297	1.200	1.185	1.052
G	0.015	1.380	1.418	1.343	1.269	1.382	1.254	1.366	1.193	1.067	1.060
H	0.014	0.000	0.010	0.014	0.014	0.015	0.011	0.014	0.010	0.005	0.010

Mean Blank =	0.055	(Only the 14 wells from the 33 ug/ml group)

	Neutral Red Stain Concentration									
Conc. (µg/mL) :	50.0			50.0				33.0		
Mean Corr. OD :	1.378	1.464	1.462	1.445	1.403	1.369	1.348	1.195	1.132	1.095
SD :	0.072	0.056	0.064	0.096	0.051	0.062	0.064	0.037	0.053	0.035
Group mean corr OD:		1.435			1.391				1.141	

Note: No crystal formation was observed in the KGM/NR prepared 1 day prior.
No ppt or crystalization observed in the wells during the NR loading of cells.

Neutral Red Stain Prepared in KGM/Filtered immediately before use
Tested in 90-100% Confluent NHK Cultures

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	
B	Blank	50 ug/ml	50 ug/ml	28 ug/ml	28 ug/ml	16 ug/ml	16 ug/ml	9 ug/ml	9 ug/ml	5 ug/ml	5 ug/ml	
C	Blank											
D	Blank											empty
E	Blank											
F	Blank											
G	Blank											
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.067	0.064	0.066	0.049	0.049	0.040	0.040	0.038	0.038	0.036	0.037	0.035
B	0.048	1.255	1.119	1.103	1.054	0.623	0.605	0.325	0.334	0.156	0.150	0.034
C	0.050	1.035	1.004	1.020	0.956	0.624	0.601	0.345	0.312	0.151	0.154	0.034
D	0.047	1.131	1.352	1.094	1.078	0.643	0.635	0.331	0.314	0.157	0.147	0.035
E	0.047	1.117	1.227	0.923	0.893	0.595	0.618	0.323	0.302	0.155	0.150	0.035
F	0.046	1.245	1.129	0.976	0.988	0.607	0.617	0.308	0.313	0.156	0.156	0.035
G	0.047	1.136	1.282	1.061	0.995	0.624	0.582	0.283	0.282	0.131	0.127	0.037
H	0.063	0.056	0.060	0.061	0.048	0.042	0.042	0.038	0.039	0.040	0.038	0.036

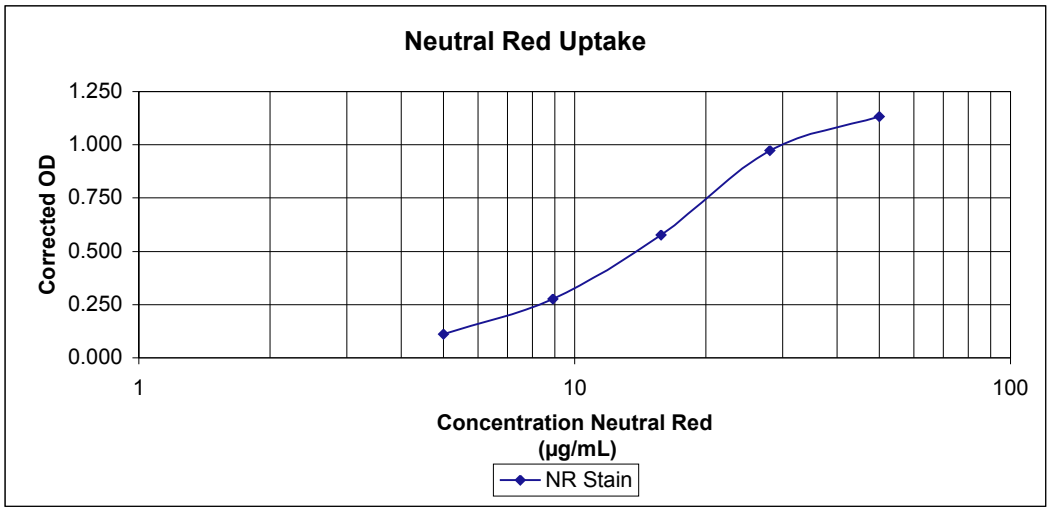
CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.017	0.014	0.016	-0.001	-0.001	-0.010	-0.010	-0.012	-0.012	-0.014	-0.013	-0.015
B	-0.002	1.205	1.069	1.053	1.004	0.573	0.555	0.275	0.284	0.106	0.100	-0.016
C	0.000	0.985	0.954	0.970	0.906	0.574	0.551	0.295	0.262	0.101	0.104	-0.016
D	-0.003	1.081	1.302	1.044	1.028	0.593	0.585	0.281	0.264	0.107	0.097	-0.015
E	-0.003	1.067	1.177	0.873	0.843	0.545	0.568	0.273	0.252	0.105	0.100	-0.015
F	-0.004	1.195	1.079	0.926	0.938	0.557	0.567	0.258	0.263	0.106	0.106	-0.015
G	-0.003	1.086	1.232	1.011	0.945	0.574	0.532	0.233	0.232	0.081	0.077	-0.013
H	0.013	0.000	0.010	0.011	-0.002	-0.008	-0.008	-0.012	-0.011	-0.010	-0.012	-0.014

Mean Blank =	0.038	(Only the 4 wells from the 5.0 ug/ml group)

	Neutral Red Stain Concentration									
Conc. (µg/mL) :	50.0	50.0	28.0	28.0	15.8	15.8	8.9	8.9	5.0	5.0
Mean Corr. OD :	1.104	1.136	0.980	0.944	0.570	0.560	0.270	0.260	0.101	0.098
SD :	0.083	0.126	0.070	0.067	0.017	0.018	0.021	0.017	0.010	0.010
Group mean corr OD:	1.120		0.962		0.565		0.265		0.100	

graph	x	50.0	28.0	15.8	8.9	5.0		
	y	1.120	0.962	0.565	0.265	0.100		



Appendix E4

Neutral Red (NR) Dye Experiments – 3T3 Cells – ECBC

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Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-1
Experiment ID : SLS-B(25ug NR/ml 1hr)

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
C	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
D	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
E	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
F	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
G	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.051	0.048	0.052	0.048	0.050	0.050	0.046	0.044	0.045	0.046	0.047
B	0.050	0.262	0.050	0.046	0.130	0.274	0.254	0.322	0.315	0.329	0.333	0.046
C	0.052	0.283	0.053	0.051	0.145	0.231	0.252	0.276	0.283	0.293	0.321	0.050
D	0.050	0.307	0.055	0.053	0.135	0.242	0.252	0.291	0.280	0.302	0.314	0.049
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

CORRECTED ABSORBANCE (Sample OD_{540} - Mean Blank OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.002	-0.001	0.003	-0.001	0.001	0.001	-0.003	-0.005	-0.004	-0.003	-0.002
B	0.001	0.214	0.001	-0.003	0.082	0.226	0.206	0.274	0.267	0.281	0.285	-0.003
C	0.003	0.235	0.004	0.002	0.097	0.183	0.204	0.228	0.235	0.245	0.273	0.001
D	0.001	0.259	0.006	0.004	0.087	0.194	0.204	0.243	0.232	0.254	0.266	0.000
E	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
F	-0.049	-0.049	0.052	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
G	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
H	-0.049	0.000	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049

Mean Blank =	0.049

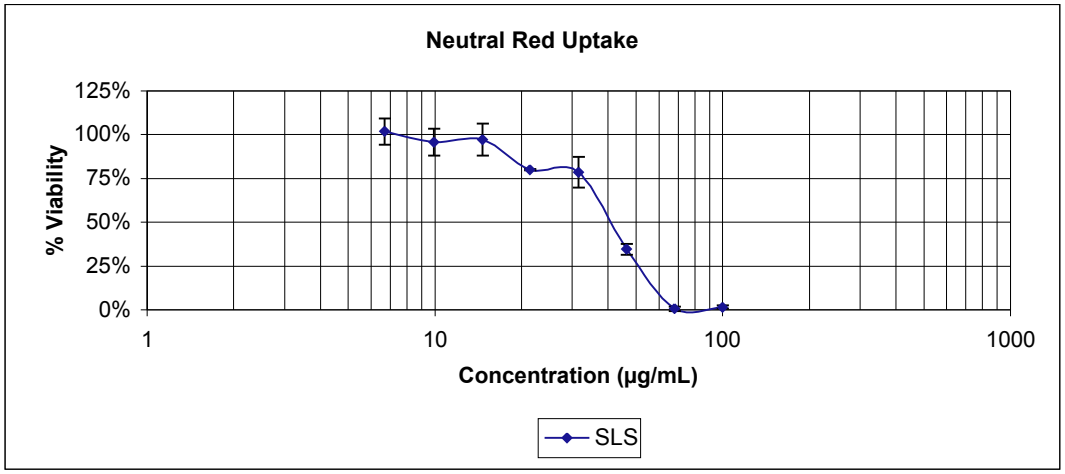
RELATIVE VIABILITY (% OF VEHICLE CONTROL)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		83.8%	0.6%	-1.0%	32.0%	88.5%	80.6%	107.3%	104.6%	110.1%	111.6%	
C		92.0%	1.8%	1.0%	37.9%	71.6%	79.9%	89.3%	92.0%	95.9%	106.9%	
D		101.4%	2.6%	1.8%	33.9%	75.9%	79.9%	95.2%	90.8%	99.5%	104.2%	
E		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
F		-19.0%	20.2%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
G		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
H												

Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-1
Experiment ID : SLS-B(25ug NR/ml 1hr)

	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2
Conc. (µg/mL) :	0.0	100.0	68.0	46.3	31.5	21.4	14.6	9.9	6.7	0.0
Mean Corr. OD :	0.236	0.004	0.001	0.088	0.201	0.204	0.248	0.244	0.260	0.274
SD :	0.023	0.003	0.004	0.008	0.022	0.001	0.023	0.019	0.019	0.010
Mean Vehicle Control :	0.255									
Mean Blank :	0.049									
% of Vehicle Control :	92.4%	1.6%	0.6%	34.6%	78.7%	80.1%	97.3%	95.8%	101.8%	107.6%
SD :	8.8%	1.0%	1.4%	3.0%	8.8%	0.5%	9.2%	7.6%	7.4%	3.8%
% CV :	9.56%	60.40%	240.37%	8.66%	11.14%	0.57%	9.47%	7.95%	7.22%	3.50%
Mean VC - VC1 (%) :	7.59%									
Mean VC - VC2 (%) :	-7.59%									
Mean Absolute OD :	0.303									



Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-2
Experiment ID : SLS-B(50ug NR/ml 1hr)

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
C	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
D	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
E	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
F	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
G	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.056	0.061	0.063	0.055	0.052	0.051	0.058	0.050	0.050	0.052	0.050	0.051
B	0.088	0.377	0.057	0.053	0.192	0.315	0.325	0.364	0.402	0.403	0.396	0.053
C	0.058	0.378	0.062	0.058	0.158	0.277	0.337	0.379	0.400	0.391	0.386	0.051
D	0.061	0.373	0.054	0.051	0.182	0.308	0.343	0.367	0.425	0.420	0.409	0.050
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

CORRECTED ABSORBANCE (Sample OD_{540} - Mean Blank OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.007	0.013	0.015	0.006	0.003	0.002	0.009	0.001	0.001	0.003	0.001	0.002
B	0.040	0.329	0.008	0.004	0.144	0.267	0.277	0.316	0.354	0.355	0.348	0.004
C	0.009	0.330	0.014	0.009	0.110	0.229	0.289	0.331	0.352	0.343	0.338	0.002
D	0.013	0.325	0.005	0.002	0.134	0.260	0.295	0.319	0.377	0.372	0.361	0.001
E	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
F	-0.049	-0.049	0.052	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
G	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
H	-0.049	0.000	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049

Mean Blank =	0.056

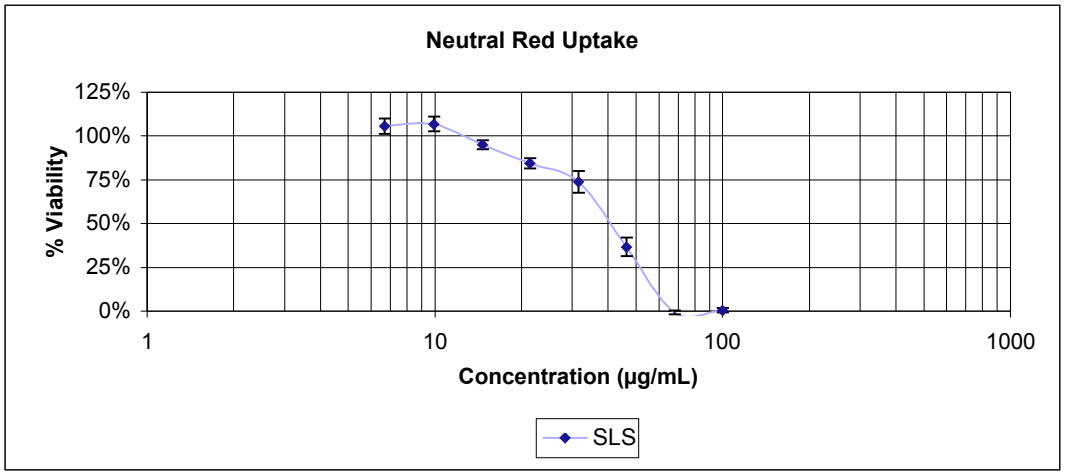
RELATIVE VIABILITY (% OF VEHICLE CONTROL)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		128.9%	3.3%	1.8%	56.3%	104.6%	108.5%	123.8%	138.7%	139.1%	136.4%	
C		129.3%	5.3%	3.7%	43.0%	89.7%	113.2%	129.7%	137.9%	134.4%	132.4%	
D		127.3%	2.2%	1.0%	52.4%	101.8%	115.6%	125.0%	147.7%	145.8%	141.5%	
E		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
F		-19.0%	20.2%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
G		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
H												

Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-2
Experiment ID : SLS-B(50ug NR/ml 1hr)

	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2
Conc. (µg/mL) :	0.0	100.0	68.0	46.3	31.5	21.4	14.6	9.9	6.7	0.0
Mean Corr. OD :	0.328	0.009	0.005	0.129	0.252	0.287	0.322	0.361	0.356	0.349
SD :	0.003	0.004	0.004	0.017	0.020	0.009	0.008	0.014	0.015	0.012
Mean Vehicle Control :	0.338									
Mean Blank :	0.056									
% of Vehicle Control :	128.5%	3.6%	2.2%	50.6%	98.7%	112.4%	126.2%	141.5%	139.8%	136.8%
SD :	1.0%	1.6%	1.4%	6.9%	7.9%	3.6%	3.1%	5.5%	5.7%	4.5%
% CV :	0.81%	44.09%	65.56%	13.56%	8.04%	3.20%	2.47%	3.85%	4.09%	3.31%
Mean VC - VC1 (%) :	3.11%									
Mean VC - VC2 (%) :	-3.11%									
Mean Absolute OD :	0.387									



Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-2
Experiment ID : SLS-B(25ug NR/ml 3hr)

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
C	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
D	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
E	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
F	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
G	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.052	0.047	0.050	0.048	0.046	0.048	0.046	0.048	0.046	0.046	0.046	0.046
B	0.049	0.559	0.047	0.050	0.175	0.387	0.506	0.474	0.580	0.489	0.610	0.048
C	0.052	0.613	0.051	0.061	0.183	0.414	0.525	0.518	0.487	0.444	0.520	0.047
D	0.052	0.554	0.052	0.052	0.195	0.364	0.507	0.523	0.527	0.555	0.485	0.057
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

CORRECTED ABSORBANCE (Sample OD_{540} - Mean Blank OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.003	-0.002	0.001	-0.001	-0.003	-0.001	-0.003	-0.001	-0.003	-0.003	-0.003	-0.003
B	0.000	0.511	-0.002	0.001	0.127	0.339	0.458	0.426	0.532	0.441	0.562	-0.001
C	0.003	0.565	0.002	0.013	0.135	0.366	0.477	0.470	0.439	0.396	0.472	-0.002
D	0.003	0.506	0.003	0.003	0.147	0.316	0.459	0.475	0.479	0.507	0.437	0.008
E	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
F	-0.049	-0.049	0.052	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
G	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
H	-0.049	0.000	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049

Mean Blank =	0.049

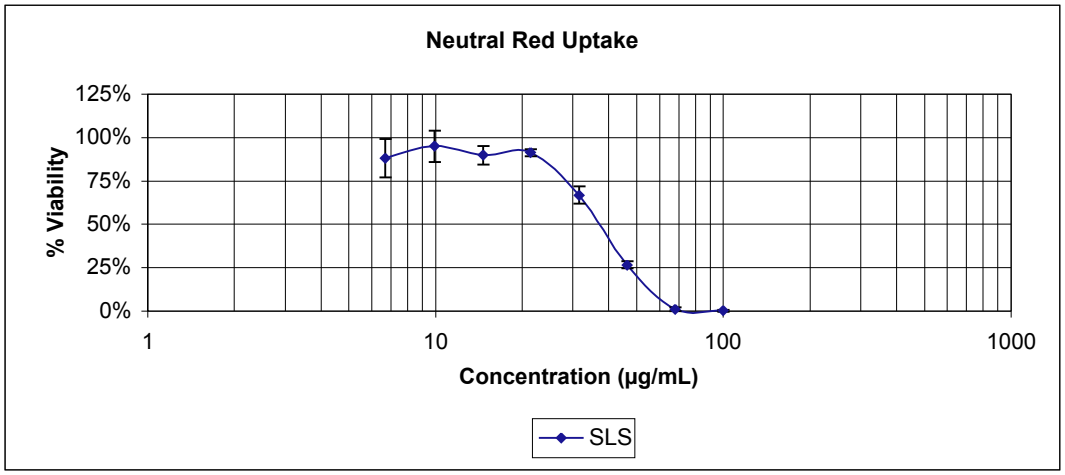
RELATIVE VIABILITY (% OF VEHICLE CONTROL)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		200.3%	-0.6%	0.6%	49.6%	132.8%	179.5%	167.0%	208.6%	172.9%	220.3%	
C		221.5%	1.0%	4.9%	52.8%	143.4%	187.0%	184.2%	172.1%	155.2%	185.0%	
D		198.4%	1.4%	1.4%	57.5%	123.8%	179.9%	186.2%	187.8%	198.8%	171.3%	
E		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
F		-19.0%	20.2%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
G		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
H												

Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-2
Experiment ID : SLS-B(25ug NR/ml 3hr)

	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2
Conc. (µg/mL) :	0.0	100.0	68.0	46.3	31.5	21.4	14.6	9.9	6.7	0.0
Mean Corr. OD :	0.527	0.001	0.006	0.136	0.340	0.464	0.457	0.483	0.448	0.490
SD :	0.033	0.003	0.006	0.010	0.025	0.011	0.027	0.047	0.056	0.064
Mean Vehicle Control :	0.508									
Mean Blank :	0.049									
% of Vehicle Control :	206.7%	0.6%	2.3%	53.3%	133.4%	182.1%	179.1%	189.5%	175.6%	192.2%
SD :	12.8%	1.0%	2.3%	4.0%	9.8%	4.2%	10.6%	18.3%	21.9%	25.3%
% CV :	6.21%	176.38%	100.45%	7.41%	7.36%	2.30%	5.91%	9.66%	12.48%	13.16%
Mean VC - VC1 (%) :	-3.64%									
Mean VC - VC2 (%) :	3.64%									
Mean Absolute OD :	0.557									



Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-2
Experiment ID : SLS-B(50ug NR/ml 3hr)

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
C	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
D	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
E	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
F	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
G	Blank	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.059	0.065	0.053	0.052	0.054	0.052	0.054	0.053	0.056	0.053	0.054	0.051
B	0.057	0.513	0.057	0.056	0.154	0.302	0.416	0.485	0.473	0.457	0.485	0.050
C	0.059	0.488	0.058	0.056	0.152	0.326	0.420	0.460	0.500	0.438	0.562	0.059
D	0.059	0.516	0.054	0.056	0.146	0.326	0.496	0.447	0.478	0.455	0.508	0.051
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

CORRECTED ABSORBANCE (Sample OD_{540} - Mean Blank OD_{540})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.011	0.017	0.004	0.003	0.005	0.003	0.005	0.004	0.007	0.004	0.005	0.002
B	0.008	0.465	0.008	0.007	0.106	0.254	0.368	0.437	0.425	0.409	0.437	0.001
C	0.011	0.440	0.009	0.007	0.104	0.278	0.372	0.412	0.452	0.390	0.514	0.011
D	0.011	0.468	0.005	0.007	0.098	0.278	0.448	0.399	0.430	0.407	0.460	0.002
E	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
F	-0.049	-0.049	0.052	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
G	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049
H	-0.049	0.000	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049	-0.049

Mean Blank =	0.055

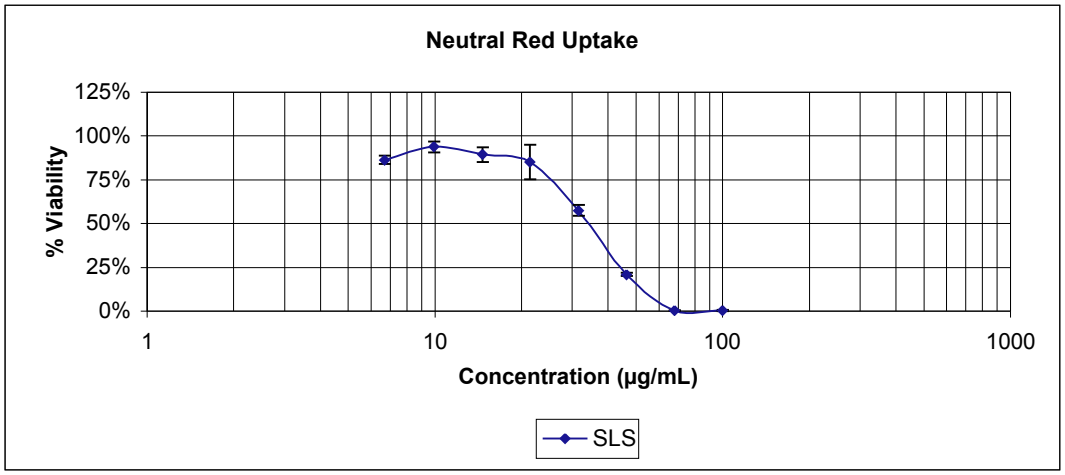
RELATIVE VIABILITY (% OF VEHICLE CONTROL)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		182.3%	3.3%	2.9%	41.4%	99.5%	144.2%	171.3%	166.6%	160.3%	171.3%	
C		172.5%	3.7%	2.9%	40.6%	108.9%	145.8%	161.5%	177.2%	152.8%	201.5%	
D		183.5%	2.2%	2.9%	38.3%	108.9%	175.6%	156.4%	168.5%	159.5%	180.3%	
E		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
F		-19.0%	20.2%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
G		-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	-19.0%	
H												

Test Facility : ECBC
Chemical Code : SLS
2nd Chem. Code*: none

Study Number.: ECBC-3T3 Ia 0#
96-Well Plate ID : 090602-2
Experiment ID : SLS-B(50ug NR/ml 3hr)

	VC1	C1	C2	C3	C4	C5	C6	C7	C8	VC2
Conc. (µg/mL) :	0.0	100.0	68.0	46.3	31.5	21.4	14.6	9.9	6.7	0.0
Mean Corr. OD :	0.457	0.008	0.007	0.102	0.270	0.396	0.416	0.435	0.402	0.470
SD :	0.015	0.002	0.000	0.004	0.014	0.045	0.019	0.014	0.010	0.040
Mean Vehicle Control :	0.464									
Mean Blank :	0.055									
% of Vehicle Control :	179.4%	3.1%	2.9%	40.1%	105.8%	155.2%	163.0%	170.8%	157.6%	184.4%
SD :	6.0%	0.8%	0.0%	1.6%	5.4%	17.7%	7.6%	5.6%	4.1%	15.5%
% CV :	3.36%	26.57%	0.00%	4.08%	5.14%	11.40%	4.65%	3.30%	2.60%	8.41%
Mean VC - VC1 (%) :	1.37%									
Mean VC - VC2 (%) :	-1.37%									
Mean Absolute OD :	0.512									



Appendix F

Reference Substance Information

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Appendix F1

NRU Test Information for the 72 Reference Substances

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Table F-1 NRU Test Information for the 72 Reference Substances

Chemical	CASRN	Purity (%)	Supplier	pH in 3T3 Medium[a]	Concentrations Tested in 3T3 Assay (μg/mL)	pH in NHK Medium[b]	Concentrations Tested in NHK Assay (μg/mL)
Acetaminophen	103-90-2	99	Sigma	8.1	4.7-1000	7.7	11.8-4000
Acetonitrile	75-05-8	99.5	Sigma	8.4	118-100000	7.9	8.12-200000
Acetylsalicylic acid	50-78-2	99.5	Sigma	7.5	9.4-2500	6.9	11.8-2500
Aminopterin	54-62-6	100.3	Fluka	8.1	0.00005-0.1	7.2	67.4-1000
5-Aminosalicylic acid	89-57-6	99	Sigma	6.7	169-2500	7.5	2.4-500
Amitriptyline HCl	549-18-8	100	Sigma	8.1	0.4-100	7.6	0.24-100
Arsenic III trioxide	1327-53-3	99.9	Sigma	7.9	0.169-100	7.5	0.46-100
Atropine sulfate monohydrate	5908-99-6	100	Fluka	7.9	4.7-1000	7.5	3.8-10000
Boric aid	10043-35-3	101.1	Fluka	7.1	4.7-10000	7.4	28.3-10000
Busulfan	55-98-1	100.2	Fluka	8.1	2.4-500	7.8	2.35-800
Cadmium II chloride	10108-64-2	99.8	Fluka	8.1	0.135-5	7.7	0.337-100
Caffeine	58-08-2	99.9	Fluka	8.3	1.6-5000	7.8	3.25-10000
Carbamazepine	298-46-4	> 99	Sigma	8.0	0.3-1000	7.9	1.88-1000
Carbon tetrachloride	56-23-5	> 99.5	Sigma-Aldrich	NA	169-7000	7.7	11.8-7000
Chloral hydrate	302-17-0	100.1	Sigma	8.4	4.7-1000	7.6	4.7-1000
Chloramphenicol	56-75-7	> 99	Fluka	8.3	4.7-2500	7.8	9.15-2500
Citric acid	77-92-9	98	Sigma	2.9	23.5-10000	4.0	23.5-10000

Table F-1 NRU Test Information for the 72 Reference Substances

Chemical	CASRN	Purity (%)	Supplier	pH in 3T3 Medium[a]	Concentrations Tested in 3T3 Assay (μg/mL)	pH in NHK Medium[b]	Concentrations Tested in NHK Assay (μg/mL)
Colchicine	64-86-8	> 98	Fluka	8.2	0	7.7	0.0014-0.10
Cupric sulfate pentahydrate	7758-99-8	99.7	Sigma	7.8	0.0059-5.0	7.4	2.4-750
Cycloheximide	66-81-9	100	Sigma	8.0	0.01-50	7.8	0.0040-100
Dibutyl phthalate	84-74-2	> 99	Sigma	8.0	3.7-2500	7.7	0.9-1000
Dichlorvos	62-73-7	99.5	Chem Service, Inc.	8.1	0.5-100	7.7	0.235-500
Diethyl phthalate	84-66-2	99.5	Aldrich	8.1	4.7-2000	7.8	2.35-2000
Digoxin	20830-75-5	98.6	Sigma	8.2	3.5-1000	7.8	0.0000047-0.100
Dimethylformamide	68-12-2	99.95	Sigma-Aldrich	8.1	236-50000	7.7	70.6-30000
Diquat dibromide monohydrate	6385-62-2	99	Chem Service, Inc.	7.9	0.03-100	7.7	0.47-500
Disulfoton	298-04-4	99.4	Chem Service, Inc.	8.0	2.4-2500	7.8	2.4-2500
Endosulfan	115-29-7	99.5	Chem Service, Inc.	8.3	0.1-100	7.8	0.67-50
Epinephrine bitartrate	51-42-3	> 99	Sigma-Aldrich	7.9	6.74-200	7.6	4.7-1000
Ethanol	64-17-5	100	Sigma-Aldrich	8.6	1011-50000	7.8	118-150000
Ethylene glycol	107-21-1	99.99	Sigma	8.4	1770-100000	7.8	1770-100000
Fenpropathrin	39515-41-8	91.8	Valent	8.3	2.4-500	7.8	0.301-100
Gibberellic acid	77-06-5	99	Acros	4.5	1348-100000	6.5	23.6-10000
Glutethimide	77-21-4	> 99	Sigma-Aldrich	8.0	19-1000	7.7	4.7-1000

Table F-1 NRU Test Information for the 72 Reference Substances

Chemical	CASRN	Purity (%)	Supplier	pH in 3T3 Medium[a]	Concentrations Tested in 3T3 Assay (μg/mL)	pH in NHK Medium[b]	Concentrations Tested in NHK Assay (μg/mL)
Glycerol	56-81-5	99.9	Sigma	8.2	4586-100000	7.8	47-101960
Haloperidol	52-86-8	99	Sigma	8.3	0.1-25	7.7	0.188-100
Hexachlorophene	70-30-4	99.2	Sigma-Aldrich	8.1	0.5-100	7.5	0.002-1
Lactic acid	50-21-5	88.6	Sigma	3.2	47.1-10000	3.0	47.1-10000
Lindane	58-89-9	100	Sigma	8.1	0.8-2500	7.7	2.35-2000
Lithium I carbonate	554-13-2	99.4	Sigma	9.3	74.3-1102.5	9.5	4.7-2000
Meprobamate	57-53-4	> 99	Sigma	8.1	9.4-2500	7.7	4.71-2500
Mercury II chloride	7487-94-7	99.5	Sigma	8.1	0.05-10	7.6	0.67-10
Methanol	67-56-1	99.97	Sigma-Aldrich	8.0	398-3500 (no toxicity)	7.6	9.42-2500
Nicotine	54-11-5	> 99.0	Fluka	8.8	94.9-1000	8.5	8.02-5000
Paraquat	1910-42-5	100	Sigma	7.9	0.5-100	7.8	2.4-1000
Parathion	56-38-2	98	Supelco	8.2	0.5-2500	7.7	0.47-1500
Phenobarbital	50-06-6	100	Spectrum	7.7	11.8-2500	7.4	7.06-3000
Phenol	108-95-2	> 99	Sigma	8.0	0.3-1500	7.7	4.7-1000
Phenylthiourea	103-85-5	98	Sigma	8.1	0.8-2500	7.7	9.42-2500
Physostigmine	57-47-6	100	Sigma	8.1	5.4-200	7.7	0.32-1000
Potassium I chloride	7447-40-7	100	Sigma	8.3	163-15000	7.8	23.5-10000

Table F-1 NRU Test Information for the 72 Reference Substances

Chemical	CASRN	Purity (%)	Supplier	pH in 3T3 Medium[a]	Concentrations Tested in 3T3 Assay (μg/mL)	pH in NHK Medium[b]	Concentrations Tested in NHK Assay (μg/mL)
Potassium cyanide	151-50-8	99.4	Mallinckrodt Baker	9.0	0.5-1500	8.2	0.401-500
Procainamide HCl	51-06-9	99.7	Sigma-Aldrich	8.3	67-1000	7.5	47-10000
2-Propanol	67-63-0	> 99.9	Sigma	8.5	1011-50000	7.7	47.1-20000
Propranolol HCl	3506-09-0	100	Sigma	7.9	1.78-1000	7.4	1.8-350
Propylparaben	94-13-3	> 99	Fluka	8.1	2.4-1000	7.7	0.47-300
Sodium arsenite	7784-46-5	> 99.0	Fluka	8.0	0.05-10.0	7.7	0.038-30
Sodium chloride	7647-14-5	99.5	Sigma	8.2	94-20000	7.9	4.71-10000
Sodium dichromate dihydrate	7789-12-0	100.4	Sigma	8.0	0.03-10.0	7.7	0.0318-100
Sodium I fluoride	7681-49-4	100	Sigma	8.1	10.1-1000	7.7	0.3-1000
Sodium hypochlorite	7681-52-9	12.9% Cl	Sigma-Aldrich	8.0	24-10000	7.7	47.1-10000
Sodium oxalate	62-76-0	99.99	Sigma-Aldrich	8.1	1.2-500	7.7	40.5-2000
Sodium selenate	13413-01-0	100	Sigma-Aldrich	8.2	6.8-300	7.8	0.47-556
Strychnine	57-24-9	99	Sigma	8.4	9.5-800	7.8	1.18-500
Thallium I sulfate	7446-18-6	99.995	Aldrich	8.3	0.1-500	7.8	0.0047-2
Trichloroacetic acid	76-03-9	> 99	Aldrich	2.3	24-10000	1.9	33.0-10000
1,1,1-Trichloroethane	71-55-6	99.78	Sigma-Aldrich	8.4	1686-50000	8.0	674-10000
Triethylenemelamine	51-18-3	98	Acros	8.0	0.02-4	7.6	0.024-10

Table F-1 NRU Test Information for the 72 Reference Substances

Chemical	CASRN	Purity (%)	Supplier	pH in 3T3 Medium[a]	Concentrations Tested in 3T3 Assay (μg/mL)	pH in NHK Medium[b]	Concentrations Tested in NHK Assay (μg/mL)
Triphenyltin hydroxide	76-87-9	~ 99.5	Sigma-Aldrich	8.0	0.0002-0.1	7.6	0.005-0.1
Valproic acid	99-66-1	100	Sigma	6.9	12-2500	6.0	11.8-2500
Verapamil HCl	152-11-4	98	Sigma-Aldrich	8.1	3.4-100	7.5	3.8-1500
Xylene	1330-20-7	99.9	Mallinckrodt Baker	6.8	398-2500	7.5	190-2000

Abbreviations:NRU=Neutral red uptake; CASRN=Chemical Abstracts Service Registry Number; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; pH=Mean pH of the highest concentration tested (of all acceptable NRU tests)

[a]3T3 Medium - Dulbecco's Modification of Eagle's Medium, with supplements.

[b]NHK medium - Keratinocyte Growth Medium (KGM® from Cambrex).

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Appendix F2

Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

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Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Acetaminophen	103-90-2	2404	151.20	Organic compound; Amide	Slightly in cold, much more in hot; 1-5 mg/mL @ 22°C	NA	0.8	NA	Liver toxin	Free?	More toxic intracellular metabolites	Covalent NAPQI binding and lipid peroxidation.
Acetonitrile	75-05-8	3798	41.05	Organic compound; Nitrile	Miscible; ≥100 mg/mL @ 22.5°C	-4.30	-0.34	81.6	CNS stimulant	Presumed	Must be metabolized to hydrogen cyanide for effect.	Assumed to be same as cyanide: General enzyme inhibition. High affinity for Fe^{+++}. Inhibits cell respiration by inhibition of cytochrome oxidase; solvent
Acetylsalicylic acid	50-78-2	1000	180.20	Organic compound; Carboxylic acid; Phenol	3.3 mg/mL @ 25°C; 4.6 mg/mL @ 25°C; <1 mg/mL @ 23°C	3.49@ 25°C	1.19	NA	Gastric irritant, CNS (encephalo-pathy), kidney toxin	Restricted	Salicylic acid is an active metabolite	General cell poison, works by uncoupling oxidation phosphorylation and inhibition of Kreb's cycle dehydrogenases.
Aminopterin	54-62-6	3 (mouse)	476.45	Organic compound; Heterocyclic compound	NA	5.5	NA	NA	Hematotoxin	Presumed to be minimal (like methotrexate)	Not expected to require metabolism for toxicity	Hypothetical: Inhibits folic acid utilization and thus cell proliferation.
5-Aminosalicylic acid	89-57-6	7749 (mouse)	153.10	Organic compound; Carboxylic acid; Phenol	2 mg/mL; <1 mg/mL @ 21°C	3.25	1.32	NA	Kidney toxin	Yes	Not activated	Unknown
Amitriptyline HCl	549-18-8	319	313.90	Organic compound; Polycyclic compound	0.0097 mg/mL @ 24°C/HCl is freely soluble	9.4	5.04	NA	Cardiotoxin	Free	Nortriptyline, a metabolite, also active	Hypothetical: Blocks norepinephrine, 5-hydroxytryptamine, and dopamine presynaptic uptake; prevents reuptake of heart norepinephrine.

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Arsenic III trioxide	1327-53-3	20	197.80	Inorganic compound; Arsenical	sparingly in cold; in 15 parts boiling; 17 mg/mL @ 16°C	NA	NA	465	CNS toxin (encephalo-pathy)	Restricted	No	Cellular poison. Multisystem failure due to uncoupling oxidative phosphorylation & inhibition of pyruvate and succinate oxidative pathways; Apoptosis induction; angiogenesis inhibition; cellular growth inhibition
Atropine sulfate monohydrate	5908-99-6	623	694.80	Organic compound; Heterocyclic compound	2.2 mg/mL	NA	1.83	NA	CNS stimulant	Free	No	Antimuscarinic, anticholinergic action. Competitive antagonism of anticholinesterase at cardiac & CNS receptor sites.
Boric aid	10043-35-3	2660	61.83	Inorganic compound; Boron compound; Acids	56 mg/mL in cold water; 10-50 mg/mL @ 19°C	NA	NA	300	Skin, kidney, liver, testicular toxin	Yes	No	Inhibits enzymes involved in metabolism and RNA synthesis.[g]
Busulfan	55-98-1	2	246.31	Organic compound; Alcohol; Acyclic hydrocarbon; Sulfur compound	Decomposes	NA	-0.52	NA	Hematotoxin	Freely (similar to plasma concentration[h]	Reactive intermediates[h]	Hypothetical: Alkylation of sufhydryl groups[i]; antineoplastic
Cadmium II chloride	10108-64-2	88	183.31	Organic compound; Cadmium compound	1400 mg/mL @ 20°C; ≥100 mg/mL @ 20°C	NA	NA	960	Kidney, liver toxin, corrosive	Yes[j]	No	Alters Ca^{++} translocation, affects membrane ATPase & mitochondrial respiration.

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Caffeine	58-08-2	192	194.20	Organic compound; Heterocyclic compound	21 mg/mL @ 25°C; 10-50 mg/mL @ 23°C	14 @ 25°C; pK_b=14 .15 @ 19°C	-0.07	17 (sublimes)	CNS stimulant	Free	No	Hypothetical: Inhibition of phosphodiesterase leading to AMP accumulation. Translocation of intracellular Ca^{++}? Adenosine receptor antagonism?; neurotoxic
Carbamazepine	298-46-4	1957	236.30	Organic compound; Heterocyclic compound	Practically insoluble	NA	2.45	NA	CNS depressant, hematotoxin	Free	10,11-epoxide metabolite as active as parent	Not known. Therapeutically decreases firing of noradrenergic neurons.
Carbon tetrachloride	56-23-5	2799	153.82	Organic compound; Halogenated hydrocarbon	0.793 mg/mL at 25°C; <1 mg/mL @ 21°C	NA	2.83	76.8	Liver, kidney toxin, CNS depressant	Free	More toxic intracellular metabolites?	Hypothetical: Covalent binding of toxic intracellular metabolites. Free radicals inducing lipid peroxidation?
Chloral hydrate	302-17-0	479	165.40	Organic compound; Alcohol	9310 mg/mL @ 25°C; ≥10 mg/mL @ 20.5°C	NA	0.99	96	CNS depressant & cardiotoxin	Freely	Active metabolite trichloroethanol is partly[f] or totally[k] responsible for CNS effect	Proposed: potentiation of $GABA_A$ receptor activity, inhibition of N-methyl-D-aspartate activity, & modulation of 5-$hydroxytryptamine_3$ receptor-mediated depolarization of the vagas nerve.[k]
Chloramphenicol	56-75-7	3393	323.14	Organic compound; Alcohol; Cyclic hydrocarbon; Nitro compound	2.5 mg/mL @ 25°C	NA	1.14	NA	Hematotoxin	Free	No	Hypothetical: Binds to mitochondrial ribosomes & inhibits enzyme syntheses (e.g., those necessary for oxidative phosphorylation)

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Citric acid	77-92-9	3000	192.10	Organic compound; Carboxylic acid	592 mg/mL @ 20°C; ≥100 mg/mL @ 22°C	1=3.128 2=4.761 3=6.396 @ 25°C	-1.72	decomposes	Acidosis	NA	NA	NA
Colchicine	64-86-8	6 (mouse)	399.45	Organic compound; Polycyclic compound	45 mg/mL; ≥100 mg/mL @ 21°C	pK=12.35 @ 20°C; pKa=1.7 & 12.4	1.03		GI, liver, kidney, hemato-, PNS toxin	No	Not expected	Depresses respiratory center.
Cupric sulfate pentahydrate	7758-99-8	300	249.70	Inorganic compound; Sulfur compound; Metal	148 & 316 mg/mL @ 0°C; 2033 mg/mL @ 100°C; 230.5 mg/mL @ 25°C; 32 mg/mL @ 20°C; ≥100 mg/mL @ 21°C	NA	NA	decomposes @ 150°C	Liver, kidney toxin	Restricted	No	Hypothetical: Copper is reduced by thiol groups in cell membranes. superoxide is formed by reoxidation of copper, inducing lipid peroxidation.
Cycloheximide	66-81-9	2	281.40	Organic compound; Heterocyclic compound	21 mg/mL @ 2°C; 10-50 mg/mL @ 20°C	NA	0.55	NA	Liver toxin	Unknown	Metabolically activated	Inhibition of protein synthesis?; metabolic inhibitor
Dibutylphthalate	84-74-2	11998	278.30	Organic compound; Carboxylic acid	0.013 mg/mL @ 25°C; 0.01 mg/mL @ 20°C; <1 mg/mL @ 20°C	NA	4.9	340	CNS depressant; pulmonary, liver, testicular toxin	Yes[p]	Monobutyl metabolite has greater toxicity than parent in rats	Peroxisome proliferator[u]
Dichlorvos	62-73-7	17	220.98	Organic compound; Organophos-phorous compound	10 mg/mL @ 20°C; 5 g/mL; 10-50 mg/mL @ 20°C	NA	1.43, 1.45	245; 140 @ 20 mmHg	CNS depressant	Assumed due to CNS effects	Rapidly inactivated by hepatic metabolism	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs; irreversible cholinesterase inhibitor

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Diethyl phthalate	84-66-2	8602	222.20	Organic compound; Carboxylic acid	<1 mg/mL @ 19°C and 25°C	NA	2.47	298	CNS depressant, liver toxin	Yes[m]	Monoethyl metabolite has greater toxicity than parent in rats	Peroxisome proliferator[u]
Digoxin	20830-75-5	18 (mouse)	780.90	Organic compound; Polycyclic compound; Carbohydrate	0.0648 mg/mL @ 25°C	NA	1.26	NA	Cardiotoxin	Restricted	Also active metabolites	Impairs ion transport & increases sarcoplasmic calcium by binding to Na^+/K^+ ATPase, increasing automaticity of cardiac cells.
Dimethylformamide	68-12-2	2800	73.10	Organic compound; Amide	Miscible; ≥100 mg/mL @ 22°C	-0.01 @ -20°C	-1.01	153	Liver, kidney toxin	NA	NA	Hepatocellular necrosis[u]
Diquat dibromide monohydrate	6385-62-2	231	362.10	Organic compound; Heterocyclic compound	700 mg/mL @ 20°C; ≥100 mg/mL @ 20°C	NA	-3.05	NA	GI, pulmonary, liver, kidney toxin	Free[n]	No[n]	Assumed to be same as Paraquat; Hypothetical: Multisystem failure due to depletion of superoxide dismutase, formation of free radicals & lipid peroxidation. Lung fibrosis due to accumulation.
Disulfoton	298-04-4	2	274.42	Organic compound; Organo-phosphorous compound; Sulfur compound	0.012 mg/mL @ 20°C	NA	4.02	132-33 @ 1.5 mmHg; 108 and 62 @ 0.01 mmHg	CNS depressant	Yes	More toxic metabolites	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs; irreversible cholinesterase inhibitor
Endosulfan	115-29-7	18	406.91	Organic compound; Heterocyclic compound; Sulfur compound	0.00053 mg/mL @ 25°C	NA	3.83	106 @ 0.7 mm, partial decom-position	CNS depressant	Yes[o]	No[o]	Affects brain neurotransmitter levels.[o]

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Epinephrine bitartrate	51-42-3	4 (mouse)	333.30	Organic compound; Alcohol; Amine	1 mg/mL @ 25°C; < 0.1 mg/mL @ 18°C (for base)	NA	-1.52	NA	Cardiovascular toxin	No	Large first pass metabolism to inactive metabolites	Adrenergic receptor stimulation.
Ethanol	64-17-5	14008	46.07	Organic compound; Alcohol	>10% why include; ≥ 100 mg/ml @ 23°C	15.9 @ 25°C	-0.31	78.5	CNS depressant	Free	Acetaldehyde, active metabolite	Hypothetical: Interferes with cell membrane fluidity, perturbing proteins such as ion channels. Depression of postsynaptic potentials in CNS; solvent
Ethylene glycol	107-21-1	8567	62.07	Organic compound; Alcohol	Miscible; ≥ 100 mg/mL @ 17.5°C	NA	-1.36	197.6 @ 760 mmHg	CNS depressant, kidney toxin	Free	Glyoxalate, glycolate, & oxalate, active metabolites	Hypothetical: Metabolites inhibit mitochondria to produce metabolic acidosis. Oxalate decreases sarcoplasmic Ca^{++}; affects kidney function; oxalic acid is toxic metabolite
Fenpropathrin	39515-41-8	18	349.43	Organic compound; Nitrile; Ester; Ether	0.00033 mg/mL @ 25°C	NA	6.0 @ 20°C	377	PNS toxin	Yes[p]	Rapidly hydrolyzed to inactive products in mammals[e,p]	Delays closure of sodium channel causing persistent depolarization of membrane.
Gibberellic acid	77-06-5	6305	346.38	Organic compound; Polycyclic compound	5 mg/mL; slightly	4	0.24	NA	NA	NA	NA	NA
Glutethimide	77-21-4	600	217.30	Organic compound; Heterocyclic compound	Practically insoluble	4.2	1.9	NA	CNS depressant	Presumed	2X active metabolite: 4-hydroxyglu-thethimide	CNS depression; anticholinergic activity
Glycerol	56-81-5	12691	92.09	Organic compound; Alcohol	Soluble in all proportions; ≥ 100 mg/mL @ 18°C	14.4	-1.76	182; 290 @ 760 mmHg, decomposes	Body fluids	No evidence found	No	Cellular dehydration; osmotic effect

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Haloperidol	52-86-8	128	375.90	Organic compound; Ketone	0.014 mg/mL	8.3	3.36	NA	CNS depressant	Presumed	No	Blocks dopamine receptors
Hexachlorophene	70-30-4	61	406.91	Organic compound; Cyclic hydrocarbon; Phenol	0.140 mg/mL @ 25°C; < 1 mg/mL @ 20°C	4.95	6.91	NA	CNS depressant	Restricted	No	Hypothetical: Uncoupling of oxidative phosphorylation. Binding to proteins in cytoplasmic membrane & cell organelles.
Lactic acid	50-21-5	3730	90.08	Organic compound; Carboxylic acid	Soluble	3.86 @ 25°C	-0.72	122 @ 14-15 mmHg	Acidosis, corrosive	Yes[g]	Unknown	Disturbance of metabolism (lactic acidosis).
Lindane	58-89-9	76	290.80	Organic compound; Halogenated hydrocarbon	0.0073 mg/mL @ 25°C; < 1 mg/mL @ 24°C	NA	3.72	323.4 @ 760 mmHg	CNS stimulant	Free	No?	CNS depression through inhibition of GABA receptor linked chloride channel at the picrotoxin binding site, leading to blockade of chloride influx into neurons?
Lithium I carbonate	554-13-2	1187 (sulfate salt; mouse)	73.89	Inorganic compound; Lithium compound; Alkalies; Inorganic carbon compound	1.5 mg/mL @ 0°C; 1.3 mg/mL @ 20°C; 1.2 mg/mL @ 40°C; 12.2 mg/mL cold; 7 mg/mL hot	NA	NA	NA	CNS depressant	Restricted (assumed same as lithium sulfate)	No	Unknown: Partial substitution for normal cations of cells may disturb energy processes?
Meprobamate	57-53-4	794	218.30	Organic compound; Carboxylic acid	3.4 mg/mL @ 20°C; 7.9 mg/mL @ 37°C; < 1 mg/mL @ 20°C	9.2	NA	NA	CNS depressant cardiotoxin	NA	Rapidly inactivated by hepatic metabolism	Unknown

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Mercury II chloride	7487-94-7	1	271.50	Inorganic compound; Mercury compound; Chlorine compound	69 mg/mL at 20°C; 5-50 mg/mL @ 22°C	NA	0.22	302	Corrosive, kidney toxin	Restricted	No	Hypothetical: Changes membrane potentials & blocks enzyme reactions in cells by targeting the sulfhydryl part of active sites of some enzymes.
Methanol	67-56-1	13012	32.04	Organic compound; Alcohol	Completely miscible at 20°C; ≥100 mg/mL @ 21°C	15.3	-0.77	64.7 @ 760 mmHg	CNS depressant	Free	Active metabolites: formadehyde, formic acid	Hypothetical: Accumulation of formic acid leads to metabolic acidosis. Lactate inhibits mitochondrial respiration; formaldehyde metabolite
Nicotine	54-11-5	50	162.20	Organic compound; Heterocyclic compound	Miscible below 60°C	pK_{b1}=6.16 @ 15°C; pK_{b2}=10.96	1.17	247	CNS stimulant	Free	No	CNS nicotinic receptor; cholinergic block causing polarization of CNS and PNS synapses.
Paraquat	1910-42-5	58	257.20	Organic compound; Heterocyclic compound	Soluble; ≥100 mg/mL @ 19°C	NA	-4.22 @ pH 7.4	175-180 @ 760 mmHg, decomposes	Pulmonary toxin	Free?	No	Multisystem failure due to depletion of superoxide dismutase, with formation of free radicals & lipid peroxidation. Lung fibrosis due to accumulation; interferes with ATP synthesis.
Parathion	56-38-2	2	291.28	Organic compound; Organo-phosphorous compound; Sulfur compound	0.011 mg/mL @ 20°C; <1 mg/mL @ 23°C	NA	3.83	375 @ 760 mm Hg	CNS depressant	Free (assumed the same as malathion)	Paraoxon is active metabolite.	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs; irreversible cholinesterase inhibitor

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Phenobarbital	50-06-6	163	232.23	Organic compound; Heterocyclic compound	1 mg/mL; 1.3 mg/mL at 25°C; <0.1 mg/mL @ 14°C	pK_1=7.3, pK_2=11.8	1.47	NA	CNS depressant	Free	No	Neurotoxic; CNS depression through inhibition of GABA synapses? Inhibits hepatic NADH cytochrome oxidoreductase;
Phenol	108-95-2	414	94.11	Organic compound; Phenol	67 mg/mL; 82.8 mg/mL @ 25°C; 93 mg/mL @ 25°C; 50-100 mg/mL @ 19°C	NA	1.46	182 @ 760 mm Hg	Corrosive; CNS depressant	Free	No	General protoplasmic poison that denatures proteins; depresses vasomotor center
Phenylthiourea	103-85-5	3.0	152.20	Organic compound; Sulfur compound; Urea	2.5 mg/mL @ 25°C; <1 mg/mL @ 21°C	NA	0.71	NA	Pulmonary toxin	NA	Humans & animals have high capacity to detoxify sulfides	Destroys cytochrome p450; interferes with pulmonary, thyroid functions.
Physostigmine	57-47-6	4.5	275.40	Organic compound; Carboxylic acid; Heterocyclic compound	Slightly soluble	NA	NA	NA	CNS depressant	Easily	None known	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs.
Potassium I chloride	7447-40-7	2602	74.55	Inorganic compound; Potassium compound; Chlorine compound	342 mg/mL @ 20°C; >100 mg/mL @ 20°C	NA	NA	1500	Cardiotoxin	Free?	No	Essential cellular electrolyte maintains normal transmembrane potential, necessary for heart conduction.
Potassium cyanide	151-50-8	10	65.12	Inorganic compound; Potassium compound; Nitrogen compound	500 mg/mL cold; 1000 mg/mL hot	NA	NA	NA	CNS stimulant, corrosive	Free	No	General enzyme inhibition. Interferes with ATP synthesis. High affinity for Fe^{+++}. Inhibits cell respiration by inhibition of cytochrome oxidase.
Procainamide HCl	51-06-9	1950	271.79	Organic compound; Carboxylic	Freely soluble	NA	NA	NA	CNS depressant, cardiotoxin	Some	Less potent[r]; active metabolite[c]	Slows impulse conduction in the heart?[r]

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
				acid; Amide								
2-Propanol	67-63-0	5843	60.10	Organic compound; Alcohol	≥100 mg/mL @ 22°C	NA	0.05	82.3	CNS depressant	Free	No.	CNS depression through membrane effects[u]
Propranolol HCl	350-60-90	470 (mouse)	295.80	Organic compound; Alcohol; Amine; Polycyclic compound	Soluble	NA	3.09	NA	Cardiotoxin	Free	No?	Unknown: Beta-adrenergic blockade?
Propylparaben	94-13-3	6326 (mouse)	180.20	Organic compound; Carboxylic acid; Phenol	0.463 mg/mL @ 25°C; <1 mg/mL @ 12°C	NA	3.04	NA	CNS depressant	NA	NA	NA
Sodium arsenite	7784-46-5	41	129.90	Inorganic compound; Arsenical; Sodium compound	Very to freely soluble	NA	NA	NA	PNS, liver, hematotoxin	Yes	Not expected	Assumed the same as arsenic trioxide - causes multisystem failure due to uncoupling of oxidative phosphorylation & inhibition of pyruvate & succinate oxidative pathways.
Sodium chloride	7647-14-5	2998	58.44	Inorganic compound; Sodium compound; Chlorine compound	357 mg/mL @ 0°C; 391.2 mg/mL @ 100°C	NA	NA	1413°C	Body fluids	Restricted	No	Acute dehydration of brain cells caused by osmotic shift of water to the outside of the blood:brain barrier.
Sodium dichromate dihydrate	7789-12-0	50	298.00	Inorganic compound; Sodium compound; Chromium compound	2380 mg/mL @ 0°C	NA	NA	decomposes @ 400	Kidney, liver toxin	Yes[s]	Less active in presence of metabolizing system	Inhibition of respiratory chain activity; carcinogenic

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Sodium I fluoride	7681-49-4	180	41.99	Inorganic compound; Sodium compound; fluorine compound	43 mg/mL @ 25˚C; 10-50 mg/mL @ 23˚C	NA	NA	NA	GI irritant, CNS depressant	Restricted	No	Hypothetical: Protoplasmic poison interfering with many enzymes. May lower sarcoplasmic Ca^{++} & induce K^{+} efflux from cells.
Sodium hypochlorite	7681-52-9	8910	74.44	Inorganic compound; Sodium compound; Oxygen compound; chlorine compound	293 mg/mL @ 0˚C	NA	NA	111	Corrosive, body fluids	NA	NA	NA
Sodium oxalate	62-76-0	155	134.00	Organic compound; Carboxylic acid	220 mg/mL @ 25˚C	NA	NA	NA	Corrosive, body fluids, kidney & cardiotoxin, CNS depressant	Restricted	No	Hypothetical: Ca^{++}-complexing action, depressing the level of ionized Ca^{++} in body fluids, but doesn't explain action on GI, vasculature, & kidney. Corrosivity not due to acidity.
Sodium selenate	13413-01-0	1.6	188.90	Inorganic compound; Sodium compound; Selenium compound	≥ 100 mg/mL @ 21˚C	NA	NA	NA	Liver, kidney toxin	Yes[t]	Not expected	Inactivates sulfhydryl enzymes for oxidative reactions in cellular respiration.[t]
Strychnine	57-24-9	2	334.40	Organic compound; Heterocyclic compound	0.16 mg/mL @ 25˚C	8.26 @ 25˚C	1.93	270 @ 5 mmHg	CNS stimulant	Expected	No	Increases glutamic acid in the CNS. Alkaloid poison.

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Thallium I sulfate	7446-18-6	29 (mouse)	504.80	Inorganic compound; Metal; Sulfur compound	48.7 mg/mL @ 15°C; 191.4 mg/mL @ 100°C	NA	NA	NA	GI irritant, CNS toxin (encephalo-pathy)	Restricted	No	Hypothetical: Enzyme inhibition by binding sulfhydryl groups of mitochondrial membranes. Interferes with oxidative phosphorylation by inhibition of Na^+/K^+ ATPase.
Trichloroacetic acid	76-03-9	4999	163.40	Organic compound; Carboxylic acid	10 g/mL @ 25°C; 1200 mg/mL @ 25°C; 13.06 g/mL @ 25°C; ≥100 mg/mL @ 22°C	NA	1.33	196	GI corrosion, acidosis	Expected	Not expected	Corrosive; possible carcinogen
1,1,1-Trichloroethane	71-55-6	10298	133.41	Organic compound; Halogenated hydrocarbon	4.4 mg/mL @ 20°C; <1 mg/mL @ 20°C	NA	2.49	76	CNS depressant; liver toxin	Free	No.	Arrhythmogenic[u]
Triethylenemelamine	51-18-3	1.0	204.23	Organic compound; Heterocyclic compound	400 mg/mL @ 26°C; <1 mg/mL @ 16°C	NA	-0.54	139 (decomposes)	Hemato-, liver, kidney toxin	Unknown	Expected since it's an alkylator	Genotoxic; binds with DNA; alkylating agent; alkylates proteins
Triphenyltin hydroxide	76-87-9	44	367.02	Organic compound; Organo-metallic compound	0.0012 mg/mL; <1 mg/mL @ 21°C	NA	NA	NA	CNS toxin (encephalo-pathy), skin & GI irritant	Rapidly	No	Affects a number of enzymes involved in cellular energy production and use. Affects immune system; causes lymphopenia; clastogenic
Valproic acid	99-66-1	670 (mouse)	144.20	Organic compound; Carboxylic acid; Lipids	2 mg/mL @ 20°C; 1.27 mg/mL; <1 mg/mL @ 22°C	NA	2.75	220	CNS depressant, liver toxin	Yes	Some metabolites may be active	Increases GABA in the CNS?

Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances

Chemical	CASRN	LD_{50} (mg/kg)[a]	MW (g/mol)	Chemical Class[b]	Water Solubility[c]	pK[d]	log Kow[c]	Boiling Point (°C)[d]	Toxic Effect Class[f]	Passage of Blood: Brain Barrier[g]	Metabolic Activation/ Inactivation[f]	Mechanism of Lethaliy[f]
Verapamil HCl	152-11-4	108	491.08	Organic compound; Amine	70 mg/mL	NA	3.79	NA	Cardiotoxin	Restricted?	Also active metabolites	Inhibition of transmembrane Ca^{++} flux in excitatory tissues. Cardiac-Ca^{++} channel blocker. Also alpha-adrenergic blockade.
Xylene	1330-20-7	4300	106.17	Organic compound; Cyclic hydrocarbon	Practically insoluble; <1 mg/mL @ 22°C	NA	3.12-3.2	136-140	CNS depressant	Free	No	Unknown: Heart failure caused by sensitization of heart to catecholamines?; solvent

Abbreviations: MW=Molecular weight; NA=No information found; NADPQI=N-acetyl-*p*-benzoquinoneimine; CNS=Central nervous system; AMP=Adenosine monophosphate; GABA=Gamma aminobutyric acid; GI=Gastrointestinal; PNS=Peripheral nervous system; NADH=Nicotine adenine dinucleotide (reduced).

[a]LD_{50} data from Registry of Cytotoxicity (Halle 1998), Hazardous Substances Data Bank (NLM 2001, 2002), or Registry of Toxic Effects of Chemical Substances® (MDL information Systems 2001, 2002). Rat data unless otherwise noted. Rounded to the nearest one.

[b]Based on the Medical Subject Heading [MeSH] index (NLM 2005).

[c]Hazardous Substances Data Bank (NLM 2001, 2002) and NTP Chemical Health and Safety Data (2001) at http://ntp-server.niehs.nih.gov/Main_Pages/Chem-HS.html. The NTP database is no longer available. NTP values can be identified by the use of the following symbols: <, >, and ≥. Conditions are reported if available.

[d]Hazardous Substances Data Bank (NLM 2001, 2002) unless otherwise specified. pK measured under the conditions specified. If no conditions were specified, none are reported.

[e]Hazardous Substances Data Bank (NLM 2001, 2002) or Material Safety Data Sheets. Boiling point measured under the conditions specified. If no conditions were specified, none are given.

[f]Ekwall et al. (1998) or Hazardous Substances Data Bank (NLM 2001, 2002) unless otherwise noted.

[g]Cosmetic Ingredient Review Panel (1983).

[h]Orphan Medical (1999).

[i]Glaxo Wellcome (2000).

[j]ATSDR (1999).

[k]EPA (2000b).

[l]ATSDR (2001).

[m]ATSDR (1995).

[n]EPA (1995).

[o]ATSDR (2000a).

[p]ATSDR (2004a).

[q]Ames (2000).

[r]Hardman et al. (1996).

[s]ATSDR (2000b).

[t]ATSDR (2004b).

[u]Casarett et al. (2001).

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Appendix F3

Candidate Reference Substances

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F.3 Candidate Reference Substances

F.3.1 Sources of Candidate Substances

The process of identifying the 72 reference substances started with the compilation of a database that ultimately contained 116 candidate substances. The intent of the SMT was to compile a database with more than 12 substances in each toxicity category that also met the other criteria, and then to prioritize the substances in each category to select the 72 reference substances to be tested. As recommended by the Workshop 2000 participants (ICCVAM 2001a), the following publicly available databases and other indicated sources were used to identify candidate chemicals:

- The MEIC program, which collected human toxicity data and *in vitro* toxicity data from 61 test methods for the first 50 chemicals (Ekwall et al. 1998). The ECVAM members of the SMT preferred these chemicals since human acute toxicity data had already been collected.
- The RC (Halle 1998, 2003), which contains a compilation of *in vitro* cytotoxicity and *in vivo* rodent LD_{50} data for 347 chemicals
- The Toxic Exposure Surveillance System (TESS) (Litovitz et al. 2000), which compiles reports of toxic human exposures from poison control centers throughout the United States
- Pesticides recommended for consideration by the EPA Office of Pesticide Programs (OPP)
- The *Guidance Document* (ICCVAM 2001b), which reported *in vitro* NRU results for 11 RC chemicals using protocols similar to those used in the NICEATM/ECVAM validation study
- The U.S. NTP test database, which contains information on the toxicity of chemicals relevant to human exposure (NTP 2002)
- The EPA High Production Volume (HPV) Challenge Program, which is a voluntary testing program to provide the public with a complete set of baseline health and environmental effects data for each chemical that is manufactured within or imported into the United States at amounts > 1 million pounds/year (EPA 2000a)

F.3.2 Selection of Candidate Substances

The 116 candidate substances consisted of the 72 reference substances selected for testing in the NICEATM/ECVAM validation study (see **Table 3-2**) and the alternate substances that were not selected for testing (see **Table F3-1**). The alternate candidate substances in **Table F3-1** are grouped by GHS acute oral toxicity classification. For each reference substance, the table provides the corresponding rat or mouse oral LD_{50} value, the database(s) or other source(s) used to identify the chemical as a potential candidate, notes on volatility and/or DEA restrictions, and the type of product and/or use for the substance. Product/use categories were identified from HSDB (NLM 2001, 2002) or RTECS® (MDL Information Systems 2001, 2002).

The final list of candidate substances, which includes the substances in **Table 3-2** and **Table F3-1**, included:

- Sixty-five MEIC chemicals. These include the first 50 chemicals evaluated by MEIC as well as another 15 chemicals that were identified for future evaluation (C. Clemedson, personal communication 2001). Twenty of these chemicals were identified for the EDIT program, a follow-on project to the MEIC study to develop supplementary toxicity and kinetic tests (to determine distribution of chemicals in the body and biotransformation of chemicals to more toxic metabolites) to improve the prediction of human toxicity by the battery of tests identified as the best predictors in the MEIC program (Clemedson et al. 2002). The EDIT chemicals were selected by excluding MEIC chemicals that were volatile, those that precipitated at the IC_{50} dose level, and those with sparse or insufficient data on human toxicity or mechanism of acute toxicity.
- Sixteen pesticides with extensive human exposure nominated by the EPA OPP. These included fenpropathrin, endosulfan, bromoxynil (phenol), fipronil, carbaryl, rotenone, metaldehyde, molinate, 1,3-dichloropropene, dichlorvos, chlorpyrifos, sodium arsenite, triphenyltin hydroxide,

cycloheximide, acrolein, and boric acid. Pentachlorophenol was also nominated, but was already on the candidate list since it was a MEIC chemical.

- Five substances associated with the highest incidence of toxic exposures reported by U.S. poison control centers participating in the TESS (Litovitz et al. 2000): hypochlorite, acetaminophen, ethanol, diphenhydramine, and isopropanol. The five chemicals with the greatest incidence of toxic exposures among children were the same, except that oxalate replaced ethanol. Most of these chemicals were already identified as candidate substances due to their inclusion in the MEIC study. Since hypochlorite (sodium salt) and diphenhydramine, were not already included, they were added to the list of candidates.
- Eleven substances recommended in the *Guidance Document* (ICCVAM 2001b) for qualifying *in vitro* cytotoxicity assays for the prediction of starting doses using the RC regression. These substances were recommended because the IC_{50} and LD_{50} data for these substances fit the RC regression line extremely well. These chemicals were sodium dichromate dihydrate, cadmium chloride, p-phenylenediamine, DL-propranolol HCl, trichlorfon, ibuprofen, nalidixic acid, salicylic acid, antipyrene, dimethylformamide, and glycerol
- Sixteen substances from the NTP database
    - Furfural, methyleugenol, and methylphenidate, scheduled for testing by the NTP National Center for Toxicogenomics (NCT) (G. Boorman, personal communication 2001), were added. Acetaminophen, another hepatotoxin to be tested by the NCT, was already a candidate substance because it was included in the MEIC study. Chromium (VI), recommended by the NTP for consideration due to the potential for human exposure via drinking water (NTP 2002) was represented in the list of candidate substances by sodium dichromate dihydrate, which was also recommended in the *Guidance Document* (ICCVAM 2001b).

  - Dibutyl phthalate, 5-aminosalicylic acid, propylparaben, gibberellic acid, and diethyl phthalate were added to increase the number of chemicals with LD_{50} values >5000 mg/kg.
  - Trichloroacetic acid was added to increase the number of substances in the $2000 < LD_{50} \leq 5000$ mg/kg category.
  - Sodium selenate was added to increase the number of chemicals in the $LD_{50} \leq 5$ mg/kg category to 12.
  - Six chemicals that were also on the HPV list were added. Lactic acid, citric acid, and acetonitrile were added to increase the number of chemicals in the $2000 < LD_{50} \leq 5000$ mg/kg category. Tert-butylamine, 2,4-dinitrophenol, and acrolein were added to increase the number of chemicals in the $5 < LD_{50} \leq 50$ mg/kg category.
- Eight additional RC substances in the $LD_{50} \leq 5$ mg/kg category. These were: triethylenemelamine, busulfan, disulfoton, parathion, aminopterin, phenylthiourea, epinephrine bitartrate, and aflatoxin B1.

The goal to identify more than 12 candidate substances for each toxicity category was unrealized for three toxicity categories. The most toxic category ($LD_{50} \leq 5$ mg/kg), and least toxic categories ($2000 < LD_{50} \leq 5000$ mg/kg, $LD_{50} > 5000$ mg/kg), contained only 12 candidate substances each. The intermediate toxicity categories ($50 < LD_{50} \leq 300$ mg/kg, $300 < LD_{50} \leq 2000$ mg/kg), however, contained two to three times the minimum number of candidate chemicals.

Table F3-1 Alternate Candidate Substances

GHS Category[1]/Chemical	Rodent Oral LD_{50}[2] (mg/kg)	Source[3]	Notes[5]	Product/Use[4]
$LD_{50} \leq 5$ mg/kg				
Aflatoxin B1	5.0	RC (outlier)	Prohibitively expensive	Food contaminant
$5 < LD_{50} \leq 50$ mg/kg				
2,4-Dinitrophenol	30	RC (outlier), NTP, HPV		Pesticide (fungicide/ insecticide) manufacturing
t-Butylamine	44[a]	EPA, NTP, HPV		Manufacturing
Acrolein	46	RC, TESS, EPA, NTP, HPV	Volatile (BP=52°C)	Pesticide (herbicide/ rodenticide/ algicide), manufacturing
$50 < LD_{50} \leq 300$ mg/kg				
Pentachlorophenol	51	MEIC, RC (outlier), NTP		Disinfectant
Amphetamine sulfate	55	MEIC, EDIT, RC (outlier), TESS, NTP	DEA	Pharmaceutical (stimulant)
Rotenone	60	RC, TESS, EPA, NTP		Pesticide (insecticide/ piscicide)
Furfural	65[a]	NTP, HPV		Solvent, food additive
p-Phenylenediamine	80	RC, GD, NTP, HPV		Dyeing
Chlorpyrifos	82[a]	TESS, EPA, NTP		Pesticide (insecticide)
Dextropropoxyphene HCl	83	MEIC, RC (outlier), TESS		Pharmaceutical (analgesic)
Methadone	86[a]	MEIC,TESS, NTP	DEA	Pharmaceutical (analgesic)
Fipronil	92[a]	EPA		Pesticide (insecticide)
Pentobarbital	125	MEIC, RC TESS	DEA	Pharmaceutical (sedative)
Bromoxynil (phenol)	190[a]	EPA		Pesticide (herbicide)
Diphenylhydantoin	199	MEIC, RC, TESS, NTP		Pharmaceutical (anticonvulsant)
Metaldehyde	227[a]	TESS, EPA		Pesticide (molluscicide)
Carbaryl	230	RC, EPA. NTP		Pesticide (insecticide)
$300 < LD_{50} \leq 2000$ mg/kg				
Ferrous sulfate	319	MEIC, RC, TESS		Food additive
Warfarin	324	MEIC, RC, TESS, EPA		Pharmaceutical (anticoagulant), pesticide
Disopyramide	333[a]	MEIC, TESS		Pharmaceutical (antiarrythmic)
Barium II nitrate	355	MEIC, RC, TESS, NTP		Pyrotechnic
Thioridazine HCl	358	MEIC, RC, TESS		Pharmaceutical (antipsychotic)
Methylphenidate	367[a]	NTP	DEA	Pharmaceutical (stimulant)
Molinate	369[a]	EPA, NTP		Pesticide (herbicide)

Table F3-1 Alternate Candidate Substances

GHS Category[1]/Chemical	Rodent Oral LD_{50}[2] (mg/kg)	Source[3]	Notes[5]	Product/Use[4]
2,4-Dichlorophenoxy-acetic acid	369	MEIC, RC, TESS, EPA, NTP, HPV		Pesticide (herbicide)
Orphenadrine HCl	425	MEIC, RC, NTP		Pharmaceutical (analgesic)
Trichlorfon	451	RC, EPA, GD, NTP		Pesticide (insecticide)
Quinidine sulfate	456	MEIC, RC, NTP (base)		Pharmaceutical (antiarrhythmic)
1,3-Dichloropropene	470[a]	TESS, EPA, NTP		Pesticide (nematocide)
Theophylline	600 [b]	MEIC, RC, TESS, NTP		Pharmaceutical (antiasthmatic)
Isoniazid	650	MEIC, RC, TESS, NTP		Pharmaceutical (antibiotic)
Diazepam	709	MEIC, EDIT, RC, TESS, NTP	DEA	Pharmaceutical (anxiolytic)
Maprotiline	760[a]	MEIC, TESS		Pharmaceutical (antidepressant)
Methyleugenol	810[a]	NTP		Food additive
Diphenhydramine HCl	855	MEIC, RC, TESS, NTP		Pharmaceutical (antihistamine)
Malathion	885	MEIC, EDIT, RC, TESS, EPA, NTP		Pesticide (insecticide)
Salicylic acid	891	RC, TESS, GD, NTP, HPV		Pharmaceutical (analgesic)
Chloroform	908	MEIC, RC, NTP, HPV	Volatile (BP=61°C)	Solvent
Chloroquine diphosphate	970	MEIC, RC		Pharmaceutical (antimalarial))
Ibuprofen	1009	RC, TESS, GD		Pharmaceutical (analgesic)
Nalidixic acid	1349	RC, GD, NTP		Pharmaceutical (antibiotic)
Dichloromethane	1597	MEIC, RC, TESS, NTP, HPV	Volatile (BP=40°C)	Solvent
Antipyrene	1800	RC, GD		Pharmaceutical (analgesic)

[1]GHS=Globally Harmonized System of Classification and Labelling of Chemicals for acute oral toxicity (UN 2005).
[2]LD_{50} data are from the Registry of Cytotoxicity (Halle 1998) and are for rats, the preferred species for oral acute toxicity studies, unless otherwise noted. Data with decimal places are rounded to the nearest one.
[3]Sources used to identify candidate chemicals: EDIT=Evaluation-guided Development of New *In Vitro* Test Batteries; EPA=Pesticides registered with the Environmental Protection Agency; EHS=EPA's Extremely Hazardous Substance list; HPV=High Production Volume chemicals (i.e., those that are imported into or produced in the United States in amounts ≥ 1,000,000 lbs/year; GD=*Guidance Document* (ICCVAM 2001b); MEIC=Multicentre Evaluation of *In Vitro* Cytotoxicity; NTP=National Toxicology Program; RC=Registry of Cytotoxicity with chemicals classified as regression outliers shown in parentheses; TESS=Toxic Exposure Surveillance System (Litovitz et al. 2000).
[4]Product/use categories from Hazardous Substances Data Bank (NLM 2002) or Registry of Toxic Effects of Chemical Substances ([RTECS®], MDL Information Systems 2002). Pharmaceutical uses from Gilman et al. (1985) or Thomson PDR® (2004).
[5]Only chemicals expected to be too volatile for the cytotoxicity assay system have "volatile" notations. BP=Boiling point. DEA (U.S. Drug Enforcement Agency) refers to Schedule II controlled substances. Chemicals with no "DEA" notation are expected to be under less strict control.
[a]RTECS® (MDL Information Systems 2002).
[b]Mouse

Appendix G

STATEMENT OF WORK (SOW)

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Appendix G1

A Validation Study For *In Vitro* Basal Cytotoxicity Testing

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STATEMENT OF WORK

A VALIDATION STUDY FOR *IN VITRO* BASAL CYTOTOXICITY TESTING

BALB/c 3T3 Neutral Red Uptake Cytotoxicity Assay
and
Normal Human Keratinocyte Neutral Red Uptake Cytotoxicity Assay

June 21, 2002

Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Based on Standard Operating Procedure Recommendations from an International Workshop Organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

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STATEMENT OF WORK

A VALIDATION STUDY FOR *IN VITRO* BASAL CYTOTOXICITY TESTING

BALB/c 3T3 Neutral Red Uptake Cytotoxicity Assay
and
Normal Human Keratinocyte Neutral Red Uptake Cytotoxicity Assay

1.0 PROJECT OBJECTIVES AND GENERAL REQUIREMENTS

1.1 Project Objectives

This Statement of Work outlines and supports the procedures for performing two *in vitro* basal cytotoxicity assays (the BALB/c 3T3 Neutral Red Uptake [NRU] assay and the Normal Human Keratinocyte [NHK] Neutral Red Uptake [NRU] assay) for analysis of test chemicals for a multi-laboratory *in vitro* Validation Study. These *in vitro* assays, recommended in *Guidance Document On Using In Vitro Data To Estimate In Vivo Starting Doses For Acute Toxicity* (ICCVAM, 2001a) use mammalian cell culture techniques to assess the basal cytotoxicity of chemicals.

A primary goal of this Validation Study is to evaluate the usefulness and effectiveness of *in vitro* basal cytotoxicity assays for reducing and refining animal use for acute oral toxicity determinations of chemicals by predicting starting doses for *in vivo* rodent acute lethality assays. Participants at an international workshop (ICCVAM, 2001b) suggested that a validation study for *in vitro* assays is needed to continue the development of alternative tests as replacements for animal testing. This is the first step to further standardization and evaluation of two test methods that may be used in conjunction with other methods as components of a test battery which may eventually replace the rodent acute oral toxicity tests.

Data will be used to:

1) Develop standardized *in vitro* basal cytotoxicity protocols with sufficient detail and instruction for distribution to other laboratories (e.g., Federal regulatory agencies) for their immediate use,
2) Evaluate the intra- and inter-laboratory reproducibility of the assays (i.e., to access test reproducibility and optimize to further enhance reproducibility),
3) Determine the reduction in the number of animals that would be used and/or killed in lethality assays compared with the conventional method of predicting starting doses, and
4) Assess the relevance of the two standardized *in vitro* cytotoxicity assays for estimating rodent oral LD50 values across the six Globally Harmonised System (GHS; OECD, 2001) categories of acute oral toxicity and estimating human lethal concentrations.

This study will test the hypothesis of the Registry of Cytotoxicity (RC) prediction model (Halle, 1998) by comparing the NRU regressions that are developed from the two assays to the RC regression. The hypothesis is that the two NRU assays will provide the same regression as the RC (i.e., comparison of IC_{50} data vs. LD_{50} data).

The proposed Validation Study will provide the means to determine IC_{20}, IC_{50}, and IC_{80} values for a test set of 72 chemicals with varying degrees of toxicity. This set of chemicals was selected separate and prior to this Statement of Work by the Study Management Team. The

basis for selection of this test set is discussed in the Study Design document prepared by the Study Management Team.

1.2 Response to the Statement of Work

The proposals submitted in response to the Statement of Work to the designated contacts shall include:

a) A timetable for project milestones
b) A cost estimate for performing all testing (both assays) in all phases of the Validation Study.
c) Cost estimates for repeating Phases Ia, Ib, and II as options, if necessary (see **Sections 4.2.2, 4.2.4, and 4.3.2**).
d) Cost for a third replicate of Phase III testing as an option, if necessary
e) Cost of software for data analysis (e.g., GraphPad PRISM® 3.0) not to exceed $500.

1.2.1 *General Capabilities*

The contracted laboratories (Testing Facilities) shall be capable of performing the following:

a) The Testing Facilities shall prepare Standard Operating Procedures (SOPs) for the 3T3 NRU assay and the NHK NRU assay (see **Section 1.4** – Definitions - SOPs)
b) The Testing Facilities shall perform the 3T3 NRU assay and the NHK NRU assay (under aseptic *in vitro* laboratory conditions) for the three phase Validation Study as identified in **Section 4.0**.
c) The Testing Facilities shall provide IC_{20}, IC_{50}, and IC_{80} values for each tested chemical and other information addressed in this document (e.g., phase reports) to the Study Management Team through the designated contacts (**Section 2.2**).
d) Testing Facilities that are compliant with Good Laboratory Practices (GLP) shall perform all aspects of the Validation Study in accordance with GLPs.
e) Testing Facilities that are not GLP-compliant shall perform all aspects of the Validation Study "in the spirit" of GLP which is defined in **Section 1.4** and addressed throughout this Statement of Work.
f) All Testing Facilities shall adhere to this Statement of Work throughout the Validation Study.

1.3 Guidelines

The Management Team and/or its representatives may inspect and audit the Testing Facilities used for this study to ensure that the Study Management Team's minimum requirements and guidelines are being followed. The contractor shall notify the Study Management Team of any changes in Key Personnel (identified in Section 3.1.1)

1.4 Definitions

Blinded/Coded Chemicals: Test chemicals supplied to the Testing Facilities that are coded (by an NIEHS/NTP-designated contractor) such that the Testing Facilities do not know the identity of the chemicals. Only the Project Officer, Management Team, and contractor know the contents of each test chemical vessel. The test chemicals will be purchased, aliquoted, coded, and distributed by a contractor under the guidance of the NIEHS Project Officer and the Management Team.

Good Laboratory Practices (GLPs): Regulations governing the conduct, procedures, and operations of toxicology laboratories; regulations to assure the quality and integrity of the data and to address such matters as organization and personnel, facilities, equipment, facility operations, test and control articles, and Validation Study protocol (Statement of Work) and

conduct (U.S. Food and Drug Administration, Title 21 CFR Part 58; Environmental Protection Agency, Title 40 CFR Part 160).

IC_X: Inhibitory concentration estimated to affect endpoint in question by X % (IC_{20} = 20 % affected; IC_{50} = 50 % affected; IC_{80} = 80 % affected).

Lead Laboratory (Protocols): A designated laboratory (identified by the Study Management Team and different from the lead laboratory for data analysis) with experience in each cytotoxicity method. The laboratory will assist the Study Management Team with troubleshooting laboratory challenges; the lead laboratory shall develop a study protocol from the Statement of Work and the Test Method Protocols that shall be used by all laboratories in the Validation Study.

Lead Laboratory (Data Analysis): A designated laboratory (identified by the Study Management Team and different from the lead laboratory for protocols) with experience in data analysis specific to the software that will be used in the study; The laboratory will assist the Study Management Team with troubleshooting data analysis challenges.

Replicate: An independent test run on different days (e.g., duplicate 96-well plates for a particular test chemical, each plate a replicate assay); replicate wells within the 96-well plate (e.g., six wells of one test chemical concentration equals six replicate wells).

Spirit of GLP: Laboratories that are non GLP-compliant shall adhere to GLP principles and other method parameters as put forth in this Statement of Work and the Test Method Protocols (provided by NIEHS/NICEATM); documentation and accountability shall be equal to GLP requirements; laboratories must make assurances that they are equal in performance criteria and that there is parity amongst the laboratories.

Standard Operating Procedures (SOPs): Written documents that describe, in great detail, the routine procedures to be followed for a specific operation, analysis, or action; consistent use of an approved SOP ensures conformance with organizational practices, reduced work effort, reduction in error occurrences, and improved data comparability, credibility, and defensibility; SOPs also serve as resources for training and for ready reference and documentation of proper procedures; each Testing Facility involved in the Validation Study shall draft SOPs specifically for its laboratories based on: protocols supplied by commercial sources specifically for cell culture products and cell lines; this Statement of Work and the Test Method Protocols provided by NIEHS/NICEATM, and the study protocol developed by the lead laboratory.

Statement of Work: A description of testing required for the *in vitro* Validation Study; defines all phases of the Validation Study and the purpose of the procedures; provides the details of the experimental design, data acquisition, data analysis, and preparation of reports; supports Test Method Protocols (equivalent to GLP protocols) and acts as a study plan.

Study Protocol: A description of the objectives and all methods for the conduct of the study (i.e., same as "protocol" according to GLP guidelines, 40 CFR 792, at http://www.ovpr.uga.edu/qau/tscatoc.html. The Study Protocol shall be developed from the Test Method Protocols for NHK and 3T3 NRU assays, which accompany this Statement of Work. The Study Protocol shall contain information such as the title and purpose of the study, name and address of the sponsor, the name and address of the testing facility at which the study is being conducted, proposed experimental start and termination dates, and other items specified in 40 CFR 792.

Test Method Protocols: Specific and detailed guides for performing the 3T3 NRU and NHK NRU cytotoxicity assays; adapted by NICEATM from protocols included in ICCVAM (2001a); equivalent to GLP protocols; protocols shall be incorporated into the SOPs specific to each Test Facility in the Validation Study.

2.0 ORGANIZATION

2.1 Validation Study Sponsors

- National Institute of Environmental Health Sciences (NIEHS)
- The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- U.S. Environmental Protection Agency (U.S. EPA)
- The European Centre for the Validation of Alternative Methods (ECVAM).

2.2 Management Team

2.2.1 *Study Management Team*

2.2.1.1 ***NIEHS/NICEATM***

Dr. William S. Stokes (NICEATM/NIEHS) – Co-chair – Study Management Team
Dr. Judy Strickland (NICEATM/ILS) – Project Coordinator
Mr. Michael Paris (NICEATM/ILS) – Assistant Project Coordinator
Dr. Ray Tice (NICEATM/ILS) – Technical Advisor

NICEATM
79 T.W. Alexander Drive
Bldg. 4401, MD-EC-17
3rd Floor, Room 3126
P.O. Box 12233
Research Triangle Park, NC 27709

2.2.1.2 ***ECVAM***

Professor Michael Balls – Co-chair – Study Management Team
Dr. Silvia Casati
Dr. Andrew Worth

European Commission
Joint Research Centre
Institute for Health and Consumer Protection
Management Support Unit - TP 202
I-21020 Ispra (VA) - Italy

2.2.2 *Project Management and Chemical Distribution Team*

Ms. Molly Vallant (NIEHS) – NIEHS Project Officer for BioReliance, Inc.
Dr. Martin L. Wenk (BioReliance, Inc., Rockville, MD) – Principal Investigator/Chemical Distribution

2.2.3 ***Contract Management***
Ms. Jackie Osgood (NIEHS) – Contracting Officer
Mr. Don Gula (NIEHS) – Contracting Officer

3.0 TESTING FACILITY AND KEY PERSONNEL

3.1 Testing Facility

The Testing Facility shall have competence in performing *in vitro* cytotoxicity assays under aseptic laboratory conditions and shall provide competent personnel, adequate facilities, equipment, supplies, proper health and safety guidelines, and satisfactory quality assurance procedures.

3.1.1 *Personnel*

3.1.1.1 ***Facility Management***

The facility management is responsible for establishing scientific guidelines and procedures, training and supervision of professional and technical staff, and evaluation of results and performance within their discipline area relative to the Study Management Team requirements. The manager must maintain records of the qualifications, training and experience, and a job description for each professional and technical individual involved in the Validation Study.

3.1.1.2 ***Study Director***

A scientist or other professional of appropriate education, training, and experience in *in vitro* cytotoxicity assay performance, or combination thereof, shall be the Study Director. The Study Director has the overall responsibility for the technical conduct of the Validation Study (e.g., GLP adherence or implementation of spirit of GLP) at the Testing Facility and shall be responsible for determining test acceptance. The Study Director shall be responsible for providing SOPs for the Validation Study and incorporating pertinent information obtained from the Statement of Work and the Test Method Protocols. Other duties include the interpretation and analysis of data, documentation of all Validation Study aspects (including maintenance of a Study Workbook), and production of all draft and final written Validation Study reports.

3.1.1.3 ***Quality Assurance (QA) Director***

For Testing Facilities that are GLP-compliant, the Quality Assurance Director shall **monitor** the Validation Study to assure conformance with GLP requirements for all aspects of the Validation Study (i.e., facilities, equipment, personnel, methods, practices, records, controls, transference of data into software, SOPs). The Quality Assurance Director or unit can be any person or organizational element, except the Study Director, designated by Testing Facility management to perform the duties relating to quality assurance of the studies. The Quality Assurance duties are not a substitute for the Study Director duties.

For Testing Facilities performing the Validation Study in the spirit of GLP, management shall appoint an individual to assure that all records, documents, raw data, reports, and specimens are available to the Management Team through the designated contacts if an inspection is requested.

3.1.1.4 ***Scientific Advisor(s)***

Scientists or other professionals of appropriate education, training, and experience in *in vitro* laboratory methods and techniques who provide scientific guidance to the Study Director and other laboratory personnel.

3.1.1.5 ***Laboratory Technician(s)***

In vitro cytotoxicity assays require personnel trained in sterile tissue culture techniques and general laboratory procedures. At least two individuals must be capable of performing the *in vitro* assays for the Validation Study. Performance of the assays requires a relatively moderate degree of technical capability and a high degree of technical accuracy. Each individual engaged in the conduct of or responsible for the supervision of a Validation Study shall have education, training, and experience, or combination thereof, to enable that individual to perform the assigned duties. The individuals in a GLP-compliant laboratory must be trained in GLP requirements and technical ability must be documented as per GLP requirements. Non GLP-compliant laboratory personnel must be able to perform all aspects of the Validation Study in the spirit of GLP.

3.1.1.6 ***Safety Officer***

A designated Safety Officer (someone not involved in the actual conduct of the Validation Study) at each participating laboratory will receive the blinded (coded) test chemicals from an NIEHS/NTP-designated contractor (BioReliance) and shall transfer the test chemicals to the Study Director without revealing the contents of the test chemical containers. A sealed health and safety information package will accompany the test chemicals and the Safety Officer shall retain the package until the completion of the Validation Study. At the end of the Validation Study, the Safety Officer shall return the unopened package to the contractor (BioReliance). If any Test Facility personnel should open the package at any time during the Validation Study, the Safety Officer shall notify the Management Team through the designated contacts.

3.1.2 *Facilities, Equipment, and Supplies*

3.1.2.1 ***Cell Culture Laboratory***

Each Testing Facility must provide a designated cell culture laboratory to ensure that *in vitro* cytotoxicity assays can be performed under clean and proper aseptic conditions. The laboratory must be located such that there is minimal through traffic to reduce possible disturbances that may compromise the cell culture assays. Room temperature of the laboratory must be easily regulated, monitored, and documented. Access to the Validation Study assays and test chemicals shall be restricted to appropriate personnel as determined by facility management.

3.1.2.2 ***Equipment***

Each Testing Facility must provide at a minimum the following equipment:

a) Laminar flow hood (biohazard type and restricted to cell culture assays)
b) Cell culture incubators
   - $37^{o}C \pm 1^{o}C$, 5 % ± 1 % CO_2, 90 ± 5 % humidified
c) Low-speed centrifuge
d) Water bath ($37^{o}C$)
e) Inverse phase microscope
f) Pippettors (multichannel pipettor, micropipettors, multichannel pipette units)

g) Spectrophotometric plate reader (equipped with a 540 nm ± 10 nm filter)
h) Computer (for data transformation and analysis)
i) Liquid nitrogen freezer (for storage of cryopreserved cells)
j) Refrigerator (4°C)
k) Freezers (-20°C and -70°C to -80°C)
l) Autoclave (for instruments and for biohazardous waste materials)
m) Balance
n) pH meter
o) Cell counting system (e.g., hemocytometer, Coulter counter)
p) General cell culture laboratory equipment (e.g., glassware, filtration systems, cell culture plasticware, etc.)
q) pH paper (wide and narrow range)

All equipment maintenance and calibration shall be routinely performed and documented as per GLP guidelines (or spirit of GLP for non GLP-compliant laboratories) and Testing Facility procedures. Additional detail is provided in **Section 10.3** and Addendum IV.

3.1.2.3 ***Supplies***

a) General cell culture materials and supplies are needed and are specifically described in the provided Test Method Protocols and in the *Guidance Document* (ICCVAM, 2001a). All cell culture reagents must be labeled so as to indicate source, identity, concentration, stability, preparation and expiration dates, and storage conditions.
b) BALB/c 3T3 mouse cells, clone 31
   - Cryopreserved (5 vials, same lot)
   - CCL-163, *LGC Reference Materials*, Customer Service, Queens Road, Teddington, Middlesex, TW110LY, UK (http://www.lgc.co.uk/atcc/)
   - CCL-163, *American Type Culture Collection (ATCC),* Manassas, VA, USA (http://www.atcc.org/)
c) Normal Human Epidermal Keratinocytes (NHK)
   - Cryopreserved (20 vials, same lot, first passage)
   - Non-transformed cells; from cryopreserved primary cells (**Clonetics #CC-2507** [pooled neo-natal keratinocytes]**)**
   - *Clonetics/BioWhittaker* [BioWhittaker, 8830 Biggs Ford Road, Walkersville, MD 21793-0127 (http://www.cambrex.com/subsidiaries/s%2Dbw%5Finc/s%2Dbiowhittaker%2Dinc%2Dcontact2.htm)
   - *BioWhittaker Europe* [BioWhittaker Europe, S.P.R.L. Parc Industriel de Petit Rechain, B-4800 Verviers, BELGIUM] (http://www.biowhittaker.be/index.htm)

3.1.3 *Health and Safety*

Each Testing Facility shall conform to all local, state, and federal statutes in effect at the time of this Validation Study. The designated Safety Officer shall be the point of contact for health and safety issues.

3.1.4 *Quality Assurance*

3.1.4.1 ***GLP-Compliant Laboratories***

GLP-compliant laboratories shall conduct this Validation Study in compliance with Good Laboratory Practice (GLP) Standards (U.S. Food and Drug Administration, Title 21 CFR Part 58; Environmental Protection Agency, Title 40 CFR Part 160). The appropriate QA unit (as per GLPs) shall review the protocol and audit the in-life phase, laboratory notebooks, and final report data.

The Final Reports for all phases of the Validation Study shall be audited by the Quality Assurance unit of the Testing Facility for GLP compliance and a QA Statement shall be provided by the Testing Facility. Each Final Report shall identify: 1) the phases and data inspected, 2) dates of inspection, and 3) dates findings were reported to the Study Director and Testing Facility management. The QA Statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study.

3.1.4.2 ***Non GLP-Compliant Laboratories***

Non GLP-compliant laboratories shall use GLP standards referenced in the ECVAM Workshop 37 Report (Cooper-Hannan, 1999) and the OECD Principles of GLP (OECD, 1998) as guidelines for conducting the Validation Study in the spirit of GLP.

At a minimum, the following laboratory parameters and equipment must be routinely documented (e.g., log books; see Addendum IV). The documents shall be archived such that they can be available to the Study Management Team through the designated contacts upon request.

Daily Documentation (value, time, and date)

- Laboratory: room temperature
- Incubators: temperature, %CO_2, %humidity
- Water bath: temperature
- Refrigerators and freezers: temperature
- Cell cultures: visual observations (see Test Method Protocols)

Per Use Documentation (value, time, and date)

- Cryogenic storage unit: amount of liquid N_2 in container; when liquid N_2 added
- Balance: standard weight used to calibrate
- pH meter: values for standards used to determine slope
- Cell counter: standard used
- Media: identification of all media and components used

Periodic Documentation

- Media and components: date of receipt; lot numbers; expiration dates
- 3T3 and NHK cells: date of receipt; lot number; storage conditions
- Plastic tissue-culture ware (sterile, disposable): stock and lot numbers
- Computer software: identification and description
- Calibration of Instruments: SOPs for laboratory equipment
  - Incubators
  - Laminar flow hoods
  - Autoclaves

Micropipettors
Balances
pH meters
Cell counters
Refrigerators
Freezers
Water baths
Spectrophotometer plate readers

A statement from the Testing Facility shall be included with each Final Report and shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study and provide assurance that all testing was done in the spirit of GLP.

4.0 TEST PHASES AND SCHEDULE

See Addendum VI for Gantt Chart of study timelines and deliverables.

4.1 Study Timeline and Deliverables

TASK	WEEK	ESTIMATED DATE
Statement of Work issued by NIEHS to the Testing Facility	0	March 29, 2002
Response /Proposal received from the Testing Facility	6	May 10, 2002
Award of Contracts	13	June 28, 2002
Submission of Study Protocol, CVs of Key Personnel and SOPs	15	July 12, 2002
Start Testing – Phase I (Phase Ia)	18	July 29, 2002
End Phase Ia	22	August 26, 2002
Begin Phase Ib	26	September 26, 2002
End Phase Ib	31	October 29, 2002
Begin Phase II	36	December 2, 2002
End Phase II	46	February 10, 2003
Begin Phase III	52	March 26, 2003
Final Report (Phase III) to SMT	89	December 9, 2003

4.1.1 Deliverables

	ESTIMATED DUE DATES			
REPORTS	**PHASE 1a**	**PHASE Ib**	**PHASE II**	**PHASE III**
Biweekly	*	*	*	*
Draft	Week 24 Sept. 9, 2002	Week 33 Nov. 11, 2002	Week 48 Feb. 25, 2003	Week 82 Oct. 24, 2003
Final	Week 33 Nov. 11, 2002	Week 42 Jan. 13, 2003	Week 57 April 28, 2003	Week 89 Dec.9, 2003
Study Workbook (Draft)	Week 24 Sept. 9, 2002	Week 33 Nov. 11, 2002	Week 48 Feb. 25, 2003	Week 82 Oct. 24, 2003
Study Workbook (Final)	Week 33 Nov. 11, 2002	Week 42 Jan. 13, 2003	Week 57 April 28, 2003	Week 89 Dec.9, 2003

* Biweekly reports shall begin at the time of implementation of the contracts and continue until the final report is submitted.

4.2 Phase I

Phase I will be the training phase for laboratory personnel. This phase includes developing a positive control database (Phase Ia) and testing three unknown chemicals (Phase Ib). SOPs for the two NRU cytotoxicity assays shall be developed by the appropriate laboratory personnel prior to implementation of test procedures (See **Section 1.4** – Definitions – SOPs). They will be submitted along with the signed protocols to the designated contacts before initiation of Phase I.

4.2.1 *Study Procedures*

4.2.1.1 ***Phase Ia: Positive Control Database***

An historical database of **IC_{50}** values for the positive control chemical (Sodium Lauryl Sulfate [SLS]) will be established and maintained for each NRU assay by performing 10 concentration-response assays (10 microtiter plates, one plate per assay) on both cell types. A range finder experiment will be performed before initiating the 10 concentration-response assays (**Section 9.3**). The Test Facility personnel shall prepare and test eight concentrations (per microtiter plate) of the positive control chemical by diluting the stock solution with a constant factor for the range finder experiment (e.g., log dilutions [1:10, 1:00, 1:1000, etc.]). For the definitive concentration-response assays, the Study Director shall use a $\sqrt[6]{10} = 1.47$ dilution scheme centered on the IC_{50} identified in the range-finding assay.

Once a range has been determined that satisfies the criteria in **Section 11.2**, then the Test Facility shall perform two tests per day (each assay) on five different days. Control limits for the positive control chemical shall be established and a draft report (including range finding data) shall be provided to the designated contacts. After evaluation of the data, the Management Team will decide when to advance to the next phase of the Validation Study.

The 95 % confidence interval (CI) of the IC_{50} of SLS will be established and defined as an acceptance criterion for test sensitivity for the 3T3 NRU and NHK

NRU assays. The confidence intervals shall be calculated using the average of the individual IC_{50} values from each positive control assay performed. An example of an historical mean IC_{50} of SLS in mammalian cultures is **93 µg /ml** and the 95 % CI is **70 - 116 µg /ml** (Spielmann et. al., 1991). An example of an historical mean IC_{50} of SLS in NHK cultures is **4.4 µg/ml ± 0.97 µg/ml [**two standard deviations] (Triglia, 1989).

The following 96-well plate configuration will be used for the positive control assays.

Figure 1. 96-Well Plate Configuration for Positive Control Assays (Phase Ia)

	1	2	3	4	5	6	7	8	9	10	11	12
A	b	b	b	b	b	b	b	b	b	b	b	b
B	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
C	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
D	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
E	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
F	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
G	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
H	b	b	b	b	b	b	b	b	b	b	b	b

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 – C_8$ = POSITIVE CONTROL (SLS) at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)

4.2.1.2 ***Reporting Positive Control Data (Phase Ia)***

Biweekly Reports: Each testing facility will provide a biweekly progress report to the designated contacts. These reports will be provided in electronic format (i.e., email with attachments) and will include raw and interim data as the study progresses. The Management Team will in turn provide a weekly progress report addressing the Validation Study as a whole to all of the Testing Facilities.

Draft Report: At the conclusion of Phase Ia, a draft report of the positive control data shall be provided by the Study Director to the designated contacts. The draft report (entitled: *In Vitro Validation Study – Phase Ia: Development of a Positive Control Database in Rodent and Human Cell Systems*) shall include everything noted in Addendum I (Draft Report – Phase Ia). If the Phase Ia data does not meet test acceptance criteria, then the Management Team (through the designated contacts) will work with the Test Facility and lead laboratory to identify problems and make corrections as needed. Once unresolved issues have been resolved, the

Validation Study will proceed. The Validation Study will advance to Phase Ib once all participating laboratories have submitted acceptable draft reports. The draft report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be copied to the designated contacts. Copies of the unaudited Study Workbook pages may be submitted as attachments in hard copy format.

Final Report: Once the draft report provides data that meets test acceptance criteria, then the Project Coordinator shall inform the Study Director to prepare a Quality Assurance audited final report for Phase Ia. The final report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be copied to the designated contacts. Copies of the audited Study Workbook pages may be submitted in hard copy format as an attachment to the report. The final report will not need to be completed to continue to Phase Ib. (See Validation Study timelines in **Section 4.1** and Report submission timelines in **Section 4.5**.)

4.2.2 *Criteria for Advancing to Phase Ib*

If there is excessive variation of ICx data within or among laboratories involved in the Validation Study, the lead laboratory for each method shall assist the Management Team (through the designated contacts) to determine the cause and recommend appropriate actions needed to reduce the variation. The Statement of Work, Test Method Protocols, and SOPs shall be revised if necessary, and testing repeated until acceptable proficiency is achieved. The Management Team will decide when all laboratories will advance to the next phase of the Validation Study. A teleconference shall be held with all of the appropriate participants of the Validation Study and the Management Team will relate information concerning the advancement of the Validation Study.

4.2.3 *Study Procedures*

4.2.3.1. ***Phase Ib: Chemical Testing***

Three blinded/coded chemicals with varying cytotoxicity (high, medium, and low) will be tested in both NRU assays. Eight concentrations of each chemical will be tested in a 96-well plate (six wells per concentration) with at least four replicates per concentration required for data analysis (**Section 12.0**). Only one test chemical will be tested on each plate. The assay setup will follow the 96-well (microtiter) plate configuration in Figure 2. A range finder experiment will be performed before initiating concentration-response assays (**Section 9.3**). After the range finding assay is completed, the concentration-response experiment shall be performed three times on three different days for each assay and each chemical. Laboratories will **calculate IC_{20}, IC_{50}, and IC_{80} values in µg/ml,** calculate confidence limits for each value, and report this and all raw data to the Management Team through the designated contacts.

Figure 2. Plate Configuration for 3T3 NRU and NHK NRU Assays (Phase Ib)

	1	2	3	4	5	6	7	8	9	10	11	12
A	b	b	b	b	b	b	b	b	b	b	b	b
B	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
C	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
D	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
E	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
F	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
G	b	VC	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	VC	b
H	b	b	b	b	b	b	b	b	b	b	b	b

VC = untreated VEHICLE CONTROL (mean viability set to 100 %)
$C_1 – C_8$ = TEST CHEMICAL at eight concentrations (C1 = highest, C8 = lowest)
b = BLANKS (contain **no** cells)

4.2.3.2 ***Reporting Test Chemical Data (Phase Ib)***

Biweekly Reports: Each testing facility will provide a biweekly progress report to the designated contacts (See Addendum I). These reports will be in electronic format (i.e., email with attachments) and will include raw and interim data as the study progresses. The Management Team will in turn provide a weekly progress report addressing the Validation Study as a whole to all of the Testing Facilities. Problems and issues shall be resolved in this manner.

Draft Report: At the conclusion of Phase Ib, a draft report of the Phase Ib test chemical data shall be provided by the Study Director to the designated contacts. The draft report (entitled: *In Vitro Validation Study – Phase Ib: Training Phase for Cytotoxicity Study of Three Coded Chemicals in Rodent and Human Cell Systems*) shall include everything noted in Addendum I (Draft Report – Phase Ib). If the Phase Ib data does not meet test acceptance criteria, then the Management Team (through the designated contacts) will work with the Test Facility and lead laboratory to identify problems and make corrections as needed. Once unresolved issues have been resolved, the Validation Study will proceed. The Validation Study will advance to Phase II once all participating laboratories have submitted acceptable draft reports. The draft report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be

copied to the designated contacts. Copies of the unaudited Study Workbook pages may be submitted as attachments in hard copy format.

Final Report: Once the draft report provides data that meets test acceptance criteria, then the Project Coordinator shall inform the Study Director to prepare a Quality Assurance audited final report for Phase Ib. The final report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be copied to the designated contacts. Copies of the audited Study Workbook pages may be submitted in hard copy format as an attachment to the report. The final report will not need to be completed to continue to Phase II. (See Validation Study timelines in **Section 4.1** and Report submission timelines in **Section 4.5**.)

4.2.4 *Criteria for Advancing to Phase II*

If there is excessive variation of ICx data within or among laboratories involved in the Validation Study, the lead laboratory for each method shall assist the Management Team (through the designated contacts) to determine the cause and recommend appropriate actions needed to reduce the variation. The Statement of Work, Test Method Protocols, and SOPs shall be revised if necessary, and testing repeated until acceptable proficiency is achieved. The Management Team will decide when all laboratories will advance to the next phase of the Validation Study. A teleconference shall be held with all of the appropriate participants of the Validation Study and the Management Team will relate information concerning the advancement of the Validation Study.

4.3 Phase II

4.3.1 *Study Procedures*

Phase II of this Validation Study is the qualification phase. This phase requires testing nine blinded/coded chemicals in the same *in vitro* cytotoxicity assays and in the same concentration-response fashion as in Phase Ib. After a range-finding assay is completed, the concentration-response experiment for each chemical shall be performed three times, once each on three different days. Laboratories will **calculate IC_{20}, IC_{50}, and IC_{80} values in µg/ml,** calculate confidence limits for each value, and report this and all raw data to the Study Management Team through the designated contacts.

4.3.1.1 ***Reporting Test Chemical Data (Phase II)***

Biweekly Reports: Each testing facility will provide a biweekly progress report to the designated contacts (See Addendum I). These reports will be in electronic format (i.e., email with attachments) and will include raw and interim data as the study progresses. The Management Team will in turn provide a weekly progress report addressing the Validation Study as a whole to all of the Testing Facilities. Problems and issues shall be resolved in this manner.

Draft Report: At the conclusion of Phase II, a draft report of the Phase II test chemical data shall be provided by the Study Director to the designated contacts. The draft report (entitled: *In Vitro Validation Study – Phase II: Qualification Phase for Cytotoxicity Study of Nine Coded Chemicals*) shall include everything noted in Addendum I (Draft Report – Phase II). If the Phase II data does not meet test acceptance criteria, then the Management Team (through the designated contacts)

will work with the Test Facility and lead laboratory to identify problems and make corrections as needed. Once unresolved issues have been resolved, the Validation Study will proceed. The Validation Study will advance to Phase III once all participating laboratories have submitted acceptable draft reports. The draft report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be copied to the designated contacts. Copies of the unaudited Study Workbook pages may be submitted as attachments in hard copy format.

Final Report: Once the draft report provides data that meets test acceptance criteria, then the Project Coordinator shall inform the Study Director to prepare a Quality Assurance audited final report for Phase II. The final report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be copied to the designated contacts. Copies of the audited Study Workbook pages may be submitted in hard copy format as an attachment to the report. The final report will not need to be completed to continue to Phase III. (See Validation Study timelines in **Section 4.1** and Report submission timelines in **Section 4.5**.)

Any solubility problems/issues with the test chemicals shall be addressed by the lead laboratory and Management Team (through the designated contacts) and resolved at the end of Phase II before proceeding to Phase III.

4.3.2 *Criteria for Advancing to Phase III*

If there is excessive variation of ICx data within or among laboratories in the Validation Study, the lead laboratory/testing facility shall assist the Management Team (through the designated contacts) to determine the cause and recommend appropriate actions needed to reduce the variation. The Statement of Work, Test Method Protocols, and SOPs shall be revised if necessary and testing repeated until acceptable proficiency and reproducibility is achieved in all participating laboratories. The Management Team will decide when all laboratories will advance to the next phase of the Validation Study. A teleconference shall be held with all of the appropriate participants of the Validation Study and the Management Team will relate information concerning the advancement of the Validation Study.

4.4 Phase III

4.4.1 *Study Procedures*

Phase III of this Validation Study requires testing 60 blinded/coded chemicals in the same manner as in Phases I and II (i.e., in the *in vitro* cytotoxicity assays in a concentration-response fashion with two - three replicate assays [see Figure 2] after completing a range-finding assay for each chemical). ***The definitive number of replicate assays will be determined based on recommendations of the Management Team and projected costs for doing replicates (see Section 1.4).*** Laboratories will **calculate IC_{20}, IC_{50}, and IC_{80} values in μg/ml,** calculate confidence limits for each value, and report this and all raw data to the Study Management Team through the designated contacts.

4.4.1.1 ***Reporting Data (Phase III)***

Biweekly Reports: Each testing facility will provide a biweekly progress report to the designated contacts of the Management Team (See Addendum I). These reports will be in electronic format (i.e., email with attachments) and will include raw and interim data as the study progresses. The Management Team will in turn provide a weekly progress report addressing the Validation Study as a whole to all of the Testing Facilities. Problems and issues shall be resolved in this manner.

Draft Report: At the conclusion of Phase III, a draft report of the Phase III test chemical data shall be provided by the Study Director to the designated contacts. The draft report (entitled: *In Vitro Validation Study – Phase III: Cytotoxicity Study of 60 Coded Chemicals in Rodent and Human Cell Systems*) must include everything noted in Addendum I Draft Report – Phase III). If the Phase III data does not meet test acceptance criteria, then the Management Team (through the designated contacts) will work with the Test Facility and lead laboratory to identify problems and make corrections as needed. Once unresolved issues have been resolved, the Validation Study will proceed. The draft report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be copied to the designated contacts. Copies of the unaudited Study Workbook pages may be submitted as attachments in hard copy format.

Final Report: Once the draft report provides data that meets test acceptance criteria, then the Project Coordinator shall inform the Study Director to prepare a Quality Assurance audited final report for Phase III. The final report shall be submitted in email and five (5) hard copy formats. File attachments in email shall be submitted in Microsoft® Word (or equivalent) and Excel format and all email correspondence shall be copied to the designated contacts. Copies of the audited Study Workbook pages may be submitted in hard copy format as an attachment to the report. (See Validation Study timelines in **Section 4.1** and Report submission timelines in **Section 4.5**.)

4.4.2 *Criteria for Completion of Phase III*

Phase III will be complete once all of the test chemicals (60) have been tested and the Study Director provides a final report to the designated contacts. The Validation Study will be complete (for all Testing Facilities) after the Study Management Team has received final reports from each Testing Facility and has statistically analyzed all of the data provided by all Testing Facilities.

4.5 Report Submission Timelines

4.5.1 *Draft Reports*

Draft reports for each phase shall be submitted to the Management Team through the designated contacts as per **Section 4.1.1**. The Management Team will respond to the Test Facility within two – four weeks after receipt of the report. If data are acceptable, then the Management Team (through the designated contacts) will instruct the Test Facility to continue to the next phase (teleconference with all participants). If the data do

not meet the criteria and adjustments to the Validation Study are needed, a new timeline will be created and relayed to the Test Facility.

4.5.2 *Final Report*

Once the Management Team (through the designated contacts) declares to a Test Facility that the Validation Study testing phase is complete, then the Test Facility shall provide a final report (electronic and hard copy) for the identified phase of the Validation Study to the Management Team through the designated contacts as per **Section 4.1.1**.

5.0 IDENTIFICATION OF TEST CHEMICALS AND CONTROL SUBSTANCES

The NIEHS/NTP designated contractor (BioReliance) will supply all test chemicals and the positive control to all Testing Facilities. Phase I chemicals will be shipped as a unit as will the Phase II chemicals. Phase III chemicals will be shipped as one unit of 60 chemicals. The Management Team will have all pertinent information for each chemical (e.g., purity, CAS #, supplier, etc.) and will make all decisions concerning any questions about or problems/issues with the chemicals.

5.1 Test Chemicals

5.1.1 *Range of Toxicities*

The chemicals proposed for the Validation Study are representative of a range of toxicities and are relevant with regard to human exposure potential. The test chemicals will represent each of the Globally Harmonized System (GHS) classification groups for rat oral LD50s: ≤ 5 mg/kg, >5 ≤ 50 mg/kg, >50 ≤ 300 mg/kg, >300 ≤ 2000 mg/kg, >2000 ≤ 5000 mg/kg, and >5000 mg/kg (OECD, 2001).

5.1.2 *Receipt of Chemicals*

Test chemicals will be packaged so as to minimize damage during transit and will be shipped to the Testing Facility according to proper regulatory procedures. Chemicals are to be packaged and shipped so as to conceal their identities. The Study Management Team and the Testing Facility shall be notified by the contractor (BioReliance) when the test chemicals are shipped so as to prepare for receipt.

Upon receipt at the facility, the test chemicals shall be stored in appropriate storage conditions as per recommendations provided by the contractor (BioReliance). The Testing Facility shall immediately notify the Project Coordinator and the contractor about receipt of chemicals. The blinded/coded test chemicals as well as a sealed health and safety information package will be shipped to the Safety Officer. The Safety Officer shall retain the package and pass the test chemicals to the Study Director. The package will contain necessary information about the chemical hazards and provide instructions for emergency actions. A disclosure key for identifying test chemicals by code will also be included. At the end of the Validation Study, the Safety Officer shall return the unopened health and safety package to the contractor (BioReliance) who supplied the chemicals (through the designated contacts). If the health and safety package must be opened by the laboratory, the Safety Officer shall immediately notify the designated contacts.

If regulatory transportation requirements dictate that each package must display a list of the chemicals it contains on the outside of the package, the list can be removed by shippers before delivery to the participating Testing Facility. If shippers have not

removed this information, the Safety Officer shall remove it prior to passing the chemicals to the Study Director.

5.1.3 ***Test Chemical Information for the Study Director***
Each test chemical will be accompanied by data sheets giving a minimum of essential information, including color, odor, physical state, weight or volume of sample, specific density for liquid test chemicals, and storage instructions (which will be the same for each chemical). The Study Director shall receive this information.

5.2 Control Materials

5.2.1 *Vehicle Control (VC)*

5.2.1.1 *3T3 NRU Assay (VC)*
Dulbecco's Modification of Eagle's Medium (DMEM) buffered with sodium bicarbonate and supplemented with (final concentrations in DMEM are quoted): 5 % NBCS, 4 mM Glutamine, 100 IU Penicillin, 100 µg/ml Streptomycin. (See specifics in Test Method Protocol) [Note: Vehicle control may also be known as negative control.]

5.2.1.2 *NHK NRU Assay (VC)*
A modified MCDB 153 formulation such as Clonetics® Keratinocyte Basal Medium (KBM®) supplemented with**:** 0.1 ng/ml Human recombinant epidermal growth factor, 5 g/ml Insulin, 0.5 g/ml Hydrocortisone, 50 g/ml Gentamicin, 50 ng/ml Amphotericin B, 0.1 mM Calcium, 2 ml 7.5 mg/ml Bovine pituitary extract. (See specifics in Test Method Protocol) [Note: Vehicle control may also be known as negative control.]

5.2.2 *Positive Control (PC)*
Sodium Lauryl Sulfate ([SLS], CAS # 151-21-3) will be the positive control for both assays. A dose-response assay of SLS dilutions will be run in one plate for each set of test chemical assays. There will be no PC in the test chemical assay plates.

5.3 Inventory of Test Chemicals
The amount of test chemical received, the amount used for specific tests, and the amount remaining shall be documented by the Testing Facility.

5.4 Disposition of Test Chemicals
After the studies are completed, the remaining test chemicals will be returned to the contractor (BioReliance) or appropriately disposed of by the Testing Facility.

5.5 Handling of Test Chemicals
Appropriate routine safety procedures shall be followed in handling the test chemicals unless the contractor (BioReliance) otherwise specifies more cautious procedures. Test Facility personnel shall be instructed to treat all blinded/coded test chemicals as ***very hazardous and potentially carcinogenic*** and to dispose of laboratory wastes as toxic wastes. The health and safety information package provided to the Testing Facility Safety Officer shall be examined by the Testing Facility only in an emergency/need-to-know situation.

5.6 Determination of Purity, Composition, and Stability of Test Chemicals

The contractor (BioReliance) will be responsible for collecting information on the analytical purity, composition, and stability of the test chemicals and the positive control material from manufacturer and supplier documentation. The contractor will provide information on chemical homogeneity in the vehicle via solubility studies. Chemicals shall be stored in an appropriate manner as stated by the contractor.

6.0 TEST SYSTEM

All testing procedures and data analyses shall follow the Test Method Protocols and Statement of Work provided by the Management Team which are based on the NIEHS Publication # 01-4500, *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* (ICCVAM, 2001a).

6.1 Neutral Red Uptake (NRU) Cytotoxicity Assay

6.1.1 *Background*

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay.

Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure, thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

6.1.2 *Sterility of the Test System*

All cell culture applications shall be conducted under aseptic conditions. The test system shall be deemed free of mycoplasmal, fungal, and/or bacterial contamination. The cell suppliers ship cryopreserved cells that have been tested for mycoplasma and are deemed mycoplasma-free. If mycoplasma contamination is suspected, then the Testing Facility shall have the cells tested in an appropriate manner. If mycoplasma is present, all old cells of the specific lot of cells shall be eliminated and new cell stocks shall be prepared or purchased. The presence of bacterial or fungal contamination in the cultures shall be determined by gross visual inspection during and at the conclusion of each assay. If bacterial or fungal contamination is present in the cultures, the Study Director shall determine the course of action.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY – 3T3 NRU ASSAY

7.1 Major Steps in the Performance of the Assay

BALB/c 3T3 cells are seeded into 96-well plates and maintained in culture for 24 hours (h) (~ 1 doubling period) to form a semi-confluent monolayer

⇓

Remove culture medium

⇓

Cells are then exposed to the test chemical in treatment medium over a range of 8 concentrations for 48 h exposure

⇓

Microscopic evaluation of morphological alterations

⇓

Remove treatment medium; wash once with PBS; add Neutral Red (NR) medium; incubate for 3 h.

⇓

Discard NR medium; wash once with PBS; add NR desorbing fixative

⇓

Shake plate for 20 minutes

⇓

Detect NR Absorption at optical density (OD) 540nm

⇓

Perform Neutral Red Uptake data calculations (% viability; calculations of IC_{20}, IC_{50}, and IC_{80} values)

7.2 Procedures for Conducting the Test

All testing procedures and data analyses shall follow the Test Method Protocols and Statement of Work provided by the Management Team and SOPs produced by the Testing Facility. All deviations from Statement of Work or SOPs shall be documented in the Study Workbook. The following abbreviated descriptions of the SOPs provide an overview of the assay, but must not be used in place of the formal SOPs.

7.2.1 *Cell Maintenance and Culture Procedures*

Ampules of cryopreserved BALB/c 3T3 cells are quickly thawed in a 37°C water bath. The cells are resuspended in cell culture medium and transferred to cell culture flasks. The thawed cells are incubated at 37°C in a 90 % humidified 5.0 % CO_2 atmosphere. Cells are passaged two to three times before using them in a cytotoxicity test. A fresh batch of cryopreserved cells should be thawed out approximately every two months (See **Section 7.2.1.1**). This period resembles a sequence of about 18 passages.

The cells are routinely grown as a monolayer in tissue culture grade flasks, at 37°C in a 90 % humidified atmosphere of 5.0 % CO_2 and are examined on a daily basis under a phase contrast microscope.

When cells approach a predetermined confluency, they must be detached from the flask by trypsinization, resuspended in culture medium, and counted using a hemocytometer or cell counter. After determination of cell number, the cell culture must be sub-cultured into other flasks or seeded into 96-well microtiter plates. Stocks of BALB/c 3T3 cells are

prepared in a medium with DMSO as a cryoprotective agent and stored in sterile, freezing tubes in a liquid nitrogen freezer for long-term storage.

7.2.1.1 ***Cryopreserved Lots of Cells***
After the initial establishment of the 3T3 cells in culture from an ampule of cryopreserved cells (from the cell supplier), laboratory personnel shall grow enough cells for cryopreservation in a number of freeze tubes (e.g., 10 – 20 tubes). These tubes will form the stock pool from which subsequent cultures will be established for use in the assays (See **Section 7.2.1**).

7.2.1.2 ***Determination of Cell Doubling Time***
A cell doubling time procedure shall be performed on the initial lot of cells that will be used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined again if there is a change in the lot of cells used. The Test Method Protocol will provide the basic procedures for this determination.

8.0 EXPERIMENTAL DESIGN AND METHODOLOGY – NHK NRU ASSAY

8.1 Major Steps in the Performance of the Assay

NHK cells are seeded into 96-well plates and maintained in culture for 24 – 72 hours (h) to form a semi-confluent (30 – 50 %) monolayer
⇓
Remove culture medium
⇓
Cells are then exposed to the test chemical in treatment medium over a range of 8 concentrations for 48 h exposure
⇓
Microscopic evaluation of morphological alterations
⇓
Remove treatment medium; wash once with PBS; add Neutral Red (NR) medium; incubate for 3 h.
⇓
Discard NR medium; wash once with PBS; add NR desorbing fixative
⇓
Shake plate for 20 minutes
⇓
Detect NR Absorption at optical density (OD) 540nm
⇓
Perform Neutral Red Uptake data calculations (% viability; calculations of IC_{20}, IC_{50}, and IC_{80} values)

8.2 Procedures for Conducting the Test

All testing procedures and data analyses shall follow the Test Method Protocols and Statement of Work provided by the Management Team and SOPs produced by the Testing Facility. All deviations from the Statement of Work or SOPs shall be documented in the Study Workbook. The following abbreviated descriptions of the SOPs provide an overview of the assay, but must not be used in place of the formal SOPs. Information specific to the keratinocytes as provided by the supplier (e.g., Clonetics) shall be considered when preparing SOPs.

8.2.1 ***Cell Maintenance and Culture Procedures***

Ampules of cryopreserved NHK cells are quickly thawed in a 37°C water bath. The cells are resuspended in cell culture medium and transferred to cell culture flasks. The thawed cells are incubated at 37°C in a 90 % humidified 5.0 % CO_2 atmosphere. NHK cells will be sustained in culture through only one passage after establishing cells in culture.

The cells are routinely grown as a monolayer in tissue culture grade flasks, at 37°C in a 90 % humidified atmosphere of 5.0 % CO_2 and are examined on a daily basis under a phase contrast microscope.

When cells approach a predetermined confluency, they must be detached from the flask by trypsinization, resuspended in culture medium, and counted using a hemocytometer or cell counter. After determination of cell number, the cell culture must be seeded into 96-well microtiter plates.

8.2.1.1 ***Determination of Cell Doubling Time***

A cell doubling time procedure shall be performed on the initial lot of cells that will be used in the first cell culture assays of Phase Ia of the Validation Study. The doubling time only needs to be determined again at the initiation of the cells in culture if there is a change in the lot of cells used. The Test Method Protocol will provide the basic procedures for this determination.

9.0 PREPARATION AND DELIVERY OF TEST CHEMICAL

9.1 Preparation of Test Chemical

The test chemical must be freshly prepared immediately prior to use. All chemicals shall be weighed on a calibrated balance (including liquid test chemicals) and added to the appropriate solvent (**Section 9.1.1**). Test chemicals must be at room temperature before dissolving and diluting. Preparation under red or yellow light may be necessary, if rapid photodegradation is likely to occur. The solutions must not be cloudy nor have noticeable precipitate.

The following hierarchy (culture medium, DMSO, ethanol) shall be followed for dissolving the test chemical.

9.1.1. ***Dissolving the Test Chemical***

9.1.1.1 ***Treatment Medium/Routine Culture Medium)***

a) Dissolve test chemical in Treatment Medium [3T3] or Routine Culture Medium [NHK] (See Test Method Protocols).
b) Gently mix. Vortex (1 –2 minutes).
c) If test chemical hasn't dissolved, use sonication (up to five minutes).
d) If sonication doesn't work, then warm solution to 37°C.

9.1.1.2 ***DMSO***

If the test chemical doesn't dissolve in the Treatment Medium/Routine Culture Medium, then follow steps a) through d) in **Section 9.1.1.1** using DMSO instead of Treatment Medium/Routine Culture Medium.

9.1.1.3 ***Ethanol***

If the test chemical doesn't dissolve in DMSO, then follow steps a) through d) in **Section 9.1.1.1** using ethanol instead of DMSO.

9.1.2. *Test Chemical Solubility*

Each test chemical will be prepared such that the highest test concentration in each range finding experiment is 100 mg/ml (100,000 μg/ml) in culture medium (10 mg/ml [10,000 μg/ml] in culture medium if DMSO or ethanol is used as a solvent). If the range finding experiment shows that 100,000 μg/ml is not high enough for the IC_{50} values in the range to meet the acceptance criteria, then higher concentrations will be used for the definitive experiment.

Solubility of the test chemical will be determined by following a modified version of EPA Product Properties Test Guidelines OPPTS 830.7840 (EPA, 1998). (See Test Method Protocols).

Dissolve the test chemical (at 200-fold the desired final concentration in the case of solvents) in an appropriate solvent. The final solvent (i.e., DMSO or ethanol) concentration should be kept at a constant level of no more than 0.5 % (v/v) in the vehicle controls and in all of the eight test concentrations (i.e., each concentration shall have the same amount of solvent). This means the test chemical is dissolved in the vehicle first, and then 1 part of this stock solution is added to 199 parts of sterile pre-warmed (37°C) medium. Check carefully to determine whether the chemical is still dissolved after the transfer from solvent stock solution to medium, and reduce the highest test concentration, if necessary.

The test chemicals selected for the Validation Study will be soluble. If an appropriate concentration cannot be achieved for the range finding experiments, then the Study Director shall contact the Study Management Team through the designated contacts. Prior to initiating any test chemical assay (and after performing solubility tests on the chemicals), the Study Director shall contact the Study Management Team (through the designated contacts) for discussion of the solvent to be used for test chemical application. The Management Team will provide direct guidance to the Study Director as to which solvent will be used for the assay.

9.1.3 *pH of Dilutions*

Measure the pH (using pH paper) of the highest concentration of the test chemical to be tested in the assay. Document the pH and note the color of the medium. Do not adjust the pH of the test chemical solutions.

9.2 Delivery of Test Chemical

The test chemical will be administered by direct addition (pipetting) to the 96-well microtiter plate with a vehicle compatible with the test system. The cells will be exposed to the test chemical for approximately 48 hours..

[Note: The 3T3 and NHK cells in the 96-well plate will have freshculture medium on the cells immediately prior to dosing with the test chemical. Each well will receive a volume of test chemical concentration therefore diluting the concentration by a factor of two.]

9.3 Range Finder Experiment

Test eight concentrations of the test chemical by diluting the stock solution with a constant factor. The initial dilution series will be log dilutions (i.e., 1:10, 1:100, 1:1000, etc.). If this dilution series meets test acceptance criteria (**Section 11.0**), then the range finding experiment dilutions can be used as the actual dilutions in the separate definitive test chemical experiment. If the dilution factor needs to be adjusted for the actual definitive experiment, then follow dilution schemes provided in **Section 9.4.**

9.4 Test Chemical Dilutions

a) A factor of $^{2}\sqrt{10} = 3.16$ could be used for covering a large range:
(e.g., 1 ⇒3.16 ⇒10 ⇒31.6 ⇒100 ⇒316 ⇒1000 ⇒3160 µg/ml).

b) The simplest geometric concentration series (i.e., constant dilution / progression factor) are **dual geometric series** (e.g., a factor of 2). These series have the disadvantage of numerical values that permanently change between logs of the series:
(e.g., *log0*-2, 4, 8; *log1*- 16, 32, 64; *log2*- 128, 256, 512; *log3*- 1024, 2048,).

c) The **decimal geometric series**, first described by Hackenberg and Bartling (1959) for use in toxicological and pharmacological studies, has the advantage that independent experiments with wide or narrow dose factors can be easily compared because they share identical concentrations. Furthermore, under certain circumstances, experiments can even be merged together:

EXAMPLE:

10						**31.6**						**100**
10				**21.5**				**46.4**				**100**
10		**14.7**		**21.5**		**31.6**		**46.4**		**68.1**		**100**
10	**12.1**	**14.7**	**17.8**	**21.5**	**26.1**	**31.6**	**38.3**	**46.4**	**56.2**	**68.1**	**82.5**	**100**

The dosing factor of **3.16** (= $^{2}\sqrt{10}$) divides a log into two equidistant steps, a factor of **2.15** (= $^{3}\sqrt{10}$) divides a decade into three steps. The factor of **1.47** (= $^{6}\sqrt{10}$) divides a log into six equidistant steps, and the factor of **1.21** (= $^{12}\sqrt{10}$) divides the log into 12 steps.

For an easier biometrical evaluation of several related concentration response experiments use decimal geometric concentration series rather than dual geometric series. The technical production of decimal geometric concentration series is simple. An example is given for factor 1.47:

Dilute 1 volume of the highest concentration by adding 0.47 volumes of diluent. After equilibration dilute 1 volume of this solution by adding 0.47 volumes of diluent...(etc.).

d) Determine which test chemical concentration is closest to the IC_{50} value (e.g., 50 % cytotoxicity). Use that value as the central concentration and adjust dilutions higher and lower in equal steps for the definitive assay.

10.0 DATA COLLECTION

10.1 Nature of Data to be Collected

After the test is performed and the NR is desorbed from the cells, measure the absorption of the resulting colored solution at 540 nm in a microtiter spectrophotometric plate reader, using the blanks as a reference. Save raw data in the file format provided by the Study Management

Team (Microsoft® Excel template [Addendum II]) for further analysis of the concentration-response (% viability calculations). Data from the OD analyses will be used for the calculation of IC_{20}, IC_{50}, and IC_{80} values (μg/ml).

10.2 Type of Media Used for Data Storage

Originals of the raw data (the Study Workbook and computer printouts of absorbance readings from the plate reader) and copies of other raw data such as instrument logs shall be collected and archived at the end of the Validation Study (under the direction of the Study Director), according to GLP-compliant procedures. The electronic files of plate reader data and any derived data shall be saved, and a backup of these electronic files shall be produced and maintained. Calculations to convert the raw data to derived data shall be performed using Microsoft® Excel (Addendum II). The derived assay data that are stored electronically shall be periodically copied, and backup files shall be produced and maintained.

10.3 Documentation

Original raw data that shall be collected shall include but are not limited to the following:

- Data recorded in the Study Workbook, which shall consist of all recordings of all activities related to preparing the 3T3 and NHK cultures and test chemicals and performing the NRU assay;
- Computer printouts of absorbance readings from the plate reader spectrophotometer;
- Other data collected as part of GLP compliance
  - Equipment logs
  - Equipment calibration records
  - Test chemical logs
  - Cryogenic freezer inventory logs
  - Cell culture media preparation logs

Addendum IV provides examples of equipment logs.

11.0 ACCEPTANCE CRITERIA FOR NRU ASSAYS

11.1 Test Acceptance Criteria

The test method protocols provide the definitive test acceptance criteria which include a specific mean OD_{540} of all vehicle controls, a set percent difference of the mean OD_{540} between two sets of vehicle controls, and a set range of the IC_{50} for SLS.

The Study Director shall decide if a test meets acceptance criteria and the Study Management Team will make decisions concerning re-testing of test chemicals.

11.2 IC_{50} Acceptance Criteria

The IC_{50} derived from the concentration-response assays shall be based on at least three responses that are ≥ 10 % and ≤ 90 % inhibition of NRU. If this is not the case, and the concentration progression factor can be easily reduced, the experiment shall be rejected and a retest shall be performed with a smaller progression factor.

The raw data output from the plate reader shall be converted into the derived data using Microsoft® Excel (Addendum II). The PC and VC from each assay shall be compared to the acceptable historical ranges as noted. If the assay is found to be valid by these criteria, then the data from that assay is considered to be acceptable. If the PC or VC values are not acceptable,

the assay shall be repeated. Results of all assays, acceptable and failed, shall be forwarded to the designated contacts via the previously identified reports.

12.0 EVALUATION OF TEST RESULTS

12.1 Cell Viability Determination

A calculation of cell viability expressed as NRU is made for each concentration of the test chemical by using the mean NRU of the six replicate values (minimum of four acceptable replicate wells) per test concentration. The Study Director shall determine if any wells do not meet expected performance criteria through visual microscopic evaluation (i.e., experimental conditions within the wells are compromised due to situations such as insufficient cell population, mechanical disruption of the monolayer, etc.). The Study Director shall decide if any of the wells of the plate need to be excluded from data analyses. If a concentration does not have a minimum of four replicate wells, then data from that concentration will not used. The test may still be acceptable if all criteria in **Section 11.1** are met (e.g., the IC_{50} derived from the concentration-response assays is backed by at least three responses ≥ 10 % and ≤ 90 % inhibition of NRU.) If any wells have bacterial or fungal contamination, the entire plate must be repeated.

The cell viability value is compared with the mean NRU of all VC values (provided VC values have met the VC acceptance criteria). Relative cell viability is then expressed as percent of untreated VC. If achievable, the eight concentrations of each chemical tested will span the range of no effect up to total inhibition of cell viability.

12.2 IC_X Determination

The concentration of a test chemical reflecting a 20 %, 50 %, and 80 % inhibition of cell viability (i.e., the IC_{20}, IC_{50}, and IC_{80}) is determined from the concentration-response and shall be done by applying a Hill function to the concentration-response data. It will not be necessary for the Testing Facilities to derive the equation. The Testing Facility shall calculate the IC_{20}, IC_{50}, and IC_{80} values for each test chemical and the confidence limits for each value using statistical software (e.g., GraphPad PRISM® 3.0) specified by the Study Management Team. In addition, the Study Management Team shall provide guidelines for calculating ICx values and confidence limits. The Testing Facility shall report data using at least three (3) significant figures and shall forward the results from each assay to the Study Management Team/biostatistician through the designated contacts in electronic format and hard copy upon completion of all testing. The Study Management Team will be directly responsible for the statistical analyses of the Validation Study data.

Hill function: a four-parameter logistic mathematical model relating the concentration of test chemical to the response being measured in a sigmoidal shape.

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{logIC50} - X)\text{HillSlope}}}$$

where Y= response, X is the logarithm of dose (or concentration), Bottom is the minimum response, Top is the maximum response, logIC50 is logarithm of X at the response midway between Top and Bottom, and HillSlope describes the steepness of the curve.

13.0 DRAFT AND FINAL REPORTS

A draft report shall be submitted to the Management Team through the designated contacts at the completion of each study phase (Ia, Ib, II, III). A Final Report for each phase of the Validation Study shall be prepared by the Testing Facility, signed by the Study Director, and provided to the Management Team through the designated contacts upon acceptance of data provided in the corresponding draft report. The submitted results shall accurately describe all methods used for generation and analysis of the data, provide a complete record of the preparation of test chemicals, and present any relevant data necessary for the assessment of the results (See Addendum I).

14.0 RECORDS AND ARCHIVES

At the end of the Validation Study, the original raw and derived assay data, as well as copies of other raw data not exclusive to this Validation Study (instrument logs, calibration records, facility logs, etc.), shall be submitted to NIEHS/NICEATM for storing and archiving according to the facility's SOP and in compliance with GLP Standards.

Originals of all raw and derived data, or copies where applicable, shall be stored and archived at NIEHS/NICEATM.

Copies of all raw and derived data shall be stored and archived at the participating Testing Facility for at least five years after completion of the Validation Study.

15.0 ALTERATIONS OF THE STATEMENT OF WORK

No changes in the Statement of Work shall be made without the consent of the Management Team. A Statement of Work Amendment detailing any change(s) and the basis for the change(s) shall be approved and prepared by the Study Director, and the amendment shall be signed and dated by the Study Director and the NIEHS representative. The amendment shall be retained with the original Statement of Work.

16.0 REFERENCES

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ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). 2001a. Guidance document on using *in vitro* data to estimate *in vivo* starting doses for acute toxicity NIH publication 01-4500. NIEHS, Research Triangle Park, North Carolina. http://iccvam.niehs.nih.gov/methods/invidocs/guidance/iv_guide.pdf

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NICEATM (The National Toxicology Program [NTP] Interagency Center for the Evaluation of Alternative Toxicological Methods). 2001. Test Method Protocol for the Normal Human Keratinocyte [NHK] Neutral Red Uptake Cytotoxicity Test. A Test for Basal Cytotoxicity for an *In Vitro* Validation Study.

OECD (Organisation for Economic Co-operation and Development). 2001. Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures as Endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals in November 1998, Part 2, p.21. OECD, Paris. http://www.oecd.org/ehs/class/HCL6htm.

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OECD (Organisation for Economic Co-operation and Development). 1992. Environment Monograph No 50, Series on Principles of Good Laboratory Practice and Compliance Monitoring: Number 6, The Application of the GLP Principles to Field Studies. Paris, France: OECD.

Spielmann, H., S. Gerner, S. Kalweit, R. Moog, T. Wirnserberger, K. Krauser, R. Kreiling, H. Kreuzer, N.P. Luepke, H.G. Miltenburger, N. Müller, P. Murmann, W. Pape, B. Siegmund, J. Spengler, W. Steiling, and F.J. Wiebel. 1991. Interlaboratory assessment of alternatives to the Draize eye irritation test in Germany. Toxicol. *In Vitro* 5: 539-542.

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17.0 APPROVAL OF STATEMENT OF WORK

Sponsor Representative	**Date**
Testing Facility Management	**Date**

ADDENDUM I

SUGGESTED REPORT FORMAT

TITLE PAGE

- **Study Title**
  - Draft/Final Report 1: *In Vitro Validation Study – Phase Ia: Development of a Positive Control Database in Rodent and Human Cell Systems*
  - Draft/Final Report 2: *In Vitro Validation Study – Phase Ib: Training Phase for Cytotoxicity Study of Three Coded Chemicals in Rodent and Human Cell Systems*
  - Draft/Final Report 3: *In Vitro Validation Study – Phase II: Qualification Phase for Cytotoxicity Study of Nine Coded Chemicals in Rodent and Human Cell Systems*
  - Draft/Final Report 4: *In Vitro Validation Study – Phase III: Cytotoxicity Study of 60 Coded Chemicals in Rodent and Human Cell Systems*

- ***In Vitro* Assay**
  - Identify the assays: 3T3 NRU and NHK NRU

c) **Test Articles**
  - Draft/Final Report 1: (Phase Ia) identify the positive control chemical
  - Draft/Final Report 2: (Phase Ib) identify the three (3) test chemicals
  - Draft/Final Report 3: (Phase II) identify the nine (9) test chemicals
  - Draft/Final Report 4: (Phase III) identify the sixty (60) test chemicals

- **Authors**
- **Study Completion Date**
- **Testing Facility**
- **Validation Study Number/Identification**

ADDENDUM I (cont.)

SUGGESTED REPORT FORMAT

SIGNATURE PAGE

- **Validation Study Initiation Date**
  Date Protocol was signed by Study Director
- **Initiation Date of Laboratory Studies**
  Actual laboratory start date
- **Validation Study Completion Date**
  Date report signed by Study Director
- **Sponsor Representative**
  The National Institute of Environmental Health Sciences (NIEHS)
  The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

  NICEATM
  79 T.W. Alexander Drive
  Bldg. 4401, MD-EC-17
  3rd Floor, Room 3126
  P.O. Box 12233
  Research Triangle Park, NC 27709
- **Study Management Team Representatives**
  Judy Strickland, Ph.D. (Project Coordinator)
  Michael Paris (Assistant Project Coordinator)
- **Testing Facility**
  Name and address
- **Archive Location**
  Name and address
- **Study Director**
  Name and signature and date
- **Key Personnel**
  Laboratory technicians, QA Director, Safety Officer
- **Facility Management**
  Name
- **Scientific Advisor**
  Name

ADDENDUM I (cont.)

SUGGESTED REPORT FORMAT

TEST CHEMICAL RECEIPT PAGE

Test Chemical Receipt Reporting Template for *In Vitro* Validation Study

Test Facility Test Chemical Identification Number	Sponsor Test Chemical Identification Number	Test Chemical Physical Description	Storage Conditions	Test Chemical Receipt Date	Test Chemical Received By	Comments

ADDENDUM I (cont.)

SUGGESTED REPORT FORMAT

DRAFT/FINAL REPORT 1

In Vitro Validation Study – Phase Ia: Development of a Positive Control Database in Rodent and Human Cell Systems

- **Table of Contents**
- **Objectives:** The reports shall provide specific objectives
- **Description of the Test System Used:** Description of 3T3 NRU assay and the NHK NRU assay
- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for range finding experiments
- **Narrative Description of the Assays:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical will be included in the description. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report as attachments. The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.
- **Statement Signed by the Study Director:** Confirm that the Validation Study was conducted in compliance with GLP (or indicating where the Study deviated from GLP), or for non GLP-compliant laboratories, confirm that the Validation Study adhered to the spirit of GLP. Confirm that the report fully and accurately reflects the raw data generated in the Validation Study.
- **Quality Assurance Statement: (For Final Report only)**
- For GLP-Compliant Laboratories: QA Statement identifying: 1) the phases and data inspected, 2) dates of inspection, and 3) dates findings were reported to the Study Director and Testing Facility management. The QA Statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study.
- For Non GLP-Compliant Laboratories: A statement from the Testing Facility shall be included with the Final Report of Phase III. This statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study and provide assurance that all testing was done in the spirit of GLP.
- **Data Analysis:** (for each NRU assay) calculate the % viability for each positive control chemical concentration (eight concentrations per assay); determine the IC_{50} values for the positive control in each assay; follow guidelines/procedures in Statement of Work and Test Method Protocols.
- **Other Information: (All copies of printouts, documents, and spreadsheets will be noted as exact duplicates of the data.)**
  - Copies of spectrometric plate reader raw data
  - Copies of the completed Microsoft® Excel spreadsheets (Addendum II) used for calculation of cytotoxicity values
  - Copies of data pages showing IC_{50} calculations for the positive control
  - Copy of the protocols
  - Deviations to the protocols, SOPs, and Statement of Work
  - Revisions/amendments to the protocols, SOPs, and Statement of Work

ADDENDUM I (cont.)

SUGGESTED REPORT FORMAT

DRAFT/FINAL REPORT 2

In Vitro Validation Study – Phase Ib: Training Phase for Cytotoxicity Study of Three Coded Chemicals in Rodent and Human Cell Systems

- **Table of Contents**

- **Objectives:** The reports shall provide specific objectives

- **Description of the Test System Used:** Description of 3T3 NRU assay and the NHK NRU assay

- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for range finding experiments

- **Narrative Description of the Assays:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical shall be included in the description. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report as attachments. The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.

- **Statement Signed by the Study Director**: Confirm that the Validation Study was conducted in compliance with GLP (or indicating where the Study deviated from GLP), or for non GLP-compliant laboratories, confirm that the Validation Study adhered to the spirit of GLP. Confirm that the report fully and accurately reflects the raw data generated in the Validation Study.

- **Quality Assurance Statement: (For Final Report only)**
- For GLP-Compliant Laboratories: QA Statement identifying: 1) the phases and data inspected, 2) dates of inspection, and 3) dates findings were reported to the Study Director and Testing Facility management. The QA Statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study.
- For Non GLP-Compliant Laboratories: A statement from the Testing Facility shall be included with the Final Report of Phase III. This statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study and provide assurance that all testing was done in the spirit of GLP.

- **Data Analysis:** (for each assay) calculate the % viability for the positive control and each test chemical concentration (eight concentrations per assay); determine the IC_{50} value for the positive control; determine the IC_{20}, IC_{50}, and IC_{80} values (and confidence limits) for each of the three (3) test chemicals.

- **Other Information: (All copies of printouts, documents, and spreadsheets shall be noted as exact duplicates of the data.)**
  - Copies of spectrometric plate reader raw data
  - Copies of the completed Microsoft® Excel spreadsheets (Addendum II) used for calculation of cytotoxicity values

- Copies of data pages showing IC_{50} calculations for the positive control and the IC_{20}, IC_{50}, and IC_{80} values (and confidence limits) for each test chemical
- Copy of the protocols
- Deviations to the protocols, SOPs, and Statement of Work
- Revisions/amendments to the protocols, SOPs, and Statement of Work

ADDENDUM I (cont.)

SUGGESTED REPORT FORMAT

DRAFT/FINAL REPORT 3

In Vitro Validation Study – Phase II: Qualification Phase for Cytotoxicity Study of Nine Coded Chemicals in Rodent and Human Cell Systems

- **Table of Contents**

- **Objectives:** The reports shall provide specific objectives

- **Description of the Test System Used:** Description of 3T3 NRU assay and the NHK NRU assay

- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for range finding experiments

- **Narrative Description of the Assays:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical will be included in the description. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report as attachments. The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.

- **Statement Signed by the Study Director:** Confirm that the Validation Study was conducted in compliance with GLP (or indicating where the Study deviated from GLP), or for non GLP-compliant laboratories, confirm that the Validation Study adhered to the spirit of GLP. Confirm that the report fully and accurately reflects the raw data generated in the Validation Study.

- **Quality Assurance Statement: (For Final Report only)**
- For GLP-Compliant Laboratories: QA Statement identifying: 1) the phases and data inspected, 2) dates of inspection, and 3) dates findings were reported to the Study Director and Testing Facility management. The QA Statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study.
- For Non GLP-Compliant Laboratories: A statement from the Testing Facility shall be included with the Final Report of Phase III. This statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study and provide assurance that all testing was done in the spirit of GLP.

- **Data Analysis:** (for each assay) calculate the % viability for the positive control and each test chemical concentration (eight concentrations per assay); determine the IC_{50} value for the positive control; determine the IC_{20}, IC_{50}, and IC_{80} values (and confidence limits) for each of the nine (9) test chemicals.

- **Other Information: (All copies of printouts, documents, and spreadsheets shall be noted as exact duplicates of the data.)**
  - Copies of spectrometric plate reader raw data
  - Copies of the completed Microsoft® Excel spreadsheets (Addendum II) used for calculation of cytotoxicity values

- Copies of data pages showing IC_{50} calculations for the positive control and the IC_{20}, IC_{50}, and IC_{80} values (and confidence limits) for each test chemical
- Copy of the protocols
- Deviations to the protocols, SOPs, and Statement of Work
- Revisions/amendments to the protocols, SOPs, and Statement of Work

ADDENDUM I (cont.)

SUGGESTED REPORT FORMAT

DRAFT/FINAL REPORT 4

- ***In Vitro Validation Study – Phase III: Cytotoxicity Study of 60Coded Chemicals in Rodent and Human Cell Systems***
- **Table of Contents**

- **Objectives:** The draft report shall provide specific objectives

- **Description of the Test System Used:** Description of 3T3 NRU assay and the NHK NRU assay

- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for range finding experiments

- **Narrative Description of the Assays:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical shall be included in the description. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report as attachments. The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.

- **Statement Signed by the Study Director:** Confirm that the Validation Study was conducted in compliance with GLP (or indicating where the Study deviated from GLP), or for non GLP-compliant laboratories, confirm that the Validation Study adhered to the spirit of GLP. Confirm that the report fully and accurately reflects the raw data generated in the Validation Study.

- **Quality Assurance Statement: (For Final Report only)**
- For GLP-Compliant Laboratories: QA Statement identifying: 1) the phases and data inspected, 2) dates of inspection, and 3) dates findings were reported to the Study Director and Testing Facility management. The QA Statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study.
- For Non GLP-Compliant Laboratories: A statement from the Testing Facility shall be included with the Final Report of Phase III. This statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Validation Study and provide assurance that all testing was done in the spirit of GLP.

- **Data Analysis:** (for each assay) calculate the % viability for the positive control and each test chemical concentration (eight concentrations per assay); determine the IC_{50} value for the positive control; determine the IC_{20}, IC_{50}, and IC_{80} values (and confidence limits) for each of the 60 (or 30) test chemicals.

- **Other Information: (All copies of printouts, documents, and spreadsheets shall be noted as exact duplicates of the data.)**
  - Copies of spectrometric plate reader raw data
  - Copies of the completed Microsoft® Excel spreadsheets (Addendum II) used for calculation of cytotoxicity values

- Copies of data pages showing IC_{50} calculations for the positive control and the IC_{20}, IC_{50}, and IC_{80} values (and confidence limits) for each test chemical
- Deviations to the protocols, SOPs, and Statement of Work
- Copy of the protocols
- A list of all SOPs used by the laboratory for the assays (SOP title and laboratory identification code)
- The Statement of Work and The Test Method Protocols

ADDENDUM I (cont.)

SUGGESTED REPORT FORMAT

BIWEEKLY REPORTS

Testing Facility:

Chemicals Received:

Chemicals Tested:
3T3 NRU Assay:
NHK NRU Assay:

Solubility Determinations: (solvents used and concentrations obtained)

Range Finding Experiments: (number performed; outcomes)

Successful Tests: (number of tests and calculated IC_{20}, IC_{50}, and IC_{80} values; include Excel® spreadsheets)

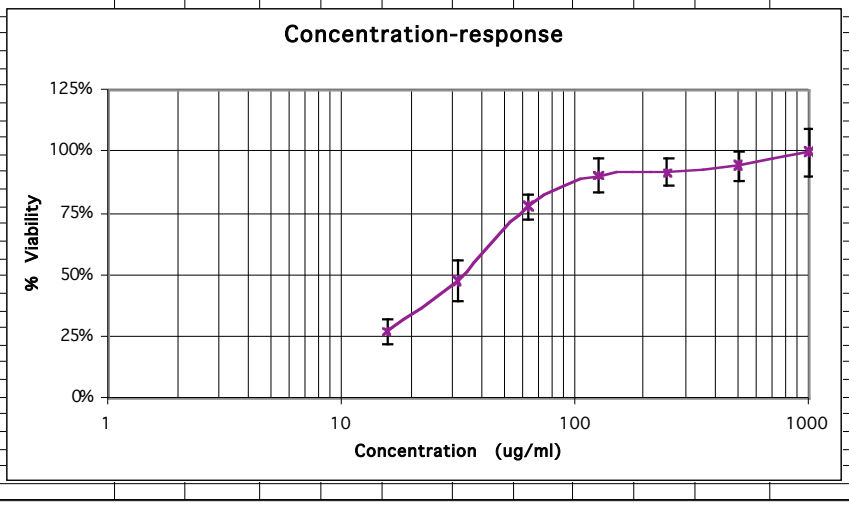
Failed Tests: (number of failed tests and reasons for failure)

Problems Encountered/Resolutions:

Projected Testing Schedule:

ADDENDUM II
EXCEL SPREADSHEET TEMPLATE FOR ASSAY DATA

Test Facility	dfdgs			Cell Line/Type		3T3						
Chemical Code	4567			Vehicle Contol		0.5% DMSO						
Plate ID	qa789											
Date Read	######											
	Plate Map											
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank	VC 1	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	VC2	Blank
C	Blank	VC 1	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	VC2	Blank
D	Blank	VC 1	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	VC2	Blank
E	Blank	VC 1	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	VC2	Blank
F	Blank	VC 1	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	VC2	Blank
G	Blank	VC 1	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	VC2	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
	Plate Data											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.004	0.006	0.036	0.004	0.028	0.019	0.023	0.029	0.012	0.003	0.004	0.011
B	0.009	0.832	0.832	0.855	0.780	0.755	0.693	0.419	0.265	0.052	0.832	0.008
C	0.014	0.894	0.894	0.916	0.884	0.83	0.73	0.368	0.213	0.105	0.935	0.012
D	-0.006	0.918	0.918	0.87	0.914	0.835	0.806	0.450	0.270	0.098	0.918	0.009
E	-0.004	0.915	0.915	0.826	0.903	0.879	0.73	0.591	0.295	0.086	0.915	0.015
F	-0.004	1.098	1.098	0.984	0.814	0.952	0.746	0.436	0.201	0.151	1.098	0.014
G	0.016	0.948	0.948	0.845	0.842	0.832	0.663	0.431	0.319	0.09	0.89	0.015
H	-0.001	-0.006	0.017	-0.005	0.009	0.004	0.002	0.014	-0.013	-0.003	-0.061	0.012
Mean blank OD	0.0068											
	Corrected OD = OD- mean blank OD											
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.825	0.825	0.848	0.773	0.748	0.686	0.412	0.258	0.045	0.825	
C		0.887	0.887	0.909	0.877	0.823	0.723	0.361	0.206	0.098	0.928	
D		0.911	0.911	0.863	0.907	0.828	0.799	0.443	0.263	0.091	0.911	
E		0.908	0.908	0.819	0.896	0.872	0.723	0.584	0.288	0.079	0.908	
F		1.091	1.091	0.977	0.807	0.945	0.739	0.429	0.194	0.144	1.091	
G		0.941	0.941	0.838	0.835	0.825	0.656	0.420	0.312	0.083	0.883	
H												
	blanks	**Vehicle Control 1**	**Conc 1**	**Conc 2**	**Conc 3**	**Conc 4**	**Conc 5**	**Conc 6**	**Conc 7**	**Conc 8**	**Vehicle Control 2**	**blanks**
Concentration [µg/ml]		0	1000	500	250	125	62.5	31.25	15.625	7.2	0	
Mean Corrected OD		0.927	0.927	0.876	0.849	0.840	0.721	0.442	0.254	0.090	0.925	
SD of Mean OD	0.0158	0.089	0.089	0.058	0.053	0.065	0.049	0.075	0.046	0.032	0.089	
Corrected Mean ------	**All VCs**	0.926										
% Viability = Mean Corrected OD/Mean Corrected VC	100%	100%	100%	95%	92%	91%	78%	48%	27%	10%	100%	
SD (% Viability) = SD OD/Mean OD All VCs		10%	10%	6%	6%	7%	5%	8%	5%	3%	10%	
%CV = SD/mean OD*100	9%	9.6%	9.6%	6.6%	6.3%	7.7%	6.8%	17.0%	18.1%	35.7%	9.7%	
Mean Vehicle Control - VC1 (%)		-0.15%										
Mean Vehicle Control - VC2 (%)		0.15%										



ADDENDUM III

SUGGESTED STANDARD TEST REPORTING TEMPLATE FOR *IN VITRO* VALIDATION STUDY WORKBOOK

TEST CHEMICAL									
Test Facility				96-Well Plate ID _____					
Chemical Code				Experiment ID _____					
PREPARATION OF TEST CHEMICAL									
Solvent _____Culture Medium _____DMSO _____Ethanol									
Highest Percent Solvent (v/v) in Dilutions _____%				Highest Concentration Tested_____µg/ml					
Aids Used to Dissolve _____Vortex _____Ultra-sonicaton _____Heat to 37°C									
pH (Highest Test Concentration)_____ Media color of test chemical solutions:									
Concentration Series (µg/ml)	C1	C2	C3	C4	C5	C6	C7	C8	
Positive Control [SLS]_____µg/ml				Vehicle Control _____% solvent					
CELL LINE/TYPE									
Name		Supplier		From Cell Lot No._____					
Total Passage No.		No. of Passages after Thawing		From:____ proliferating ____frozen					
CELL CULTURE CONDITIONS									
Name of Medium		Supplier/ID		Lot No./Lab I.D.					
Name of Serum		Supplier/ID		Lot No.					
Serum Concentration		During Growth: _____%		During Exposure: _____%					
TEST ACCEPTANCE CRITERIA									
VC: Mean Absolute OD_{540}				Mean OD =_____		____Accept		____Reject	
VC: Difference Between Col.2 and Col. 10				Difference =_____%		____Accept		____Reject	
PC: IC_{50} of Concurrent SLS Test				IC_{50} =_____µg/ml		____Accept		____Reject	
TIMELINE									
Assay Start Date (cells to plates)		Application of Test Chemical Date		NRU/OD_{540} Measurement Date					

ADDENDUM IV

EXAMPLES OF LABORATORY EQUIPMENT LOGS

INCUBATOR

INCUBATOR I.D._____

MONTH:_____ YEAR:_____ LOCATION:_____

DATE	TIME	INITIALS	CO_2 %	RH %	TEMP. (^{O}C.)	CO_2 TANK (PSI)	CO_2 TANK (NEW)
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							

FYRITE CHECK OF CO2:

ADDITION OF WATER:

TOTAL INCUBATOR DISINFECTION:

ADDENDUM IV (cont.)

EXAMPLES OF LABORATORY EQUIPMENT LOGS

pH METER

pH METER I.D._____

MONTH:_____ YEAR:_____ LOCATION:_____

DATE	TIME	INITIALS	pH STD. 7.00	pH STD. 10.00	pH STD. 4.00	pH STD. 7.40	SLOPE
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							
pH STANDARDS		7.00	10.00	4.00	7.40		
SUPPLIER/I.D.							
LOT NUMBER							
EXPIRATION DATE							

NOTES:

ADDENDUM IV (cont.)

EXAMPLES OF LABORATORY EQUIPMENT LOGS

MONTH_____ YEAR_____			RERIGERATOR I.D. NUMBER_____ LOCATION_____	FREEZER I.D. NUMBER_____ LOCATION_____
DATE	**TIME**	**INITIALS**	**TEMPERATURE (^{O}C.)**	**TEMPERATURE (^{O}C.)**
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
NOTES:				

ADDENDUM V

SUGGESTED STANDARD TEST REPORTING TEMPLATE FOR STUDY WORKBOOK

[1]SOLUBILITY TESTING
Test Chemicals for the *In Vitro* Validation Study

Study No._____

Test Chemical_____ Test Chemical Code_____ CAS #_____

Physical Description_____ Liquid Density_____

Solubility Determined by_____
Date_____

Solvent	Amount of Test Chemical	Volume Added	Total Volume	pH and medium color	Vortex (V) Sonication (S) Heating-37°C (H)	Comments
Treatment Medium (3T3 NRU)		0.1ml				
		0.5ml				
		1.0ml				
Routine Culture Medium (NHK NRU)		0.1ml				
		0.5ml				
		1.0ml				
DMSO		0.1ml				
Ethanol		0.1ml				

Reference Color of Treatment Medium_____

Reference Color of Routine Culture Medium_____

Balance I.D._____
Treatment Medium and Routine Culture Medium: minimum concentration of 100mg/ml.
DMSO and Ethanol: minimum concentration of 1000mg/ml.

[1] Adaptation of Institute of In Vitro Sciences (IIVS) form – 350 [2/2002]

ADDENDUM VI

GANTT CHART OF STUDY TIMELINES AND DELIVERABLES

In Vitro Cytotoxicity Validation Study			MARCH 2002	APRIL 2002	MAY 2002	JUNE 2002	JULY 2002	AUGUST 2002	SEPTEMBER 2002	OCTOBER 2002	NOVEMBER 2002	DECEMBER 2002	JANUARY 2003	FEBRUARY 2003	MARCH 2003	APRIL 2003	MAY 2003	JUNE 2003	JULY 2003	AUGUST 2003	SEPTEMBER 2003	OCTOBER 2003	NOVEMBER 2003	DECEMBER 2003
TASK	**START**	**FINISH**																						
Statement of Work Issued by NIEHS		3/29/02	29																					
Proposal received		5/10/02			10																			
Contracts Awarded		6/29/02				29																		
Submission of Study Protocol, CVs of Key Personnel, and SOPs		7/12/02					12																	
Phase Ia Positive control	7/29/02	8/26/02					July 29 Aug. 26																	
Phase Ia Draft Report		9/9/02					Sept. 9																	
Phase Ia Final Report		11/11/02					Nov. 11																	
Phase Ib 3 chemicals	9/26/02	10/29/02							Sept. 26 Oct. 29															
Phase Ib Draft Report		11-11/02							Nov. 11															
Phase Ib Final Report		1/13/03							Jan. 13															
Phase II 9 chemicals	12/2/02	2/10/03										Dec. 2 Feb. 10												
Phase II Draft Report		2/25/03										Feb. 25												
Phase II Final Report		4/28/03										April 28												
Phase III 60 chemicals	3/26/03	12/9/03													Mar. 26 Dec. 9									
Phase III Draft Report		10/24/03													Oct. 24									
Phase III Final Report		12/9/03													Dec. 9									
Biweekly Reports	7/10/02	12/9/03				July 10, 2002 – December 9, 2003																		

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Appendix G2

Procedures for Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals for a Validation Study for *In Vitro* Basal Cytotoxicity Testing

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NOTE: This Statement of Work shall not be cited, quoted, nor distributed to any Testing Facility participating in the In Vitro Validation Study. Confidentiality must be maintained to ensure that test chemicals remain unknown to the Testing Facilities.

STATEMENT OF WORK

Procedures for Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals for a Validation Study for *In Vitro* Basal Cytotoxicity Testing

April 26, 2002
Revision 1: May 8, 2002
Revision 2: June 21, 2002
Revision 3: September 17, 2002
Revision 4: October 11, 2002
Prepared by

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences (NIEHS)
National Institutes of Health (NIH)
U.S. Public Health Service
Department of Health and Human Services

Table of Contents

NOTE: Revisions in this document are identified by footnotes, strike-out text (i.e., ~~deleted~~), and added verbiage (i.e., *italicized text*).

[3] Revised 9/17/02

STATEMENT OF WORK

Procedures for Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals for a Validation Study for *In Vitro* Basal Cytotoxicity Testing

1.0 PROJECT OBJECTIVES AND GENERAL REQUIREMENTS

1.1 Project Objectives

This Statement of Work outlines and supports the procedures that the Contractor will initiate for the acquisition, preparation, solubility testing, and distribution of the test chemicals needed to perform two *in vitro* basal cytotoxicity assays (the BALB/c 3T3 Neutral Red Uptake [NRU] assay and the Normal Human Keratinocyte [NHK] Neutral Red Uptake [NRU] assay) for a multi-laboratory Validation Study. These assays, recommended in *Guidance Document On Using In Vitro Data To Estimate In Vivo Starting Doses For Acute Toxicity* (ICCVAM, 2001), use mammalian cell culture techniques to assess the basal cytotoxicity of chemicals.

A primary goal of this Validation Study is to evaluate the usefulness of the BALB/c 3T3 Neutral Red Uptake (NRU) and the Normal Human Keratinocyte (NHK) NRU assays for reducing and refining animal use for acute oral toxicity determinations of chemicals by predicting starting doses for *in vivo* rodent acute lethality assays.

The proposed Validation Study will determine IC_{20}, IC_{50}, and IC_{80} values for a test set of 72 chemicals with varying degrees of toxicity. This set of chemicals was selected separate and prior to this Statement of Work by the Study Management Team. The basis for selection of this test set is discussed in the Study Design document prepared by the Study Management Team.

The Contractor shall perform the following activities:

- Acquire 73 high quality and high purity (99% or greater when economically feasible) chemicals from reputable commercial sources
- Perform solubility tests on all chemicals using solvents and procedures that have been recommended to the test laboratories
- Repackage chemicals into multiple smaller units
- Code chemicals with a unique identification number so that chemicals can be provided to testing laboratories in a blinded fashion
- Distribute chemicals and health and safety information to the Testing Facilities
- Provide draft and final reports of these activities.

1.2 Response to the Statement of Work

Proposals submitted in response to this Statement of Work shall include:

a) A Work Plan
b) A timetable for project milestones
c) A cost estimate based on chemical acquisition, performance of solubility tests for all test chemicals, chemical coding, repackaging, and distribution to two U. S labs and one U. K. lab.

1.2.1 *General Capabilities*

The Contractor shall be capable of performing the following:

a) Prepare/provide Standard Operating Procedures (SOPs) for the performance of the activities outlined in **Section 1.1** (see **Section 1.4** – Definitions - SOPs)

b) Perform all aspects of the Test Chemical Preparation in accordance with Good Laboratory Practices (GLP).
c) Adhere to this Statement of Work throughout the Validation Study.

1.3 Guidelines

The Project Officer and/or her/his representatives (e.g., Study Management Team) may inspect and audit the Contractor to ensure that the Project Officer's minimum requirements and guidelines are being followed.

1.4 Definitions

Blinded/Coded Chemicals: Test chemicals supplied to the Testing Facilities that are coded and distributed by the Contractor such that only the Project Officer, Management Team, and the Contractor have knowledge of the contents of each test chemical vessel. The test chemicals will be purchased, aliquoted, coded, and distributed by the Contractor under the guidance of the NIEHS/NTP Project Officer and the Management Team.

Contractor: Facility that will initiate the acquisition, preparation, solubility testing, and distribution of the test chemicals needed to perform two *in vitro* basal cytotoxicity assays for a multi-laboratory *in vitro* Validation Study.

Good Laboratory Practices (GLPs): Regulations governing the conduct, procedures, and operations of toxicology laboratories; regulations to assure the quality and integrity of the data and to address such matters as organization and personnel, facilities, equipment, facility operations, test chemicals, and study protocol (Statement of Work) and conduct (U.S. Food and Drug Administration, Title 21 CFR Part 58; Environmental Protection Agency, Title 40 CFR Part 160).

Standard Operating Procedures (SOPs): Written documents that describe, in great detail, the routine procedures to be followed for a specific operation, analysis, or action; consistent use of an approved SOP ensures conformance with organizational practices, reduced work effort, reduction in error occurrences, and improved data comparability, credibility, and defensibility; SOPs also serve as resources for training and for ready reference and documentation of proper procedures;

Statement of Work: A description of test chemical preparation required for the *in vitro* Validation Study; defines all phases of the Validation Study and the purpose of the procedures; provides the details of test chemical acquisition, preparation, solubility testing, and distribution; provides guidance for the preparation of reports

Testing Facility: A laboratory that has been designated to participate in the *In Vitro* Validation Study; facilities identified in **Section 2.2.4**.

2.0 ORGANIZATION

2.1 Validation Study Sponsors

- National Institute of Environmental Health Sciences (NIEHS)
- The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- U.S. Environmental Protection Agency (U.S. EPA)
- The European Centre for the Validation of Alternative Methods (ECVAM).

2.2 Management Team

2.2.1 *Project Management and Chemical Distribution Team*

Ms. Molly Vallant (NIEHS) – NIEHS Project Officer for BioReliance, Inc.
NIEHS
MD E1-03
P.O. BOX 12233
RTP, NC 27709

Dr. Martin L. Wenk (BioReliance, Inc.) – Chemical acquisition, preparation, solubility testing, and distribution
BioReliance Corporation
14920 Broschart Road
Rockville, Maryland 20850-3349

2.2.2 *Contract Management*

Ms. Jackie Osgood (NIEHS) – Contracting Officer
Mr. Don Gula (NIEHS) – Contracting Officer

2.2.3 *Study Management Team*

2.2.3.1 ***NIEHS/NICEATM***

Dr. William S. Stokes (NICEATM/NIEHS) – Co-chair – Study Management Team
Dr. Judy Strickland (NICEATM/ILS) – Project Coordinator
Mr. Michael Paris (NICEATM/ILS) – Assistant Project Coordinator
Dr. Ray Tice (NICEATM/ILS) – Technical Advisor

NICEATM
79 T.W. Alexander Drive
Bldg. 4401, MD-EC-17
3rd Floor, Room 3126
P.O. Box 12233
Research Triangle Park, NC 27709

2.2.3.2 ***ECVAM***

Professor Michael Balls – Co-chair – Study Management Team
Dr. Silvia Casati
Dr. Andrew Worth

European Commission
Joint Research Centre
Institute for Health and Consumer Protection
Management Support Unit - TP 202
I-21020 Ispra (VA) - Italy

2.2.4 *Testing Facilities*

XXX, Safety Officer
Institute for *In Vitro* Sciences (IIVS)
21 Firstfield Road
Suite 220
Gaithersburg, MD 20878

Bill Cappuccio, Safety Officer
5183 Blackhawk Rd
E3330/Room 278
Aberdeen Proving Ground-EA, MD 21010
410-436-7462

Rodger Dainty, Safety Officer
School of Biomedical Sciences
University of Nottingham Medical School
Queen's Medical Centre
Nottingham, NG7 2UH UK

3.0 CONTRACTOR AND KEY PERSONNEL

3.1 Contractor

The Contractor shall have competence in chemical acquisition, preparation, solubility testing, and distribution and shall provide competent personnel, adequate facilities, equipment, supplies, proper health and safety guidelines, and satisfactory quality assurance procedures.

3.1.1 *Personnel*

3.1.1.1 ***Facility Management***

The facility management is responsible for establishing scientific guidelines and procedures, training and supervision of professional and technical staff, and evaluation of results and performance within their discipline area relative to the Project Officer's stated requirements. The manager must maintain records of the qualifications, training and experience, and a job description for each professional and technical individual involved in test chemical acquisition, preparation, solubility testing, and distribution.

3.1.1.2 ***Study Director***

A scientist or other professional of appropriate education, training, and experience in chemical acquisition, preparation, solubility testing, and distribution, or combination thereof, shall be the Study Director. The Study Director has the overall responsibility for the technical conduct of chemical acquisition, preparation, solubility testing, and distribution for the Validation Study (e.g., GLP adherence) and shall be responsible for determining test acceptance. The Study Director shall be responsible for providing SOPs that incorporate pertinent information obtained from the Statement of Work. Other duties include the interpretation and analysis of test chemical solubility data, documentation of all study aspects (including maintenance of a Study Workbook), and production of all draft and final written reports.

3.1.1.3 ***Quality Assurance (QA) Director***

The Quality Assurance Director shall **monitor** all tasks and assure conformance with GLP requirements (i.e., facilities, equipment, personnel, methods, practices, records, controls, transference of data into software, SOPs). Quality Assurance Director or unit can be any person or organizational element, except the Study Director, designated by Contractor management to perform the duties relating to quality assurance of the studies and tasks. The Quality Assurance duties are not a substitute for the Study Director duties.

3.1.1.4 ***Scientific Advisor(s)***
Scientists or other professionals of appropriate education, training, and experience in chemical acquisition, preparation, solubility testing, and distribution who provide scientific guidance to the Study Director and other laboratory personnel.

3.1.1.5 ***Laboratory Technician(s)***
Each individual engaged in the conduct of or responsible for the supervision of a study shall have education, training, and experience, or combination thereof, to enable that individual to perform the assigned duties. The individuals must be trained in GLP requirements and technical ability must be documented as per GLP requirements.

3.1.1.6 ***Safety Officer***
The Contractor shall designate a Safety Officer who will provide a sealed health and safety information package that will accompany the test chemicals to the Test Facilities. A duplicate package will be provided to the Project Officer and Management Team.

3.1.2 ***Facilities, Equipment, and Supplies***

3.1.2.1 ***Laboratory***
The Contractor must provide a designated laboratory/area to ensure that test chemical preparation and solubility testing can be performed under clean conditions. Potential for cross-contamination of chemicals should be minimal.

3.1.2.2 ***Equipment***
The Contractor must provide at a minimum the following equipment:
a) Water bath (37^{o}C)
b) Sonication unit
c) Vortex unit
d) Pippettors (micropipettors,)
e) Computer (for data transformation and analysis)
f) Balance
g) pH meter

All equipment maintenance and calibration shall be routinely performed and documented as per GLP guidelines and Contractor procedures

3.1.2.3 ***Supplies***
All cell culture reagents must be labeled so as to indicate source, identity, concentration, stability, preparation and expiration dates, and storage conditions.
a) Dulbecco's Modification of Eagle's Medium (DMEM) without L-Glutamine; should have Hanks' salts and high glucose [4.5gm/l] (e.g., ICN-Flow Cat. No. 12-332-54)
b) L-Glutamine 200 mM (e.g., ICN-Flow # 16-801-49)
c) New Born Calf Serum (NBCS) (e.g., Biochrom # SO 125)
d) Dimethyl sulfoxide (DMSO), U.S.P. analytical grade. DMSO shall be stored under nitrogen at –20^{o}C.
e) Ethanol (ETOH), U.S.P. analytical grade (100%, non-denatured)

f) Keratinocyte Basal Medium without Ca^{++} (KBM®, Clonetics CC-3104) that is completed by adding the *KBM® SingleQuots®* ~~Bullet Kit®~~[2] (Clonetics CC-4131) to achieve the proper concentrations of epidermal growth factor, insulin, hydrocortisone, antimicrobial agents, bovine pituitary extract, and calcium (e.g., Clonetics Calcium SingleQuots®, CC-4202)*.

g) Penicillin/streptomycin solution (e.g. ICN-Flow # 16-700-49)

* BioWhittaker, 8830 Biggs Ford Road, Walkersville, MD 21793 (http://www.cambrex.com/subsidiaries/s%2Dbw%5Finc/s%2Dbiowhittaker%2Dinc%2Dcontact2.htm)

3.1.3 *Health and Safety*

The Contractor shall conform to all local, state, and federal statutes in effect at the time of this study.

3.1.4 *Quality Assurance*

The Contractor shall conduct the acquisition, preparation, solubility testing, and distribution of test chemicals in compliance with Good Laboratory Practice (GLP) Standards (U.S. Food and Drug Administration, Title 21 CFR Part 58; Environmental Protection Agency, Title 40 CFR Part 160). The appropriate QA unit (as per GLPs) shall audit the procedures and final report.

The Final Report shall be audited by the Quality Assurance unit of the Contractor for GLP compliance and a QA Statement shall be provided by the Contractor. The Final Report shall identify: 1) the phases and data inspected, 2) dates of inspection, and 3) dates findings were reported to the Study Director and Contractor management. The QA Statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the study.

4.0 TEST PHASES AND SCHEDULE

4.1 Study Timeline

The following timeline is for the **laboratory testing aspect** of the *In Vitro* Validation Study. The Contractor shall provide the required chemicals in a timely fashion so that each phase of the study can start on the appointed date.

[2] Revised 6/21/02

TASK	WEEK	ESTIMATED DATE
Statement of Work issued by NIEHS to the Testing Facility	0	March 29, 2002
Response /Proposal received from the Testing Facility	6	May 10, 2002
~~Award of Contracts~~[2]	~~9~~[2]	~~May 29, 2002~~[2]
Submission of *Study Protocol, CVs of Key Personnel,* SOPs[2]	11	June 12, 2002
Award of Contracts[2]	*13*[2]	*June 28, 2002*[2]
Start Testing – Phase I (Phase Ia)	~~14~~*18*[2]	July ~~1~~*29*[2], 2002
End Phase Ia	~~18~~*22*[2]	~~July~~ *August* 2~~6~~9[2], 2002
Begin Phase Ib	~~22~~*26*[2]	~~August~~ *September* ~~29~~*26*[2], 2002
End Phase Ib	~~27~~*31*[2]	October ~~1~~*29*[2], 2002
Begin Phase II	~~31~~*36*[2]	~~October~~ *December* 2~~9~~[2], 2002
End Phase II	~~42~~*46*[2]	~~January~~ *February* ~~13~~*10*[2], 2003
Begin Phase III	~~48~~*52*[2]	~~February~~ *March*[2] 26, 2003
Final Report (Phase III) to SMT	~~85~~*89*[2]	~~November~~ *December* ~~11~~*9*[2], 2003

4.2 Deliverables

The following schedule of deliverables is for **the acquisition, preparation, solubility testing and distribution of test chemicals**.

	ESTIMATED DUE DATES (to Project Officer)			
Submission of SOPs for **Section 1.1** activities	**Week 11**		**June 12, 2002**	
REPORTS	**PHASE Ia**	**PHASE Ib**	**PHASE II**	**PHASE III**
Biweekly Reports	a	a	a	a
Draft Phase Reports	Week ~~13~~*17* ~~June~~ *July* 2~~4~~6[2], 2002 [b]		Week ~~29~~*33* ~~Oct~~*Nov.* ~~1~~6*13*[2], 2002 [b]	Week ~~44~~*48* ~~Jan~~*Feb.* ~~29~~*26*[2], 2003 [b]
Draft Final Report (all phases combined)	Week ~~48~~*52* *March*~~Feb.~~[2] 26, 2003 [c]			
Final Report (all phases combined)	Week ~~50~~*54* ~~March~~ *April* 9~~12~~[2], 2003 [d]			

a Biweekly reports shall begin at the time of implementation of the contracts and continue until the final report is submitted.

b Draft Phase Reports shall be submitted to the Project Officer no later than the dates provided (at least two weeks before shipment of chemicals to the Test Facilities).

c Draft Final Report shall be submitted to the Project Officer no later than the date provided (at the most one month after final shipment of chemicals to the Test Facilities).

d Final Report shall be submitted to the Project Officer no later than the date provided (at the most one month after the Project Officer receives the Draft Final Report.

[2] Revised 6/21/02

The following schedule is for the **distribution of test chemicals** to the Testing Facilities.

	ESTIMATED DUE DATES (to Testing Facilities)			
CHEMICAL SHIPPING TO TESTING FACILITIES[a]	**PHASE Ia**	**PHASE Ib**	**PHASE II**	**PHASE III**
Positive Control (SLS)	Before July ~~1~~*29*[2], 2002	---	---	---
Phase Ib (3 chemicals)	---	Before ~~August~~ *September* ~~29~~*26*[2], 2002	---	---
Phase II (9 chemicals)	---	---	Before ~~October~~ *December* 2~~9~~[2], 2002	---
Phase III (60 chemicals)	---	---	---	Before ~~February~~ *March*[1] 26, 2003

a Dates for chemical shipments are to ensure that the Testing Facilities receive Test Chemicals prior to the start dates of each lab testing phase. Phase III chemicals shall be shipped as one group of 60 chemicals. Chemicals for each phase are identified in Addendum IV.

4.3 *In Vitro* Validation Study Phases

Phase I: The training phase for laboratory personnel. This phase includes developing a positive control database (Phase Ia) and testing three unknown chemicals (Phase Ib).
Phase II: The qualification phase. This phase requires testing nine blinded/coded chemicals in the same *in vitro* cytotoxicity assays and in the same concentration-response fashion as in Phase Ib.
Phase III: Testing 60 blinded/coded chemicals in the same manner as in Phases I and II.

4.4 Report Submission Timelines

4.4.1 *Draft Reports*

Draft reports for each phase shall be submitted to the Project Officer as per **Section 4.2**.

4.4.2 *Final Report*

The Final report shall be submitted to the Project Officer as per **Section 4.2**.

5.0 ACQUISITION, PREPARATION, AND DISTRIBUTION OF TEST CHEMICALS

5.1 Test Chemicals

5.1.1 *Range of Toxicities*

The chemicals proposed for the Validation Study are representative of a range of toxicities and are relevant with regard to human exposure potential. The test chemicals

[2] Revised 6/21/02

will represent each of the Globally Harmonized System (GHS) classification groups for rat oral LD50s: ≤ 5 mg/kg, >5 ≤ 50 mg/kg, >50 ≤ 300 mg/kg, >300 ≤ 2000 mg/kg, >2000 ≤ 5000 mg/kg, and >5000 mg/kg (OECD, 2001). Addenda III and IV provide the list of test chemicals for the *In Vitro* Validation Study.

5.1.2 ***Procurement of Test Chemicals***
The Contractor shall purchase 73 chemicals specified in Addenda III and IV (72 "test chemicals" and one "positive control") from commercial manufacturers. Chemical purity shall be 99% or greater when economically feasible. Chemical information from the manufacturers shall be collected as specified in **Section 7.1.2** and reported as indicated in Addendum I. Chemicals shall be stored as recommended by the manufacturer.

5.1.3 ***Dispensing Chemicals***
While preparing the purchased chemicals for distribution to the Testing Facilities, only one bulk substance shall be dispensed at any time. All test samples shall be sealed and labeled before dispensing the next substance. Once test samples have been dispensed into aliquots, they shall be returned to appropriate storage conditions until they are dispatched.

During dispensing, all test chemicals, with the exception of the positive control, will be randomly blinded/coded so that testing by the Testing Facilities will be conducted on chemicals with a masked identity. Each chemical shall have a code that is unique for each Testing Facility (i.e., no chemical shall have the same code in any Testing Facility). The Contractor shall dispense 4 g of test chemical/Testing Facility (see Addendum V for assumptions used to determine the amount of chemical/Testing Facility) into clean, sterile containers, and assign unique code identifiers, and archive two additional samples. About 100 g of the positive control shall be distributed to each lab and one additional sample shall be archived.

5.1.4 ***Shipment of Chemicals***
After dispensing and labeling chemical aliquots with unique codes, the Contractor shall ship a set of the test chemicals, including the positive control, to the each of three Testing Facilities. Two Facilities will be in the US and one will be in the United Kingdom. The Contractor will package test chemicals so as to minimize damage during transit and will ship them to each Testing Facility according to proper regulatory procedures. Except for the positive control in Phase Ia, chemicals are to be packaged and shipped so as to conceal their identities. Test chemicals shall be shipped under conditions that will preserve the integrity of the chemicals. The Contractor shall notify the Testing Facilities (and the Project Officer) when the test chemicals are shipped so as to prepare for receipt.

The Contractor will retain the archived chemicals, which may be required for retesting or purity analysis, until the completion of the Validation Study.

5.1.4.1 ***Distribution Phases***
Phase Ia: For Phase I, the positive control chemical identified in Addendum III shall be distributed to all three Testing Facilities.
Phase Ia: For Phase Ib, the three (3) blinded/coded chemicals identified in Addendum III shall be distributed to all three Testing Facilities.
Phase II: Nine (9) blinded/coded chemicals identified in Addendum III shall be distributed to all three Testing Facilities.

Phase III: Sixty (60) blinded/coded chemicals identified in Addendum III shall be distributed to the Test Facilities. Chemicals will be shipped –as a group of 60 chemicals.

5.1.5 ***Receipt of Chemicals by the Testing Facilities***

With the exception of the positive control shipment, which shall be shipped directly to the Study Director, the chemical shipments shall be addressed to the Testing Facility Safety Officers and accompanied by a sealed information packet containing the appropriate health and safety procedures for use (i.e., Material Safety Data Sheets (MSDS) or equivalent documentation with proper protection, procedures for accidental ingestion or contact with skin or eyes, and procedures for containing and recovering spills) and a disclosure key for identifying test chemicals by code. The shipment shall include instructions for the Testing Facility Safety Officer to:

1) Immediately notify the Contractor and Study Project Coordinator upon receipt of chemicals,
2) Retain the health and safety package and pass the test chemicals to the Study Director without revealing the identities of the test chemicals,
3) Notify the Management Team if Test Facility personnel open the health and safety packet at any time during the Validation Study, and
4) Return the unopened health and safety package to the Contractor after testing is complete. The Contractor shall immediately notify the Project Officer regarding chemical receipt.

If regulatory transportation requirements dictate that each package must display a list of the chemicals it contains on the outside of the package, the Contractor shall direct the Testing Facility Safety Officer to remove it prior to passing the chemicals to the Study Director.

5.1.6 ***Test Chemical Information for the Study Director***

The Contractor shall supply, with each test chemical, data sheets giving a minimum of essential information, including color, odor, physical state, weight or volume of sample, specific density for liquid test chemicals, and storage instructions. The Study Director shall receive this information from the Safety Officer.

5.2 Handling of Test Chemicals

Appropriate routine safety procedures shall be followed in handling the test chemicals. The Contractor shall include instructions to the Test Facilities to treat all blinded/coded test chemicals as ***very hazardous and potentially carcinogenic.*** After the studies are completed, the remaining test chemicals will be returned by the Testing Facilities to the Contractor.

5.3 Determination of Purity, Composition, and Stability of Test Chemicals

As indicated in **Section 7.1.2**, the Contractor will be directly responsible for collecting information (from manufacturer and supplier documentation) on the analytical purity, composition, and stability of the test chemicals and the positive control material, and their homogeneity (via Contractor solubility studies) in the vehicle.

6.0 SOLUBILITY DETERMINATION OF TEST CHEMICALS

The Contractor shall determine solubility of the test chemicals in the same manner as recommended to the Testing Facilities (i.e., by following the hierarchy below).

6.1 Cell Culture Media and Control Material

6.1.1 *Test Chemical Medium Solvents*

6.1.1.1 ~~*Treatment*~~ *Chemical Dilution*[3] *Medium (BALB/c 3T3 NRU)*

Serum-free[3] Dulbecco's Modification of Eagle's Medium (DMEM) [see **Section 3.1.2.3.a**] buffered with sodium bicarbonate and supplemented with (final concentrations in DMEM are quoted):

~~5% NBCS~~[3]
4 mM Glutamine
~~100~~ *200* IU/*mL*[3] Penicillin
~~100~~ *200* µg/ml[3] Streptomycin

This *serum-free*[3] medium is used in the assay for ~~application of~~*dissolving*[3] test chemicals *prior to application*[3] to the 3T3 cells.

6.1.1.2 *Routine Culture Medium (NHK NRU)*

KBM® (Clonetics CC-3104) supplemented with KBM® SingleQuots® (Clonetics CC-4131) and Clonetics Calcium SingleQuots® (CC-4202) to make 500ml of medium. Final concentration of supplements in medium are: ~~A modified MCDB 153 formulation such as Clonetics® Keratinocyte Basal Medium (KBM®) supplemented with (final concentrations in KBM® are quoted):~~[2]

0.*000*1 ng/ml[2] Human recombinant epidermal growth factor
5 *µ*g/ml[2] Insulin
0.5 g/ml[2] Hydrocortisone
~~50~~ *30 µ*g/ml[2] Gentamicin
~~50~~ *15* ng/ml[2] Amphotericin B
0.10 mM Calcium
~~2 ml 7.5 mg/ml~~*30 µg/ml*[2] Bovine pituitary extract.

This medium is used in the assay as the routine culture medium and for application of test chemicals to the NHK cells. *Complete media should be kept at 4°C and stored for no longer than two weeks.*[2]

NOTE: KBM® SingleQuots® contain the following stock concentrations and volumes:[2]

0.1 ng/ml	*hEGF*	*0.5 ml*[2]
5.0 mg/ml	*Insulin*	*0.5 ml*[2]
0.5 mg/ml	*Hydrocortisone*	*0.5 ml*[2]
30 mg/ml	*Gentamicin, 15 ug/ml Amphotericin-B*	*0.5 ml*[2]
7.5 mg/ml	*Bovine Pituitary Extract (BPE)*	*2.0 ml*[2]

Clonetics Calcium SingleQuots® are 2 ml of 300mM concentration of calcium.[2] *165 ul of solution per 500 ml calcium-free medium equals 0.10 mM calcium in the medium.*[2]

[3] Revised 9/17/02
[2] Revised 6/21/02

6.1.2 ***Positive Control (PC)***
Sodium Lauryl Sulfate ([SLS], CAS # 151-21-3) will be the positive control material for the *In Vitro* Validation Study.

6.2 Preparation of Test Chemical

All chemicals (including the positive control [SLS]) shall be weighed on a calibrated balance (including liquid test chemicals) and added to the appropriate solvent (**Section 6.2.1**). Test chemicals must be at room temperature before dissolving. Preparation under red light or yellow light may be necessary, if rapid photodegradation is likely to occur. The solutions must not be cloudy nor have noticeable precipitate.

6.2.1 ***Dissolving the Test Chemical***[3]
The hierarchy specified in **Sections 6.2.1.1 to 6.2.1.3** (i.e., culture medium, DMSO, ethanol) shall be followed for dissolving the test chemicals and positive control. Both assay-specific culture media specified in **Section 6.1.1** *(i.e., Chemical Dilution Medium for 3T3 cells and Routine Culture Medium for NHK cells)* must be tested.

Approximately 100 mg (100,000 μg) of the test chemical will be weighed into a glass tube and the weight will be documented. Assay-specific media will be added to the vessel so that the concentration is 200,000 μg/ml (200 mg/mL) (i.e., approximately 0.5 mL). The solution is mixed as specified in Section 6.2.1.1. If complete solubility is achieved, then additional solubility procedures are not needed. If only partial solubility is achieved, follow the test chemical dissolving steps in Table 1, derived from EPA (1998), to add additional medium in steps until the concentration is a minimum of 2,000 μg/mL (2 mg/mL). If complete solubility at 2,000 μg/mL in medium can't be attained, then repeat the solubility steps using the other solvent(s) in the solubility hierarchy. Test chemicals that are only soluble in DMSO or ethanol will be prepared at 500,000 μg/mL as the highest concentration of stock solution.

Table 1: Determination of Solubility in Media

STEP	***1***	***2***	***3***	***4***	***5***
Total Volume of Medium	*0.5 mL*	*2.5 mL*	*5.0 mL*	*2.0 mL*	*10.0 mL*
Concentration of Test Chemical (Add 100 mg to a tube. Add the first volume of medium. Dilute with subsequent volumes if necessary.)	*200,000 μg/mL (200 mg/mL)*	*40,000 μg/mL (40 mg/mL)*	*20,000 μg/mL (20 mg/mL)*		
Concentration of Test Chemical (Add 20 mg to a large tube. Add the first volume of medium. Dilute with subsequent volume if necessary.)				*10,000 μg/mL (10 mg/mL)*	*2,000 μg/mL (2.0 mg/mL)*
If test chemical is insoluble in medium at 2000 μg/mL, then attempt to dissolve chemical in DMSO. Actual volume of solution can be determined after test chemical is dissolved and solution is measured using a calibrated instrument (e.g., micropipettor, or serological pipette). The actual stock concentration can be calculated accordingly.					

Example: If complete solubility is not achieved in 0.5 mL medium (Step 1) using the mixing procedures specified in ***Section 6.2.1.1, b-d,*** *then 2.0 mL must be added to obtain a total volume of 2.5 mL (Step 2). Chemical and medium are again mixed as prescribed in* ***Section 6.2.1.1*** *in an attempt to dissolve. If solubility is not achieved at Step 2, then 2.5 mL medium is added in Step 3.*

[3] Section 6.2.1 replaced 9/17/02

*Chemical and medium are again mixed as prescribed in Section **6.2.1.1** in an attempt to dissolve. No additional weighing of the chemical is required until Step 4.*

6.2.1.1 Chemical Dilution Medium/Routine Culture Medium

a) *Dissolve test chemical in Chemical Dilution Medium and Routine Culture Medium as in Step 1 of **Table 1**.*
b) *Gently mix. Vortex for 1-2 minutes.*
c) *If test chemical hasn't dissolved, use sonication for up to five minutes.*
d) *If sonication doesn't work, then warm solution to 37°C.*
e) *Proceed to Step 2 (and Steps 3-5, if necessary) of **Table 1** and repeat procedures b-d.*

6.2.1.2 DMSO

*If the test chemical doesn't dissolve in the Chemical Dilution Medium or Routine Culture Medium, then follow the dilution steps in **Table 1A** and mixing steps a) through e) in **Section 6.2.1.1** using DMSO instead of Chemical Dilution Medium/Routine Culture Medium.*

6.2.1.3 Ethanol

*If the test chemical doesn't dissolve in DMSO, then follow the dilution steps in **Table 1A** and mixing steps a) through e) in **Section 6.2.1.1** using ethanol instead of DMSO.*

Table 1A: Determination of Solubility in DMSO and Ethanol

Steps	***1***	***2***	***3***	***4***	***5***	***6***
Total Volume of DMSO or Ethanol	*0.2 mL*	*0.5 mL*	*2.5 mL*	*5.0 mL*	*2.0 mL*	*10.0 mL*
Concentration of Test Chemical (Add 100 mg to a tube. Add the first volume of solvent. Dilute with subsequent volumes if necessary.)	*500,000 µg/mL (500 mg/mL)*	*200,000 µg/mL (200 mg/mL)*	*40,000 µg/mL (40 mg/mL)*	*20,000 µg/mL (20 mg/mL)*		
Concentration of Test Chemical (Add 20 mg to a tube. Add the first volume of solvent. . Dilute with subsequent volume if necessary.)					*10,000 µg/mL (10 mg/mL)*	*2,000 µg/mL (2.0 mg/mL)*
If test chemical is insoluble in DMSO at 2000 µg/mL, then attempt to dissolve chemical in ethanol. Actual volume of solution can be determined after test chemical is dissolved and solution is measured using a calibrated instrument (e.g., micropipettor, or serological pipette). The actual stock concentration can be calculated accordingly.						

*If the test chemical does not dissolve in Chemical Dilution Medium/Routine Culture Medium, DMSO, or ethanol, at 2 mg/mL, then repeat the entire solubility procedure with each solvent (in the order of Chemical Dilution Medium/Routine Culture Medium, DMSO, and ethanol) using the dilution steps in **Table 1B** and mixing steps a) through e) in **Section 6.2.1.1**.*[4]

[4] Added 10/11/02

Table 1B: Further Determination of Solubility in Chemical Dilution Medium/Routine Culture Medium, DMSO, or Ethanol[4]

STEP	*6*	*7*	*8*	*9*	*10*
Total Volume of Solvent	*5 mL*	*10 mL*	*20 mL*	*40 mL*	*100 mL*
Concentration of Test Chemical (Add 5 mg to a tube. Add the first volume of solvent. Dilute with subsequent volumes if necessary.)	*1,000 µg/mL (1 mg/mL)*	*500 µg/mL (0.5 mg/mL)*	*250 µg/mL (0.25 mg/mL)*	*125 µg/mL (0.125 mg/mL)*	*50 µg/mL (0.05 mg/mL)*
If test chemical is insoluble in medium at 50 µg/mL, then attempt to dissolve chemical in DMSO and then ethanol. Actual volume of solution can be determined after test chemical is dissolved and solution is measured using a calibrated instrument. The concentration can be calculated accordingly.					

~~Approximately 100 *200* mg (100*200*,000 µg)[2] of the test chemical will be weighed into a glass tube and the weight will be documented. Assay-specific culture media will be added to the vessel so that the concentration is 1*2*,000,000 µg/ml (1000 *2000* mg/ml)[2] (i.e., approximately 0.1 ml). If complete solubility is achieved, then additional solubility procedures are not needed. If only partial solubility is achieved, follow the test chemical dissolving steps in Table 1, derived from EPA (1998), to add additional medium in steps until the concentration is a minimum of 100*200*,000 µg/ml (100 *200* mg/ml)[2]. If complete solubility at 100,000 µg/ml in culture medium can't be attained, then repeat the solubility steps using the other solvent(s) in the solubility hierarchy. Test chemicals that are only soluble in DMSO or ethanol will be prepared at 1*2*,000,000 µg/ml[2] as the highest concentration of stock solution.~~

~~Table 1: Determination of Solubility~~

~~Solubility Data~~	**~~Step 1~~**	**~~Step 2~~**	**~~Step 3~~**
~~Total volume of medium added (ml)~~	~~0.1~~	~~0.5~~	~~1.0~~
~~Total volume of DMSO or ethanol added (ml)~~	~~0.1~~	~~***0.5~~[2]	~~***1.0~~[2]
~~Approximate solubility (µg/ml)~~	~~≥ 1*2*,000,000~~[2]	~~200*400*,000~~[2]	~~100*200*,000~~[2]

~~**6.2.1.1 *Treatment Medium/Routine Culture Medium)***~~

~~*a)f)* Dissolve test chemical in Treatment Medium and Routine Culture Medium~~
~~*b)g)* Gently mix. Vortex for 5-10 seconds*1-2 minutes*.[2]~~
~~*c)h)* If test chemical hasn't dissolved, use sonication (up to five minutes).~~
~~*d)i)* If sonication doesn't work, then warm solution to 37°C.~~

~~**6.2.1.2 *DMSO***~~

~~If the test chemical doesn't dissolve in the Treatment Medium/Routine Culture Medium, then follow steps a) through d) in **Section 6.2.1.1** using DMSO instead of Treatment Medium/Routine Culture Medium.~~

6.2.1.3 *Ethanol*

~~If the test chemical doesn't dissolve in DMSO, then follow steps a) through d) in **Section 6.2.1.1** using ethanol instead of DMSO.~~

[2] Revised 6/21/02
[2] Revised 6/21/02

6.2.2 *pH of Solutions*

Measure the pH (using pH paper) of the highest concentration of test chemical dissolved in the culture media. Document the pH and note the color of each test chemical concentration in medium.

7.0 DATA COLLECTION

7.1 Nature of Data to be Collected

7.1.1 *Solubility Studies*

The Contractor shall record all information pertinent to the solubility of the test chemical:

a) *Approximate t*~~T~~[3]est chemical solubility in *all solvents tested (i.e., media, DMSO, and/or ethanol) in weight per unit volume (i.e. mg/mL) estimated by following the step-wise solubility protocol* ~~culture medium at a minimum of 100~~*200*,000[2] ~~µg/ml~~[3]
b) pH of test chemical in culture medium; color of culture medium
~~c) Test chemical solubility in DMSO or ethanol at 1~~*2*,000,000[2] ~~µg/ml~~[3]
d) Need of vortexing, sonication, and/or heating

The Contractor shall provide this information to the Study Management Team via the Project Officer by the avenues described in Section 8. **This information shall NOT be provided to the Testing Facilities.** Information to be provided to the Testing Facilities is specified in Sections 5.1.5 and 5.1.6.

7.1.2 *Chemical Information*

The Contractor shall supply at a minimum the following information about each test chemical and report as specified in Addendum I.

a) Purity
b) CAS #
c) Supplier
d) Specification sheets
e) Certificates of analysis
f) Material Safety Data Sheet (MSDS)
g) Color
h) Odor
i) Physical state
j) Weight or volume of sample distributed to the Testing Facility
k) Specific density for liquid test chemicals
l) Storage instructions
m) Chemical hazards
n) Special handling instructions
o) Amount of material archived

[Note: Much of the information will be in the MSDS.]

7.2 Type of Media Used for Data Storage

Originals of the raw data (the Study Workbook) and copies of other raw data such as instrument logs shall be collected and archived at the end of the study (under the direction of the Study Director), according to GLP-compliant procedures. Data that are stored electronically shall be periodically copied, and backup files shall be produced and maintained.

[2] Revised 6/21/02
[3] Revised 9/17/02

7.3 Documentation

Original raw data that shall be collected shall include but are not limited to the following:

- Data recorded in the Study Workbook, which shall consist of all recordings of all activities related to acquisition, preparation, solubility testing, and distribution of the test chemicals;
- Other data collected as part of GLP compliance
  - Equipment logs
  - Equipment calibration records

8.0 DRAFT AND FINAL REPORTS

Biweekly Reports: The Contractor will provide a biweekly progress report to the Project Officer and copied to the Project Coordinators of the Study Management Team (See **Section 4.2** and Addendum I). These reports will include raw and interim data as the study progresses. These reports will be in electronic format (i.e., email with Microsoft® Word (or equivalent) or Excel attachments).

Draft Reports: A draft report shall be submitted to the Project Officer for each Validation Study phase (See **Section 4.2** and Addendum I). A Draft Final Report detailing the Contractor's involvement in all phases of the Validation Study shall be prepared by the Contractor, signed by the Study Director, and provided to the Project Officer. The submitted results shall accurately describe all methods used for generation and analysis of the data, provide a complete record of the preparation of test chemicals, and present any relevant data necessary for the assessment of the results (See Addendum I).

Final Report: The Draft Final Report shall be revised according to comments from the Project Officer and submitted as the Final Report (**See Section 4.2** and Addendum I).

9.0 RECORDS AND ARCHIVES

At the conclusion of the Contractor's participation in the distribution of chemicals for the Validation Study, the original raw and derived data, as well as copies of other raw data not exclusive to this Validation Study (instrument logs, calibration records, facility logs, etc.), shall be submitted to NIEHS/NICEATM (via the Project Officer) for storing and archiving according to the facility's SOP and in compliance with GLP Standards.

Originals of all raw and derived data, or copies where applicable, shall be stored and archived at NIEHS/NICEATM.

10.0 ALTERATIONS OF THE STATEMENT OF WORK

No changes in the Statement of Work shall be made without the consent of the Project Officer and Study Management Team. A Statement of Work Amendment detailing any change(s) and the basis for the change(s) shall be approved and prepared by the Study Director, and the amendment shall be signed and dated by the Study Director and the NIEHS representative. The amendment shall be retained with the original Statement of Work.

11.0 REFERENCES

Clonetics Normal Human Keratinocyte Systems Instructions for Use, AA-1000-4-Rev.03/00. (http://www.clonetics.com).

EPA Product Properties Test Guidelines. OPPTS 830.7840. 1998. Water Solubility: Column Elution Method; Shake Flask Method. United States Environmental Protection Agency. Prevention, Pesticides and Toxic Substances (7101). EPA 712-C-98-041. March 1998.

National Toxicological Program, September 2000, Attachment 2 revised. Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological and Physical Agents in Laboratory Animals for the National Toxicology Program (NTP).

NICEATM (The National Toxicology Program [NTP] Interagency Center for the Evaluation of Alternative Toxicological Methods). 2001. Test Method Protocol for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test. A Test for Basal Cytotoxicity for an *In Vitro* Validation Study.

NICEATM (The National Toxicology Program [NTP] Interagency Center for the Evaluation of Alternative Toxicological Methods). 2001. Test Method Protocol for the Normal Human Keratinocyte [NHK] Neutral Red Uptake Cytotoxicity Test. A Test for Basal Cytotoxicity for an *In Vitro* Validation Study.

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). 2001. Guidance document on using *in vitro* data to estimate *in vivo* starting doses for acute toxicity NIH publication 01-4500. NIEHS, Research Triangle Park, North Carolina.

OECD (Organisation for Economic Co-operation and Development). 2001. Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures as Endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals in November 1998, Part 2, p. 21. OECD, Paris. http://www.oecd.org/ehs/class/HCL6htm.

12.0 APPROVAL OF STATEMENT OF WORK

_____ _____

Sponsor Representative **Date**

_____ _____

Testing Facility Management **Date**

ADDENDUM I

SUGGESTED REPORT FORMAT

TITLE PAGE

- **Study Title**
  - Draft Report 1: *Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Phase I of the In Vitro Validation Study*
  - Draft Report 2: *Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Phase II of the In Vitro Validation Study*
  - Draft Report 3: *Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Phase III of the In Vitro Validation Study*
  - Draft/Final Report: *Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Final Report for the In Vitro Validation Study*
- **Test Articles**
  - Draft Report 1: Identify the positive control chemical of Phase Ia and the three (3) test chemicals of Phase Ib
  - Draft Report 2: Identify the nine (9) test chemicals of Phase II
  - Draft Report 3: Identify the sixty (60) test chemicals of Phase III
  - Draft/Final Report: Identify all seventy-two (72) test chemicals and positive control of the *In Vitro* Validation Studies
- **Authors**
- **Study Completion Date**
- **Contract Facility**
- **Study Number/Identification**

SIGNATURE PAGE

- **Study Initiation Date:** Date Statement of Work was signed
- **Initiation Date of Laboratory Studies:** Actual laboratory start date
- **Study Completion Date:** Date report signed by Study Director
- **Sponsor Representative:**
  Ms. Molly Vallant – Project Officer
  The National Institute of Environmental Health Sciences (NIEHS)
- **Study Management Team Representatives**
  Judy Strickland, Ph.D. (Project Coordinator)
  Michael Paris (Assistant Project Coordinator)
- **Contractor Facility:** Name and address
- **Archive Location:** Name and address
- **Study Director:** Name and signature and date
- **Key Personnel:** Laboratory technicians, QA Director, Safety Officer
- **Facility Management:** Name
- **Scientific Advisor:** Name

ADDENDUM I (cont.)

DRAFT REPORT 1

Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Phase I of the In Vitro Validation Study

- **Table of Contents**
- **Objectives :**The report shall provide specific objectives
- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for the positive control (SLS) and the three (3) Phase Ib chemicals.
- **Narrative Description of the Solubility Studies:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical will be included in the description. Provide the information requested in **Section 7.1.1**. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report (as attachments). The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.
- **Statement Signed by the Study Director:** Confirm that the solubility studies, acquisition, preparation, and distribution of the test chemicals were conducted in compliance with GLP (or indicating where the Study deviated from GLP). Confirm that the report fully and accurately reflects the raw data generated in the Study.
- **Other Information:** (All copies of documents will be noted as exact duplicates of the data.)
  - Information requested in **Section 7.1.2**
  - Deviations to the protocols, SOPs, and Statement of Work
  - Revisions/amendments to the protocols, SOPs, and Statement of Work

DRAFT REPORT 2

Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Phase II of the In Vitro Validation Study

- **Table of Contents**
- **Objectives:** The report shall provide specific objectives
- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for the nine (9) Phase II chemicals.
- **Narrative Description of the Solubility Studies:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical shall be included in the description. Provide the information requested in **Section 7.1.1**. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report (as attachments). The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.
- **Statement Signed by the Study Director**: Confirm that the solubility studies, acquisition, preparation, and distribution of the test chemicals were conducted in compliance with GLP (or indicating where the Study deviated from GLP). Confirm that the report fully and accurately reflects the raw data generated in the Study.
- **Other Information:** (All copies of printouts, documents, and spreadsheets shall be noted as exact duplicates of the data.)
  - Information requested in **Section 7.1.2**
  - Deviations to the protocols, SOPs, and Statement of Work
  - Revisions/amendments to the protocols, SOPs, and Statement of Work

ADDENDUM I (cont.)

DRAFT REPORT 3

Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Phase III of the In Vitro Validation Study

- **Table of Contents**
- **Objectives:** The report shall provide specific objectives
- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for sixty (60) Phase III chemicals.
- **Narrative Description of the Solubility Studies:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical will be included in the description. Provide the information requested in **Section 7.1.1**. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report (as attachments). The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.
- **Statement Signed by the Study Director:** Confirm that the solubility studies, acquisition, preparation, and distribution of the test chemicals were conducted in compliance with GLP (or indicating where the Study deviated from GLP). Confirm that the report fully and accurately reflects the raw data generated in the Study.
- **Other Information:** (All copies of printouts, documents, and spreadsheets shall be noted as exact duplicates of the data.)
  - Information requested in **Section 7.1.2**
  - Deviations to the protocols, SOPs, and Statement of Work
  - Revisions/amendments to the protocols, SOPs, and Statement of Work

DRAFT/FINAL REPORT

Acquisition, Preparation, Solubility Testing, and Distribution of Test Chemicals: Draft/Final Report for the In Vitro Validation Study

- **Table of Contents**
- **Objectives:** The draft/final report shall provide specific objectives
- **Summary of the Findings:** Referenced to the raw data where appropriate; Include all information for the seventy-two (72) test chemicals and the positive control (SLS).
- **Narrative Description of the Solubility Studies:** Describe any problems that were encountered and how such problems were solved. Justifications for solvents used for each test chemical shall be included in the description. Provide the information requested in **Section 10.1.1**. Deviations from the protocols, SOPs, and/or the Statement of Work shall be addressed in this section. Copies of appropriate sections of the Study Workbook shall be included with the report (as attachments). The draft report will include unaudited Study Workbook pages. The final report will include a copy of the audited Study Workbook with a statement (signed and dated by the Study Director) on the front of it stating that it is an exact copy of the original audited workbook.
- **Statement Signed by the Study Director:** Confirm that the acquisition, preparation, solubility studies, and distribution of the test chemicals were conducted in compliance with GLP (or indicating where the Study deviated from GLP). Confirm that the report fully and accurately reflects the raw data generated in the Study.
- **Quality Assurance Statement: (For Final Report only)**
  QA Statement identifying: 1) the phases and data inspected, 2) dates of inspection, and 3) dates findings were reported to the Study Director and Testing Facility management. The QA

Statement shall identify whether the methods and results described in the Final Report accurately reflect the raw data produced during the Study.

- **Other Information:** (All copies of printouts, documents, and spreadsheets shall be noted as exact duplicates of the data.)
  - Deviations to the protocols, SOPs, and Statement of Work
  - A list of all SOPs used by the laboratory (SOP title and laboratory identification code)
  - The Statement of Work

BIWEEKLY REPORTS

Contract Facility:

Chemicals Acquired:

Chemicals Tested for Solubility:

Results of Solubility Tests:

Chemicals Shipped to Testing Facilities:

Date of Shipping:

Problems Encountered/Resolutions:

Projected Shipping Schedule:

ADDENDUM II
SUGGESTED STANDARD TEST REPORTING TEMPLATE FOR STUDY WORKBOOK

[1]SOLUBILITY TESTING
Test Chemicals for the *In Vitro* Validation Study

Study No._____

Test Chemical_____ Test Chemical Code_____ CAS #_____

Physical Description_____ Liquid Density_____

Solubility Determined by_____
Date_____

Solvent	Amount of Test Chemical	Volume Added	Total Volume	pH and medium color	Vortex (V) Sonication (S) Heating-37°C (H)	Comments
Treatment Medium (3T3 NRU)		0.1ml				
		0.5ml				
		1.0ml				
Routine Culture Medium (NHK NRU)		0.1ml				
		0.5ml				
		1.0ml				
DMSO		0.1ml				
Ethanol		0.1ml				

Reference Color of Treatment Medium_____

Reference Color of Routine Culture Medium_____

Balance I.D._____
Treatment Medium and Routine Culture Medium: minimum concentration of 100mg/ml.
DMSO and Ethanol: minimum concentration of 1000mg/ml.

[1] Adaptation of Institute of In Vitro Sciences (IIVS) form – 350 [2/2002]

ADDENDUM III
TEST CHEMICALS FOR THE *IN VITRO* VALIDATION STUDY (ALPHABETICAL)

[NOTE: TESTING FACILITIES MUST NOT SEE THIS LIST OF CHEMICALS]

CHEMICAL	CAS NO.
1,1,1-Trichloroethane	71-55-6
2-Propanol	67-63-0
5-Aminosalicylic acid	89-57-6
Acetaminophen	103-90-2
Acetonitrile	75-05-8
Acetylsalicylic acid	50-78-2
~~**To be determined**~~[1]	
Aminopterin	54-62-6
Amitriptyline *HCl*[3]	~~50-48-6~~*549-18-8*[3]
Arsenic III trioxide	1327-53-3
Atropine sulfate *monohydrate*[3]	~~55-48-1, (~~*17108-73-5*~~)~~*73791-47-6*[3]
Boric aid	10043-35-3
Busulphan	55-98-1
Cadmium II chloride	10108-64-2
Caffeine	58-08-2
Carbamazepine	298-46-4
Carbon tetrachloride	56-23-5
Chloral hydrate	302-17-0
Chloramphenicol	56-75-7
Citric Acid	77-92-9
Colchicine	64-86-8
Cupric sulfate * 5 H2O	7758-99-8
Cycloheximide	66-81-9
Dibutylphthalate	84-74-2
Dichlorvos (DDVP)	62-73-7
Diethyl phthalate	84-66-2
Digoxin	20830-75-5
Dimethylformamide	68-12-2
Diquat	2764-72-9
Disulfoton	298-04-4
Endosulfan	115-29-7
Epinephrine bitartrate	51-42-3
Ethanol	64-17-5
Ethylene glycol	107-21-1
Fenpropathrin	39515-41-8
Gibberellic acid	77-06-5
Glutethimide	77-21-4
Glycerol	56-81-5
Haloperidol	52-86-8
Hexachlorophene	70-30-4
Lactic acid	50-21-5
Lindane	58-89-9

[1] Revised 5/23/02
[3] Revised 9/17/02

ADDENDUM III (CONT.)

CHEMICAL	CAS NO.
Lithium I ~~sulfate~~*carbonate*[3]	*554-13-2*~~10377-48-7~~[3]
Meprobamate	57-53-4
Mercury II chloride	7487-94-7
Methanol	67-56-1
Nicotine	54-11-5
Paraquat	1910-42-5, (*3765-78-4,57593-74-5,65982-50-5,136338-65-3,205105-68-6,247050-57-3*)[3]
Parathion	56-38-2
Phenobarbital	50-06-6
Phenol	108-95-2
Phenylthiourea	103-85-5
Physostigmine[1]	*57-47-6*[1]
Potassium cyanide	151-50-8
Potassium I chloride	7447-40-7
Procainamide *HCl*[3]	~~51-06-9~~*614-39-1*[3]
Propranolol HCl	318-98-9, (*3506-09-0, 146874-86-4*)[1]
Propylparaben	94-13-3
Sodium arsenite	7784-46-5
Sodium chloride	7647-14-5
Sodium dichromate dihydrate	7789-12-0
Sodium hypochlorite	8007-59-8, (*7681-52-9*)[3]
Sodium I fluoride	7681-49-4
Sodium oxalate	62-76-0
Sodium selenate~~*10 H20~~[1]	~~13413~~*13410*-01-0[1]
Strychnine	57-24-9
Thallium I sulfate	7446-18-6
Trichloroacetic acid	76-03-9
Triethylene melamine	51-18-3
Triphenyltin hydroxide	76-87-9
Valproic acid	99-66-1
Verapamil HCl	152-11-4
Xylene	1330-20-7

[3] Revised 9/17/02
[1] Revised 5/23/02

ADDENDUM IV
TEST CHEMICALS FOR THE *IN VITRO* VALIDATION STUDY BY STUDY PHASE

PHASE Ia	
Sodium laurel sulfate	151-21-3
PHASE Ib	
Arsenic III trioxide	1327-53-3
Ethylene glycol	107-21-1
Propranolol HCl	318-98-9, (*3506-09-0, 146874-86-4*)[1]
PHASE II	
Aminopterin	54-62-6
Chloramphenicol	56-75-7
Colchicine	64-86-8
Cupric sulfate * 5 H2O	7758-99-8
Lithium I ~~sulfate~~*carbonate*[3]	*554-13-2*~~10377-48-7~~[3]
Potassium I chloride	7447-40-7
2-Propanol	67-63-0
Sodium I fluoride	7681-49-4
Sodium selenate~~*10 H20~~[1]	~~13413~~*13410*-01-0[1]
PHASE III	
1,1,1-Trichloroethane	71-55-6
5-Aminosalicylic acid	89-57-6
Acetaminophen	103-90-2
Acetonitrile	75-05-8
Acetylsalicylic acid	50-78-2
~~**To be determined**~~[1]	
Amitriptyline *HCl*[3]	*549-18-8*~~50-48-6~~[3]
Atropine sulfate *monohydrate*[3]	*73791-47-6*~~55-48-1, (17108-73-5)~~[3]
Boric aid	10043-35-3
Busulphan	55-98-1
Cadmium II chloride	10108-64-2
Caffeine	58-08-2
Carbamazepine	298-46-4
Carbon tetrachloride	56-23-5
Chloral hydrate	302-17-0
Citric Acid	77-92-9
Cycloheximide	66-81-9
Dibutylphthalate	84-74-2
Dichlorvos (DDVP)	62-73-7
Diethyl phthalate	84-66-2
Digoxin	20830-75-5
Dimethylformamide	68-12-2
Diquat	2764-72-9
Disulfoton	298-04-4
Endosulfan	115-29-7
Epinephrine bitartrate	51-42-3

[3] Revised 9/17/02
[1] Revised 5/23/02

ADDENDUM IV (CONT.)

PHASE III (cont.)

Ethanol	64-17-5
Fenpropathrin	39515-41-8
Gibberellic acid	77-06-5
Glutethimide	77-21-4
Glycerol	56-81-5
Haloperidol	52-86-8
Hexachlorophene	70-30-4
Lactic acid	50-21-5
Lindane	58-89-9
Meprobamate	57-53-4
Mercury II chloride	7487-94-7
Methanol	67-56-1
Nicotine	54-11-5
Paraquat	1910-42-5, (*3765-78-4,57593-74-5,65982-50-5,136338-65-3,205105-68-6,247050-57-3*)[3]
Parathion	56-38-2
Phenobarbital	50-06-6
Phenol	108-95-2
Physostigmine[1]	*57-47-6*[1]
Phenylthiourea	103-85-5
Potassium cyanide	151-50-8
Procainamide *HCl*[3]	~~51-06-9~~*614-39-1*[3]
Propylparaben	94-13-3
Sodium arsenite	7784-46-5
Sodium chloride	7647-14-5
Sodium dichromate dihydrate	7789-12-0
Sodium hypochlorite	8007-59-8, (*7681-52-9*)[3]
Sodium oxalate	62-76-0
Strychnine	57-24-9
Thallium I sulfate	7446-18-6
Trichloroacetic acid	76-03-9
Triethylene melamine	51-18-3
Triphenyltin hydroxide	76-87-9
Valproic acid	99-66-1
Verapamil HCl	152-11-4
Xylene	1330-20-7

[1] Revised 5/23/02
[3] Revised 9/17/02

ADDENDUM V

ASSUMPTIONS FOR CALCULATION OF AMOUNT OF TEST MATERIAL NEEDED FOR EACH TESTING FACILITY

	Chemical Amount	Assumption
Phase I		
Test in 3 solvents	300 mg	Chemical must be tested in all 3 solvents
Test in 3 replicate assays	300	3 replicate assays must be performed
Repeat 3 times	300	3 replicate assays must be repeated 3 times
Phase I Amount/Testing Facility	900 mg	
x 3 Testing Facilities	2700	Assumes 3 labs participate in study
2 Archive samples (3 solubility + 3 assays)	1200	Archive samples use same amount of chemical as testing sample
Total Phase I Amount	3900 mg	
Phase II		
Test in 3 solvents	300 mg	Chemical must be tested in all 3 solvents
Test in 3 replicate assays	300	3 replicate assays must be performed
Repeat 2 times	200	2 replicate assays must be repeated 3 times
Phase II Amount/Testing Facility	800 mg	
x 3 Testing Facilities	2400	Assumes 3 labs participate in study
2 Archive samples (3 solubility + 3 assays)	1200	Archive samples use same amount of chemical as testing sample
Total Phase II Amount	3600 mg	
Phase III		
Test in 3 solvents	300 mg	Chemical must be tested in all 3 solvents
Test in 3 replicate assays	300	3 replicate assays must be performed
Phase III Amount/Testing Facility	600 mg	
x 3 Testing Facilities	1800	Assumes 3 labs participate in study
2 Archive samples (3 solubility + 3 assays)	1200	Archive samples use same amount of chemical as testing sample
Total Phase III Amount	3000 mg	

Specification of 4 g of chemical per Testing Facility in **Section 5.1.3** was chosen to allow a generous amount of error (in the direction of the Testing Facilities being provided with more chemical than necessary) in the calculations and assumptions made here.

Appendix H

Candidate Reference Oral LD_{50} Data

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Appendix H1

Rat and Mouse Oral LD_{50} Database

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Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
1,1,1-Trichloroethane	9600	9600	7384 - 12480	NA	rats	NA	oral	NA	NA	reference in Russian	NA	Paligov VI, Khananaev LI, Goinatskii MG, Gavrilyuk VM. 1990. Hygienic substantiation of content of methylchloroform in water bodies. Gigiena Naselennykh Mest 29:45-49. **(RTECS REFERENCE)**
1,1,1-Trichloroethane	9600	10300	8270 - 12800 (95% CL)	Thompson method of moving averages	Wistar white rats; 175 - 250 g	female	oral; stomach tube	single dose; undiluted; no more than 7 cc administered	all surviving rats observed up to 2 weeks; 35 rats used	NA	NA	Torkelson TR, Oyen F, McCollister DD, Rowe VK. 1958. Toxicity of 1,1,1-trichloroethane as determined on laboratory animals and human subjects. Am Ind Hyg Assoc J 19:353-362. *The Dow Chemical Company, Midland, MI*
1,1,1-Trichloroethane	9600	12300	11000 - 13700 (95% CL)	Thompson method of moving averages	Wistar white rats; 175 - 250 g	male	oral; stomach tube	single dose; undiluted; no more than 7 cc administered	all surviving rats observed up to 2 weeks; 35 rats used	this compound is an inhibited form	NA	Torkelson TR, Oyen F, McCollister DD, Rowe VK. 1958. Toxicity of 1,1,1-trichloroethane as determined on laboratory animals and human subjects. Am Ind Hyg Assoc J 19:353-362. *The Dow Chemical Company, Midland, MI*
1,1,1-Trichloroethane	9600	12600	926 - 17100 (CL)	Litchfield and Wilcoxon	Holtzman, Sprague-Dawley albino rats; 215-330 g; adult	male	oral; gastric intubation	single dose; undiluted; 464, 1000, 2150, 4660, 10000, 21500 mg/kg doses	observations recorded at 1, 4, 24 hours, daily thereafter for 7 days; 5 dead at highest dose; depression, ataxia, labored respiration, salivation, ptosis, excessive urination, diarrhea	3-4 hour fasting period; stabilized 1,1,1-trichloroethane; inhibited formulation	NA	from EPA TSCATS database; Acute Oral Administration-Rats Acute Dermal Application-Rabbits Acute Eye Irritation-Rabbits Primary Skin Irritation-Rabbits Subacute (Four-Week) Inhalation; 1969. EPA Doc. No. 878210366, Fiche No. OTS0205891; *Ethyl Corp.*
1,1,1-Trichloroethane	9600	12627	5356 - 29765 (CL)	Litchfield and Wilcoxon	Holtzman, Sprague-Dawley albino rats; 215-330 g; adult	male	oral; gastric intubation	single dose; undiluted; 464, 1000, 2150, 4660, 10000, 21500 mg/kg doses	observations recorded at 1, 4, 24 hours, daily thereafter for 7 days; 5 dead at highest dose; depression, ataxia, labored respiration, salivation, ptosis	3-4 hour fasting period; stabilized 1,1,1-trichloroethane; inhibited formulation	NA	from EPA TSCATS database; Acute Oral Administration-Rats Acute Dermal Application-Rabbits Acute Eye Irritation-Rabbits Primary Skin Irritation-Rabbits Subacute (Four-Week) Inhalation; 1969. EPA Doc. No. 878210366, Fiche No. OTS0205891; *Ethyl Corp.*
1,1,1-Trichloroethane	9600	16000	no CL ("all-or-none" response)	Litchfield and Wilcoxon	Holtzman, Sprague-Dawley albino rats; 215-330 g; adult	male	oral; gastric intubation	single dose; undiluted; 464, 1000, 2150, 4660, 10000, 21500 mg/kg doses	observations recorded at 1, 4, 24 hours, daily thereafter for 7 days; 4 dead at highest dose; depression, ataxia, labored respiration, excessive urination, diarrhea, ruffled fur, salivation, piloerection	3-4 hour fasting period; unstabilized 1,1,1-trichloroethane	NA	from EPA TSCATS database; Acute Oral Administration-Rats Acute Dermal Application-Rabbits Acute Eye Irritation-Rabbits Primary Skin Irritation-Rabbits Subacute (Four-Week) Inhalation; 1969. EPA Doc. No. 878210366, Fiche No. OTS0205891; *Ethyl Corp.*
2-Propanol	5045	4074 (5.19 mL/kg; sp. density = 0.78505; convert LD50 to mg/kg)	3015 - 5503	moving average method	Wistar rats; 90-120 g; 3-4 weeks old	male	oral; stomach intubation	doses differ by a factor of 2 in a geometric series	14 day observation; dose, number of dead/total: 16 mL/kg -- 3/3; 8 mL/kg -- 5/5; 4 mL/kg -- 1/5	non-fasted; tested in 1975; 13 rats used	NA	from EPA TSCATS database; Range Finding Toxicity Studies With Isopropanol Recovery Column, Side Stream Decanter Make With Cover Letter Dated 020987; EPA Document No. 86870000097 Fiche No. OTS0513282; *Union Carbide Corp.; Carnegi Mellon 1976*
2-Propanol	5045	4396 (5.6 mL/kg; sp. density = 0.78505; convert LD50 to mg/kg)	3297 - 5809 (95% CL; 4.2 - 7.4 mL/kg; sp. density = 0.78505; convert LD50 to mg/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 16-50 g; 14 days	male and female	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; 6-12 rats of both sexes used for studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
2-Propanol	5045	4500	3500 - 5800 (95% CL)	UDP	Sprague-Dawley rats; ~ 7 weeks	female	oral gavage	undiluted dose (g/kg) 3.5, 4.5, 5.8, 7.5	clinical observations: soft stools, diarrhea, decreased limb tone, hypoactivity, hypothermia, lacrimation, pinna and pain reflex absent, red-stained nose, mouth, and eyes, dyspnea, brown-stained urogenital or anal region, bradypnea and piloerection, ataxia; dose (g/kg), rats dead: 3.5-0/2; 4.5-2/4; 5.8-2/2; 7.5-1/1	18-20 hour fasted rats; 1-4 rats per dose; GLP study	NA	from EPA TSCATS database; Acute Oral Toxicity (Up/Down Method) Report with Cover Letter Dated 020987; 1983. EPA Document No. 86870000160, Fiche No. OTS0513345; *Hazelton Labs;* Hazelton 1983
2-Propanol	5045	4710 (6.0 mL/kg; sp. density = 0.78505; convert LD50 to mg/kg)	4082 - 5495 (95% CL; 5.2 - 7.0 mL/kg; sp. density = 0.78505; convert LD50 to mg/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 80-160 g; young adult (4-6 weeks according to Taconic Farms)	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
2-Propanol	5045	5045	4650 - 5400	NA	rats	female?	oral	NA	NA	reference in Russian	NA	Antonova VI, Salmina ZH. 1978. The maximal permissible concentration of isopropyl alcohol in water bodies with due regards for its action on the gonads and the progeny. Gigiena i Sanitariya 43(1):9-11. **(RTECS REFERENCE)**
2-Propanol	5045	5087 (6.48 mL/kg; sp. density = 0.78505; convert LD50 to mg/kg)	3768 - 6877	moving average method	Wistar rats; 90-120 g; 3-4 weeks old	male	oral; stomach intubation	doses differ by a factor of 2 in a geometric series	14 day observation;dose, number of dead/total: 10mL/kg - 5/5; 5 mL/kg - 1/5	non-fasted; tested in 1971; 10 rats used	NA	from EPA TSCATS database; Isopropanol, Anhydrous Range Finding Toxicity Studies with Cover Letter Dated 020987, (1971), EPA Document No. 86870000102, Fiche No. OTS0513287; *Carnegie-Mellon Inst. of Res. 1971*
2-Propanol	5045	5300	4100 - 7000 (95% CL)	UDP	Sprague-Dawley rats; ~ 7 weeks	male	oral gavage	undiluted dose (g/kg) 4.5, 5.8, 7.5, 9.8	clinical observations: soft stools, diarrhea, ataxia, decreased limb tone, hypoactivity, hypothermia, lacrimation, pinna and pain reflex absent, red-stained nose, mouth and eyes, brown-stained urogenital or anal region, dyspnea, bradypnea and piloerection; dose (g/kg), rats dead:4.5-0/2; 5.8-2/3; 7.5-3/3; 9.8-1/1	18-20 hour fasted rats; 1-3 rats per dose; GLP study	NA	from EPA TSCATS database; Acute Oral Toxicity (Up/Down Method) Report with Cover Letter Dated 020987, (1983), EPA Document No. 86870000160, Fiche No. OTS0513345; *Hazelton Labs;* Hazelton 1983
2-Propanol	5045	5338 (6.8 mL/kg; sp.density is 0.78505; convert LD50 to mg/kg)	4161 - 6908 (95% CL; 5.3 - 8.8 mL/kg; sp. density = 0.78505; convert LD50 to mg/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 300-470 g; older adult (9-18 weeks according to Taconic Farms)	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
2-Propanol	5045	5840	NA	based on assumption that probit mortality vs log dose has same slope as similar chemical	Sherman rats; 90 -120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; doses (in g/kg) differ by 1 log to bracket LD50, then refine LD50 with doses in a series of antilog 1.1, 1.3, 1.5, etc.	LD50 based on mortalities during a 14 day period	6 rats/dose at doses that differ by 1 log to bracket LD50 (given 1 week apart); then refined LD50 with 10 rats/dose in a dose series of antilog 1.1, 1.3, 1.5, etc.; assumed to use materials/methods of Smyth & Carpenter (1944) except for reported changes	reagent grade	Smyth HF Jr, Carpenter CP. 1948. Further experience with the range finding test in the industrial toxicology laboratory. J Ind Hyg Toxicol 30: 63-68. (LD50 value) Smyth HF Jr, Carpenter CP. 1944. The place of the range-finding test in the industrial toxicology laborotory. J Ind Hyg Toxicol 26:269-273. (most materials/methods).
5-Aminosalicylic acid	2800	2800	1781 - 3819 (95% CL)	Miller and Tainter (1944)	CDR Sprague-Dawley albino rats; male 288-346 g; 9-12 weeks old	male	oral; intubation	single dose; 2500, 3500, 5000 mg/kg doses; conc. 250, 350, 500 mg/mL; 10 mL dose vol.; methylcellulose vehicle	14 day observation; initial checks at 1, 2, and 4 hours after administration; 2 daily thereafter	15 rats used (five/dose level); fasted overnight; GLP	Monsanto Company	from EPA TSCATS database; Acute Toxicity Study in Rats Administered 10 Materials (final report) with Cover Letter dated 062669, (1969), EPA Doc. No. 40-6942188, Fiche No. OTS0519234; *Monsanto Co./Bio/dynamics*
5-Aminosalicylic acid	2800	3450	2513 - 4387 (95% CL)	Miller and Tainter (1944)	CDR Sprague-Dawley albino rats; male 288-346 g; female 225-267 g; 9-12 weeks old	male and female (equal numbers)	oral; intubation	single dose; 2500, 3500, 5000 mg/kg doses; conc. 250, 350, 500 mg/mL; 10 mL dose vol.; methylcellulose vehicle	14 day observation; initial checks at 1, 2, and 4 hours after administration; 2 daily thereafter	30 rats used (five/sex/dose level); fasted overnight; GLP; used same animals as 2800 and 4200 mg/kg values from Monsanto 1969	Monsanto Company	from EPA TSCATS database; Acute Toxicity Study in Rats Administered 10 Materials (final report) with Cover Letter dated 062669, (1969), EPA Doc. No. 40-6942188, Fiche No. OTS0519234; *Monsanto Co./Bio/dynamics*
5-Aminosalicylic acid	2800	4200	2863 - 5537 (95% CL)	Miller and Tainter (1944)	CDR Sprague-Dawley albino rats; female 225-267 g; 9-12 weeks old	female	oral; intubation	single dose; 2500, 3500, 5000 mg/kg doses; conc. 250, 350, 500 mg/mL; 10 mL dose vol.; methylcellulose vehicle	14 day observation; initial checks at 1, 2, and 4 hours after administration; 2 daily thereafter; toxicologic signs: soft stool, hyponea, hypoactivity; urinary and fecal staining	15 rats used (five/dose level); fasted overnight; GLP	Monsanto Company	from EPA TSCATS database; Acute Toxicity Study in Rats Administered 10 Materials (final report) with Cover Letter dated 062669, (1969), EPA Doc. No. 40-6942188, Fiche No. OTS0519234; *Monsanto Co./Bio/dynamics*
Acetaminophen	1944	1944	NA	Litchfield and Wilcoxon	Wistar rats; 130-150 g	male and female	stomach tube	5 mL/kg bw in 1% carboxymethyl-cellulose	observed 3 weeks	fasted 18 hours before dosing	NA	Kammerer F-J, Schleyerbach R. 1987. U.S. Patent 4,636,513. Isoxazole derivatives and medicaments containing these compounds (January 13, 1987). **(RTECS REFERENCE)**
Acetaminophen	1944	2404	+/- 95 (S.E.)	Miller and Tainter (1944)	Charles River CD and Sprague-Dawley rat strains; >100 g; adult	NA	oral intubation	up to 50 mL/kg	rats observed for 7 days; observed up to 14 days when heavy metals or other compounds that produce latent death were investigated	fasted overnight	NA	Yeary RA, Benish RA, Finkelstein M. 1966. Acute Toxicity of Drugs in Newborn Animals. Journal of Pediatrics 69(4):663-667. *Dept. of Veterinary Preventive Medicine, Ohio State University, Columbus, OH*
Acetonitrile	2460	157 (0.2 mL/kg; sp. density = 0.7857; convert LD50 to mg/kg	79 - 236 (95% CL; 0.1 - 0.3 mL/kg; sp. density = 0.7857; convert LD50 to mg/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 16-50 g; 14 days	male and female	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; 6-12 rats of both sexes used for studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Acetonitrile	2460	1320 (1.68 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	972 - 1799 (1.24 - 2.27 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	NA	Carworth Farms Wistar or Nelson albino rats; 90-112g	male	oral gastric intubation	undiluted cmpd; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	1453	1123 - 1879 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	male	oral gavage	single dose	14 day observation; toxicity symptoms: ptosis, posture, respiratory effects, lethargy, ataxia, convulsions; time to onset of signs < 1 day; duration of signs 5 days; 5 rats dead (average per test)	3 dose levels (5 male each); 15 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Acetonitrile	2460	1623 (2.07 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg	1050 - 2524 (1.34 - 3.22 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg	NA	Carworth Farms Wistar or Nelson albino rats; 90-112g	male	oral gastric intubation	undiluted cmpd; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	1730	1100 - 2720	NA	Carworth Farms Wistar or Nelson albino rats; 90-112g	female	oral gastric intubation	0.1 in corn oil; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	> 2000	NA	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	female	oral gavage	single dose	14 day observation; toxicity symptoms: Ptosis, posture, respiratory effects, lethargy, ataxia, convulsions; time to onset of signs < 1day; duration of signs 5 days; 5 rats dead (average per test)	3 dose levels (5 female each); 15 rats used; OECD TG401 (1981) followed for experimental procedures		Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28: (7) 469-482.
Acetonitrile	2460	2230	1900 - 2620	NA	Carworth Farms Wistar or Nelson albino rats; 30-54 g; weanlings	female	oral gastric intubation	0.1 in 1% aqueous Tergitol 7; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Acetonitrile	2460	2340	2030 - 2700	NA	Carworth Farms Wistar or Nelson albino rats; 90-112g	female	oral gastric intubation	0.1 in 1% aqueous Tergitol 7; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	2460	1600 - 2780	NA	Carworth Farms Wistar or Nelson albino rats; 90-120g	male	oral gastric intubation	0.1 in water; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	2460	NA	NA	rat	NA	oral	NA	NA	Duplicate record. Assumed to be the same values from Pozzani et al. (1959), Mellon Institute and Union Carbide.	NA	UCDS** Bibliographic Data: Union Carbide Data Sheet. (Union Carbide Corp., 39 Old Ridgebury Rd., Danbury, CT 06817) (see Pozzani et al. 1959) **(RTECS REFERENCE)**
Acetonitrile	2460	2830	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels=2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Acetonitrile	2460	3064 (3.9 mL/kg; sp. density = 0.7857; convert LD50 to mg/kg)	2593 - 3614 (95% CL; 3.3 - 4.6 mL/kg; sp. density = 0.7857; convert LD50 to mg/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 80-160 g; young adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Acetonitrile	2460	3360	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels=2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Acetonitrile	2460	3457 (4.4 mL/kg; sp. density = 0.7857; convert LD50 to mg/kg)	2200 - 5343 (95% CL; 2.8 - 6.8 mL/kg; sp. density = 0.7857; convert LD50 to mg/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 300-470 g; older adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Acetonitrile	2460	3504 (4.47 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	2187 - 5613 (2.79 - 7.16 mL/kg; sp. density is 0.7839; convert LD50 to mg/kg)	NA	Carworth Farms Wistar or Nelson albino rats; 84-114 g	male	oral gastric intubation	undiluted cmpd; single dose	NA	fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	3520 (4.49 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	1419 - 8748 (1.81 - 11.16 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	NA	Carworth Farms Wistar or Nelson albino rats; 90-120g	male	oral gastric intubation	undiluted cmpd; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	3570	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels=2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Acetonitrile	2460	3717 (4.49 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	1921 - 6436 (2.45 - 8.21mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	NA	Carworth Farms Wistar or Nelson albino rats; 250 - 318 g; yearlings	female	oral gastric intubation	undiluted cmpd; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	3800	NA	based on assumption that probit mortality vs log dose has same slope as similar chemical	Sherman rats; 90 -120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; doses (in g/kg) differ by 1 log to bracket LD50, then refine LD50 with doses in a series of antilog 1.1, 1.3, 1.5, etc	LD50 based on mortalities during a 14 day period	6 rats/dose at doses (in g/kg) that differ by 1 log to bracket LD50 (given 1 week apart); then refined LD50 with 10 rats/dose in a dose series of antilog 1.1, 1.3, 1.5, etc.; assumed to use materials/methods of Smyth & Carpenter (1944) except for reported changes. RC reference	reagent grade	Smyth HF Jr, Carpenter CP. 1948. Further experience with the range finding test in the industrial toxicology laboratory. J Ind Hyg Toxicol 30:63-68. *(RC and 1983/84 RTECS LD50 value)* Smyth HF Jr, Carpenter CP. 1944. The place of the range-finding test in the industrial toxicology laborotory. J Ind Hyg Toxicol 26:269-273. (most materials/methods)
Acetonitrile	2460	4050		Litchfield and Wilcoxon (1948)	Sprague-Dawley rats; 175-260 g		oral	undiluted; 3220 - 4970 mg/kg doses	observatons recorded frequently on the day of dosing, daily thereafter for 14 days; tremors, clonic/tonic convulsions, weight loss; clinical signs appeared within 3 hour after dosing and progessed to death in 24-72 hour	overnight fasted; groups of at least 5 rats per dose	99+%; Aldrich Chemical Co.	Freeman JJ, Hayes EP. 1985. Acetone potentiation of acute acetonitrile toxicity in rats. J Toxicol Environ Hlth 15:609-621. *Rutgers University, Piscataway, NJ*
Acetonitrile	2460	4240	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight		Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Acetonitrile	2460	4490	2460 - 8210	NA	Carworth Farms Wistar or Nelson albino rats; 240-425 g; yearlings	female	oral gastric intubation	0.1 in 1% aqueous Tergitol 7; single dose		non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	4850	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight		Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Acetonitrile	2460	5244 (6.69 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	3222 - 8545 (1.34 - 3.22 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	NA	Carworth Farms Wistar or Nelson albino rats; 82-109 g	male	oral gastric intubation	undiluted cmpd; single dose	NA	fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	5450	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Acetonitrile	2460	5900	4580 - 7220	NA	rats; 220 +/- 40 g	NA	oral; intragastric	NA	NA	(source of information not provided)	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Acetonitrile	2460	6498 (8.27 mL/kg; sp. density = 0.7857; convert LD50 to mg/kg)	NA	Thompson method; Weil tables	Carworth-Wistar rats; 90 - 120 g; 4 - 5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 10 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF, Weil CS, West JS, Carpenter CP. 1970. An exploration of joint toxic action:II. Equitoxic versus equivolume mixtures. Toxicol Appl Pharmacol 17:498-503. (LD50 value) Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., Striegel, JA. And Nycum, JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30:470-476. *Carnegie-Mellon University, Pittsburgh, PA.* Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., and Striegel, JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters).
Acetonitrile	2460	6500	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Acetonitrile	2460	6687 (8.53 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	4797 - 9328 (6.12 - 11.9 mL/kg; sp. density = 0.7839; convert LD50 to mg/kg)	NA	Carworth Farms Wistar or Nelson albino rats; 90-114 g	female	oral gastric intubation	undiluted cmpd; single dose	NA	non-fasted	Union Carbide Chemicals Company	Pozzani UC, Carpenter CP, Palm PE, Weil CS, Nair JH. 1959. An investigation of the mammalian toxicity of acetonitrile. J Occup Med 1: 634-642. *Mellon Institute, Pittsburgh, PA*
Acetonitrile	2460	8120	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Acetylsalicylic acid	200	200	NA	NA	NA	NA	NA	NA	NA	RTECS reference for 200 mg/kg (from Deichman 1969) is a typo; this is a secondary reference which cites Caldwell and Boyd 1966; the value should be 920 mg/kg	NA	Toxicology of Drugs and Chemicals. Deichmann, W.B., New York, Academic Press, Inc., 1969 **(RTECS REFERENCE)**
Acetylsalicylic acid	200	616	+/- 46 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 100 days	female	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Acetylsalicylic acid	200	920	+/- 43 (S.E.)	Croxton (1953) and Waugh (1952)	Wistar albino rats; 213 +/- 16 g; 3-5 months	female	oral; stomach tube	single dose; suspension of cmpd in 0.2% gum tragcanth solution in distilled water; 15 mL/kg dose; dose (mg/kg), rats per dose: 0-14; 750-10; 875-10; 1000-10;1125-10; 1250-2; 1500 2; 2000-2	within 1 hour of dosing rats were drowsy, withdrawn, hearing and vision impared, confused, tense, liquid stool, nasal bleeding, convulsionsrespiratory failure, cardiovascular shock	fasted overnight (16 hour); 60 rats used; 26/46 rats dead from compound	USP grade	Boyd EM. 1959. The acute oral toxicity of acetylsalicylic acid. Toxic Appl Pharmac 1: 229-239. *Queen's University, Ontario, Canada*
Acetylsalicylic acid	200	1360	NA	Reed and Muench (1938)	Wistar albino rats	male and female (75% male)	oral; stomach tube	single dose; solution in 2% acaia in physiological saline; volume of dose is 10 mL/kg	observed for one week; more than 80% of fatalities occurred within 48 hour	182 rats used; fasted for 18 hour	G.D. Searle and Co.	Eagle E, Carlson AJ. 1950. Toxicity, antipyretic and analgesic studies on 39 compounds including aspirin, phenacetin and 27 derivatives of carbazole and tetrahydrocarbazole. J Pharm Exp Ther 99:450-457. *University of Chicago, Chicago, IL*
Acetylsalicylic acid	200	1430	1065 - 1921 (95% CL)	Litchfield and Wilcoxon method (1949)	HLA strain albino rats; 95-180 g (mean wt. 122 g)	male	oral intubation	10-20 mL/kg in 10% gum acacia suspension; 4 doses	toxic effects included neurological abnormality; LD50 at 168 hour (7days); same result at 96 hour; observed at 24 & 48 hour with higher LD50	rats fasted 15-17 hours before dosing and for 6 hours after intubation; 40 rats used (10/dose)	NA	Boxill GC, Nash CB, Wheeler AG. 1958. Comparative pharmacological and toxicological evaluation of N-acetyl-p-aminophenol, salicylamide, and acetylsalicylic acid. J Am Pharm Assoc 47:479-487.
Acetylsalicylic acid	200	1430	1065 - 1921 (95% CL)	Litchfield and Wilcoxon method (1949)	HLA strain albino rats; 95-180 g (mean wt. 122 g)	male	oral intubation	10-20 mL/kg in 10% gum acacia suspension; 4 doses	toxic effects included neurological abnormality; this LD50 at 96 hour (same as 158 hour); observed at 24 & 48 hour with higher LD50	rats fasted 15-17 hours before dosing and for 6 hours after intubation; 40 rats used (10/dose)		Boxill GC, Nash CB, Wheeler AG. 1958. Comparative pharmacological and toxicological evaluation of N-acetyl-p-aminophenol, salicylamide, and acetylsalicylic acid. J Am Pharm Assoc 47:479-487.

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Acetylsalicylic acid	200	1459 (value converted from mM/kg to mg/kg)	1009 - 2108 (95% CL)	Weil (1952)	Homozygous Gunn rat (Gunn strain bred from mutant Wistar stock); 137-230 g	male	oral; gastric lavage	single dose; solution in 0.5 - 1.0% (w/v) aqueous methyl cellulose; 10 mL/kg dose vol.; low dose (mg/kg): 176.6, 281.1, 450.4, 720.7, 1153.1; high dose (mg/kg): 450.4, 720.7, 1153.1, 1844.9, 2951.2	low dose experiment observed for 3 days; high dose observed for 7 days; LD50 determined at 7 days; dose (mg/kg), rats dead per dose: 176.6-0/6; 281.1-0/6; 450.4-0/12; 720.7-1/12; 1153.1-1/12; 1844.9-5/6; 2951.2-5/6	fasted overnight (16 hour); 6 rats per dose; 60 rats used	NA	Axelsen RA. 1976. Analgesic-induced renal papillary necrosis in the Gunn rat: the comparative nephrotoxicity of aspirin and phenacetin. J Path 120:145-150. *University of Queensland, Queensland, Australia*
Acetylsalicylic acid	200	1500	NA	determined graphically	rats	NA	oral; stomach tube	aqueous with gum ragacanth (cmpd at 5 - 10% concen)	rats dead within 48 hours considered for determination of LD50	15 rats used	NA	Hart ER. 1947. The toxicity and analgetic potency of salicylamide and certain of its derivatives as compared with established analgetic-antipyretic drugs. J Pharmacol Exp Ther 89:205-209. *Jefferson Medical College, Philadelphia, PA*
Acetylsalicylic acid	200	1500	NA	Litchfield and Wilcoxon	Wistar rats; 130-150 g	male and female	stomach tube	5 mL/kg bw in 1% carboxymethylcellulose	observed 3 weeks	Fasted 18 hour before dosing	NA	Kammerer F-J, Schleyerbach R. 1987. U.S. Patent 4,636,513. Isoxazole derivatives and medicaments containing these compounds (January 13, 1987). **(RTECS REFERENCE)**
Acetylsalicylic acid	200	1523	NA	NA	Upjohn Sprague-Dawley strain albino rats; ~140 g; young	male	oral	single dose; cmpd suspended in 1% aqueous carboxymethylcellulose; 13 dose groups from 400 - 2500 mg/kg	observed for 7 days post-treatment; most deaths occurred during the first day; frequently, animals observed in convulsions prior to death	fasted overnight (12+ hour); 5 rats per dose; 65 rats used	NA	Gray JE, Jones PM, Feeenstra ES. 1960. Comparative effect of acetylsalicylic acid and acetylsalicylic acid anhydride on the non-glandular portion of the stomach. Toxic Appl Pharmac 2:514-522. *The Upjohn Company, Kalamazoo, MI*
Acetylsalicylic acid	200	1528	+/- 156 (S.E.)	Miller and Tainter (1944)	Charles River CD and Sprague-Dawley rat strains; > 100 g	NA	oral intubation	dose up to 50 mL/kg	rats observed for 7 days; observed up to 14 days when heavy metals or other compounds that produce latent death were investigated	fasted overnight	NA	Veary RA, Benish RA, Finkelstein M. 1966. Acute Toxicity of Drugs in Newborn Animals. Journal of Pediatrics. 69 (4):663-667. *Dept. of Veterinary Preventive Medicine, Ohio State University, Columbus, OH*
Acetylsalicylic acid	200	1600	1194 - 2144 (95% CL)	Litchfield and Wilcoxon method (1949)	HLA strain albino rats; 95-180 g (mean wt. 122 g)	male	oral intubation	10-20 mL/kg in 10% gum acacia suspension; 4 doses	toxic effects included neurological abnormality; this LD50 at 24 hour (same as 48 hour); observed at 96 & 168 hour with lower LD50	rats fasted 15-17 hours before dosing and for 6 hours after intubation; 40 rats used (10/dose)	NA	Boxill GC, Nash CB, Wheeler AG. 1958. Comparative pharmacological and toxicological evaluation of N-acetyl-p-aminophenol, salicylamide, and acetylsalicylic acid. J Am Pharm Assoc 47:479-487.
Acetylsalicylic acid	200	1600	1194 - 2144 (95% CL)	Litchfield and Wilcoxon method (1949)	HLA strain albino rats; 95-180 g (mean wt. 122 g)	male	oral intubation	10-20 mL/kg in 10% gum acacia suspension; 4 doses	toxic effects included neurological abnormality; this LD50 at 48 hour (same as 24 hour); observed at 96 & 168 hour with lower LD50	rats fasted 15-16 hours before dosing and for 6 hours after intubation; 10 rats used	NA	Boxill GC, Nash CB, Wheeler AG. 1958. Comparative pharmacological and toxicological evaluation of N-acetyl-p-aminophenol, salicylamide, and acetylsalicylic acid. J Am Pharm Assoc 47:479-487.
Acetylsalicylic acid	200	1761	+/- 162 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 100 days	male	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Acetylsalicylic acid	200	1880	1528 - 2312 (95% CL; slope = 1.27)	Litchfield and Wilcoxon method (1949)	Wistar SPF rats; 150-200 g	female	oral	cmpd suspended in a solution of 10% gum arabic in distilled water	observed for 7 days post-treatment	10 animals per dose	NA	Zapatero J, Sanfeliu C, Bruseghini L. 1981. Toxicological studies of Plafibride Part 1: Acute toxicity and its determination after several administrations of plafibride. Arsneim Forsch 31:1816-1819. *Chemical Pharmaceutical Research Centre, Barcelona, Spain*
Acetylsalicylic acid	200	1960	1441 - 2666 (95% CL; slope = 1.64)	Litchfield and Wilcoxon method (1949)	Wistar SPF rats; 150-200 g;	male	oral	cmpd suspended in a solution of 10% gum arabic in distilled water	observed for 7 days	10 animals per dose	NA	Zapatero J, Sanfeliu C, Bruseghini L. 1981. Toxicological studies of Plafibride Part 1: Acute toxicity and its determination after several administrations of plafibride. Arsneim Forsch 31:1816-1819. *Chemical Pharmaceutical Research Centre, Barcelona, Spain*
Acetylsalicylic acid	200	1992	1692 - 2345 (95% CL; slope = 1.45)	Litchfield and Wilcoxon method (1949)	Wistar SPF rats; 150-200 g;	male and female	oral	cmpd suspended in a solution of 10% gum arabic in distilled water	observed for 7 days post-treatment	10 animals per dose	NA	Zapatero J, Sanfeliu C, Bruseghini L. 1981. Toxicological studies of Plafibride Part 1: Acute toxicity and its determination after several administrations of plafibride. Arsneim Forsch 31:1816-1819. *Chemical Pharmaceutical Research Centre, Barcelona, Spain*
Acetylsalicylic acid	200	> 2000		Litchfield and Wilcoxon method (1949)	Sprague-Dawley SPF rats (Charles River, France); 100-110 g	male	oral	suspended in 0.25% carboxymethylcellulose with 0.2% polysorbate 80; doses in geometrical progression	observed for 7 days post-treatment; rats presented no signs	10 animals per dose; fasted 6 h prior to dosing	NA	Glomot R, Chevalier B, Vannier B. 1976. Toxicological studies on floctafenine. Toxicol Appl Pharmac 36:173-185.
Acetylsalicylic acid	200	> 2000		Litchfield and Wilcoxon method (1949)	Sprague-Dawley SPF rats (Charles River, France); 100-110 g	female	oral	suspended in 0.25% carboxymethylcellulose with 0.2% polysorbate 80; doses in geometrical progression	observed for 7 days post-treatment; rats presented no signs	10 animals per dose; fasted 6 h prior to dosing	NA	Glomot R, Chevalier B, Vannier B. 1976. Toxicological studies on floctafenine. Toxicol Appl Pharmac 36:173-185.
Acetylsalicylic acid	200	2840	2075 - 3890 (95% CL)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley CD strain albino rats	male	oral; gavage	single dose; 5 mL/kg dose; min. of 3 dose levels; cmpd suspended in solution of 1% gum acacia vehicle	observed for 7 days post-treatment; LD50 based on number of deaths at 7 days	20 animals per dose level; 60 animals used; not fasted	Aldrich Chemical Company	Sofia RD. 1977. Alteration of hepatic microsomal enzyme systems and the lethal action of non-steroidal anti-arthritic drugs in acute and chronic models of inflammation. Agents and Actions 7: 289-297. *Wallace Laboratories, Cranbury, NJ*
Aminopterin	NA	7	NA	Maximum likelihood estimation using log probit model (BMDS by US EPA)	Wistar albino rats; 100-200 g	male and female	oral	used measured samples neutralized before drying or added 2 molar eq NaHCO3 to weighed amounts of free acid; in 09% NaCl at 1 mL/100 g bw	observed for 14 days; deaths delayed until 3rd day; moderate weight loss by 1st day; intoxicated animals lost 20% by 3rd day; severe, watery diarrhea after 48 hour; yellowish brown feces, terminally, grossly stained with blood; deaths/dose: 40 mg/kg-5/6 (3 at 3-4 days, 2 at 5-7 days), 20 mg/kg-5/6 (2 at 3-4 days, 2 at 5-7 days, 1 at 8-14 days), 10 mg/kg-4/6 (3 at 3-4 days, 1 at 5-7 days); 5 mg/kg-2/6 (1 at 3-4 days, 1 at 8-14 days), 2.5 mg/kg-2/6 (2 at 3-4 days), 1.25 mg/kg-0/6	LD50 calculated by NICEATM; 36 rats used	ampuled and bulk samples from Lederle Laboraotries	Philips FS, Thiersch JB. 1949. Studies of the actions of 4-amino-pteroylglutamic acid in rats and mice. J Pharmacol Exp Ther 95:303-311.
Amitriptyline	320	320	286 - 359	Litchfield and Wilcoxon method (1949)	rats	NA	oral	NA	lethality counted after 7 days	40-50 rats used; reference in German	NA	Ribbentrop VA, Schaumann W. 1965. Pharmakologische Untersuchungen mit Doxepin, einem Antidepressivum mit zentral anticholinerger und sedierender Wirkung. Arzneimittel-Forschung 15:863-868. *Aus den Pharmakologischen Laboratorien der Firma C.F. Boehringer & Soehne Gmbh, Mannheim, Germany* **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Amitriptyline	320	380	300 - 486 (95% CL)	Litchfield and Wilcoxon method (1949)	Wistar strain rats; 200 -300 g	male	oral	NA	72 hour observations	8 rats per group used; hydrochloride salt	NA	Tobe A, Yoshida Y, Ikoma H, Tonomura S, Kikumoto R. 1981. Pharmacological evaluation of 2-(4-methylaminobutoxy)diphenylmethane hydrochloride (MCI-2016), a new psychotropic drug with antidepressant activity. Arzneimittelforschung 31(8):1278-85.
Arsenic III trioxide	14.6	13	NA	NA	rats		oral; stomach tube	NA	violent gastroenteritis, diarrhea, rice water stools	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletine (Association of Food and Drug Officials of the United States). Vol.15:122-133. *U.S. FDA*
Arsenic III trioxide	14.6	14.6	NA	NA	rats	male	oral	NA	no clinical picture given	reference is in Russian; not translated	NA	Tulakino NV, Novikov JV. 1987. On the question of reglameutation of arsenic in drinking water of different hardness. Gigiena i Sanitariya. 52 (1):21 -24. **(RTECS REFERENCE)**
Arsenic III trioxide	14.6	19.9 (15.1 mg As/kg)	+/- 2.4 (reported as +/- 1.8 mg As/kg)	de Beer (1945)	Sprague-Dawley Albino rats; 125 - 200 g	male	oral; intra-esophageally	pure arsenic trioxide dissolved in distilled water; 0.03 mL per g of bw; max volume 8 mL; dose range 10 - 50 mg As/kg	observed over 96 hours for LD50; experiment lasted 2 weeks; no significance between male or female; 95 dead at 24 hour; No of deaths/dose at 96 hour (male): 10 mg As/kg - 9/30; 20 mg As/kg - 20/30; 30 mg As/kg - 27/30; 40 mg As/kg - 28/30; 50 mg As/kg - 30/30	rats fasted 24 hour before dosing; 5 groups of 30 rats each (150 total); male and female rats tested; results and information given for male	99.999% pure	Harrison JWE, Packman EW, Abbott DD. 1958. Acute oral toxicity and chemical and physical properties of arsenic trioxides. AMA Arch ind Health, 17:118-123. *LaWall and Harrison Research Laboratories*
Arsenic III trioxide	14.6	32.6	28.4 - 36.7 (95% CI)	Finney (1971). Probit Analysis.	NA	male	intubated; single dose	dissolved in distilled water; administered by gavage in volume of 2mL/kg	rats dosed with one of 5 or 6 doses of chemical; deaths recorded daily for 7 days	animals acclimated to environment for 2 weeks before testing; used only healthy rats; all rats assigned to one of 5 to 6 groups of 8 to 10 rats each	Mallinckrodt	Pryor GT, Uyeno ET, et al. 1983. "Assessment of chemicals using a battery of neurobehavioral tests: a comparative study." Neurobehav Toxicol Teratol 5(1): 91-117. *SRI International, Menlo Park, CA; NIEHS, Research Triangle Park, NC*
Arsenic III trioxide	14.6	81.5	70.5 - 94.3	Bliss-Probit method	Sprague-Dawley rats; 5 weeks	male	oral gavage	dissolved in saline; range (mg/kg) of doses 51.2, 66.5, 86.5, 112.5, 146.2	rats observed at 6 hours after dosing and a once a day for 1 - 2 weeks; most rats found dead within 3 days; 27 of 50 rats died; toxic symptoms: vomiting and diarrhea; No of deaths/dose (mg As/kg) at 14 days: 51.2 mg - 0/10; 66.5 mg - 2/10; 86.5 mg - 6/10; 112.5 mg - 9/10; 146.2 mg - 10/10	animals acclimated to environment for 1 week before testing; 5 groups of10 rats each; fasted 16 hours before dosing; 100% lethal dose = 143.2 mg/kg; 0% lethal dose = 51.2 mg/kg	Kishida Chemical Co., Ltd.	Kitagawa H, Saito H, Sugimoto T, Yanaura S, Kitagawa H, Hosokawa T, Sakamoto K. 1982. Effects of diispropyl-1,3-dithiol-2-ylidene malonate (NKK-105) on acute toxicity of various drugs and heavy metals. J Toxicol Sci 7(2):123-34. *Chiba University; Hoshi College of Pharmacy; Showa University -- Japan*
Arsenic III trioxide	14.6	138	+/- 13 (standard error)	Litchfield and Fetig (1941)	wild Norway rats (trapped in Baltimore, MD); 148-493 g (ave = 253 g), adult	male and female	oral gavage	chemical suspended in 10% acacia solution; received appropriate doses in 1mL per 100 g bw	rats survived from 6 - 72 hours	41 rats used (approx. equal number of male and female); overnight fasting before dosing; assays performed in winter, repeated in summer; LD50s from combined information; final LD50 higher than winter LD50; attributed to not having enough rats in winter.	Merck U.S.P.	Dieke SH, Richter CP. 1946. Comparative assays of rodenticides on wild Norway rats. I. Toxicity. Publ. Health Rep 61:672-679. *Johns Hopkins Hospital, Baltimore, MD*
Arsenic III trioxide	14.6	140	NA	statistical formula based on mortality rates	wild Norway rats	unknown	oral, stomach tube; single dose	a number of individual doses of a cmpd, each dose at a different conc level are given to an equal number of test animals	enteritis and neuritis	NA	NA	Peardon DL, Kilbourn E, et al. 1972. "New selective rodenticides." Soap Cosmet Chem Spec 48(12):6. *Rohm and Haas Company, Philadelphia, PA*
Arsenic III trioxide	14.6	191.8 (145.2 mg As/kg)	+/- 11.5 (reported as +/- 8.7 mg As/kg)	de Beer (1945)	Sprague-Dawley Albino rats; 125- 200 g	male	oral	pure arsenic trioxide incorporated into 3 g rat Purina chow; rats consumed meal in 1 hour; dose range 301 - 338 mg As/kg	observed over 96 hours for LD50; 2 week experiment; no significance between male or female; 76 dead at 24 hour; No of deaths/dose (mg As/kg) at 96 hour: 301 mg - 0/20; 91 mg - 2/20; 1281 mg - 6/20; 1809 mg -12/20; 2078 mg -18/20; 269 mg - 20/20; 338 mg - 20/20	rats fasted 24 hour before dosing; 7 groups of 20 rats each (140 total); male and female rats tested; results and information given for male	99.999% pure	Harrison JWE, Packman EW, Abbott DD. 1958. Acute oral toxicity and chemical and physical properties of arsenic trioxides. AMA Arch ind Health 17:118-123. *LaWall and Harrison Research Laboratories*
Arsenic III trioxide	14.6	385	350 - 424 (95% CL)	Litchfield and Wilcoxon method	Holtsman rats; 300 -500 g; 100-300 days old (13 - 41 weeks)	male and female	oral; gelatin capsules	20, 50, 100, 250, 500, 750, 1000 (all in mg/kg)	rats dosed under light anesthesia; death occurred within 4 days	approximately 70 rats used; 24 hour fasting before dosing	Baker Analyzed Reagent with 0.02% impurities	Done AK and Peart AJ. 1971. Acute Toxicities of Arsenical Herbicides. Cinical Toxicology, 4(3):343 - 355. *University of Utah, Salt Lake City, UT*
Atropine sulfate	600	600	530 - 675	Litchfield and Wilcoxon method	rats	NA	oral	NA	NA	reference in German	NA	Wirth W, Gosswald R. 1965. Pharmakologische Untersuchungen in der Reihe der Diphenylcarbamidsaurethioester. Arch Int Pharmacodyn 155 (2):393 - 417. **(RTECS REFERENCE)**
Atropine sulfate	600	622	+/- 36	NA	Sprague-Dawley rats; from Charles River; adult	male	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185 -207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.*
Atropine sulfate	600	698.7	629.2 - 776.0	Bliss-Probit method	Sprague-Dawley rats; 5 weeks	male	oral gavage	dissolved in saline; range (mg/kg) of doses 500, 625, 781, 977	rats observed at 6 hours after dosing and a once a day for 1 - 2 weeks; most rats dead within 3 days; 20 of 40 rats died; toxic symptoms: decrease of spontaneous movement, myasthenia and coma observed at 10 minutes; stretching of the limbs, abdominal posture, anaerosis and cardiac arrest after convulsions; dose (mg/kg), dead rats per dose: 500 - 1/10; 625 -- 4/10; 781 -- 6/10; 977 -- 10/10	animals acclimated to environment for 1 week before testing; 4 groups of 10 rats each; fasted 16 hours before dosing; 100% lethal dose = 977 mg/kg; 0% lethal dose = 500 mg/kg	Tokyo Kasei Kogyo Co.	Kitagawa H, Saito H, Sugimoto T, Yanaura S, Kitagawa H, Hosokawa T, Sakamoto K. 1982. Effects of diispropyl-1,3-dithiol-2-ylidene malonate (NKK-105) on acute toxicity of various drugs and heavy metals. J Toxicol Sci 7(2):123-34. *Chiba University; Hoshi College of Pharmacy; Showa University -- Japan*
Atropine sulfate	600	840	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	200, 400, 800, 1000, 1600 mg/kg	200 mg/kg: 0/3 dead; 400 mg/kg: 0/3 dead; 800 mg/kg: 1/3 dead; 1600 mg/kg: 3/3 dead; 4 of 12 rats dead; LD50 based on 12 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 1000 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 900 mg/kg	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group, 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Atropine sulfate	600	874	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	200, 400, 800, 1000, 1600 mg/kg	200 mg/kg: 0/5 dead; 400 mg/kg: 0/5 dead; 800 mg/kg: 1/5 dead; 1600 mg/kg: 5/5 dead; 6 of 20 rats dead; LD50 based on 20 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 1000 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 950 mg/kg	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group; 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Atropine sulfate	600	878	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	200, 400, 800, 1000, 1600 mg/kg	200 mg/kg: 0/11 dead; 400 mg/kg: 0/11 dead; 800 mg/kg: 2/11 dead; 1600 mg/kg: 11/11 dead; 13 of 44 rats dead; LD50 based on 44 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 1000 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 900 mg/kg	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group; 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Atropine sulfate	600	1135	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	200, 400, 800, 1000, 1600 mg/kg	200 mg/kg: 0/1 dead; 400 mg/kg: 0/1 dead; 800 mg/kg: 0/1 dead; 1600 mg/kg: 1/1 dead; 1 of 4 rats dead; LD50 based on 4 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 1000 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 950 mg/kg	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group; 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Atropine sulfate	600	1136	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	200, 400, 800, 1000, 1600 mg/kg	200 mg/kg: 0/2 dead; 400 mg/kg: 0/2 dead; 800 mg/kg: 0/2 dead; 1600 mg/kg: 2/2 dead; 2 of 8 rats dead; LD50 based on 8 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 1000 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 950 mg/kg	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group; 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Boric acid	2662	2660	+/- 220 (S.E.; slope = 7.7)	Litchfield and Fetig (1941)	rats	NA	oral	NA	NA	45 rats used	NA	Pfeiffer CC, Hallman LF, Gersh IG. 1945. Boric Acid Ointment. A study of possible intoxication in the treatment of burns. Journal of the American Medical Association 128:266 - 274. *National Naval Medical Center, Bethesda, MD* **(RTECS REFERENCE)**
Boric acid	2662	2660	+/- 200 (S.E.)	NA	rats; 220 +/- 40 g		oral; intragastric	NA	NA	(source of information not provided); reference in Russian;	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Boric acid	2662	3160 (estimate)	NA	NA	Long Evans rats from Diablo Laboratories; 85-118 g	male	oral; stomach intubation	50% w/v in distilled water suspension	observed for 14 days; signs included depression, ataxia, convulsion and death	fasted rats; 6 groups of 5 rats each; total of 30 rats	NA	Weir RJ Jr, Fisher RS. 1972. Toxicologic studies on borax and boric acid. Toxicol Appl Pharmac 23:351-364.
Boric acid	2662	3450	2950-4040 (CL)	NA	Albino Sprague-Dawley rats (Charles River SPF); 267-310 g	male	oral; stomach intubation	50% w/v in 0.5% aqueous methylcellulose suspension	observed for 14 days; signs included depression, ataxia, convulsion and death	fasted rats; 6 groups of 5 rats each; total of 30 rats	NA	Weir RJ Jr, Fisher RS. 1972. Toxicologic studies on borax and boric acid. Toxicol Appl Pharmac 23:351-364.
Boric acid	2662	4080	3640-4560 (CL)	NA	Albino Sprague-Dawley rats (Charles River SPF); 206-248 g	female	oral; stomach intubation	50% w/v in 0.5% aqueous methylcellulose suspension	observed for 14 days; signs included depression, ataxia, convulsion and death	fasted rats; 6 groups of 5 rats each; total of 30 rats	NA	Weir RJ Jr, Fisher RS. 1972. Toxicologic studies on borax and boric acid. Toxicol Appl Pharmac 23:351-364.
Boric acid	2662	5140	4750 - 5580 (range is +/- 1.96 S.D.)	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 200 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum, JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30: 470-476. *Carnegie-Mellon University, Pittsburgh, PA* (LD50 value) Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)
Busufan	110 (mouse) no rat oral value	2	NA	NA	NA	NA	NA	NA	NA	Value used by RC from 1983/84 RTECS. No rat oral LD50 in current RTECS. This study treated rats with 0.13 mg/kg busulfan, which was 7% LD50. LD50 = 1.9 mg/kg	NA	Schmahl D, Osswald H. 1970. Experimental studies on the carcinogenic effects of anticancer chemotherapeutics and immunosuppressive agents. Arzneimittelforschung. Oct;20(10):1461-1467.
Busufan	110 (mouse) no rat oral value	14	6 (SE)	probit method Finney (1962)	JO13 strain rats; 170-250 g; 10-12 weeks	male and female	oral	as aqueous emulsion with tragacanth powder	30 day observation	fasted rats; rats from CEN Breeding Centre Mol, Belgium from former L strain of Institute of Cancer	NA	Dunjic A, Cuvelier A-M. 1973. Survival of rat bone marrow cells after treatment with Myleran and Endoxan. Experimental Hematology 1:11-21.
Busufan	110 (mouse) no rat oral value	28	21 - 38 (95% CL)	NA	Sprague-Dawley strain rats	male	oral	doses (mg/kg): 20, 30, 40, 50, 100, 150, 200	observed for 14 days; doses (mg/kg, deaths at 14 days: 20 -- 1/5; 30 -- 2/5; 40, 50, 100, 150, and 200 -- 5/5	5 rats per dose; 35 rats used	NA	Kiso to Rinsho. Clinical Report. 1971. (Yubunsha Co., Ltd., 1-5, Kanda Suda-Cho, Chiyoda-ku, KS Bldg., Tokyo 101, Japan. 5(12): 1894. **(RTECS REFERENCE)**
Busufan	110 (mouse) no rat oral value	29	23 - 38 (95% CL)	NA	Sprague-Dawley strain rats	female	oral	doses (mg/kg): 10, 30, 40, 50, 100, 150, 200	observed for 14 days; doses (mg/kg, deaths at 14 days: 10 -- 1/5; 30 -- 2/5; 40 -- 4/5; 50, 100, 150, and 200 -- 5/5	5 rats per dose; 35 rats used	NA	Kiso to Rinsho. Clinical Report. 1971. (Yubunsha Co., Ltd., 1-5, Kanda Suda-Cho, Chiyoda-ku, KS Bldg., Tokyo 101, Japan. 5(12): 1894. **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Cadmium II chloride	88	47	43 - 51 (95% CL)	Thompson and Weil (1952); method of moving averages	albino rats; 2 weeks	male and female	oral; stomach tube	1 mL/200 g bw	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Cadmium II chloride	88	88	NA	NA	rats	NA	oral; stomach tube	NA	salivation, vomiting, diarrhea; onset within 30 minutes	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals	NA	Quarterly Bulletin--Association of Food and Drug Officials of the United States. (Denver, CO) V.3-38, 1939-74. Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletine (Association of Food and Drug Officials of the United States). Vol. 15:122 - 133. *U.S. Food and Drug Administration* **(RTECS REFERENCE)**
Cadmium II chloride	88	109	86 - 136 (95% CL)	Thompson and Weil (1952); method of moving averages	albino rats; 54 weeks	female	oral; stomach tube	1 mL/200g bw	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used;	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Cadmium II chloride	88	132	109.4 - 159.3 (95% CL)	Bliss-Probit method	Sprague-Dawley rats; 5 weeks	male	oral gavage	dissolved in saline; range (mg/kg) of doses 66.5, 86.5, 112.5, 146.2, 190.1, 247.1	rats observed at 6 hours after dosing and a once a day for 1 - 2 weeks; most rats found dead within 3 days; 29 of 60 rats died; toxic symptoms: drooling, diarrhea, nasal bleeding; dose (mg/kg), rats dead per dose: 66.5-0/10; 86.5-1/10; 112.5-3/10; 146.2-6/10; 190.1-9/10; 247.1-10/10	animals acclimated to environment for 1 week before testing; 6 groups of10 rats each; fasted 16 hours before dosing; 100% lethal dose = 247.1 mg/kg; 0% lethal dose = 66.5 mg/kg	MITSUWA Chemical Co., Ltd.	Kitagawa H, Saito H, Sugimoto T, Yanaura S, Kitagawa H, Hosokawa T, Sakamoto K. 1982. Effects of diospropyl-1,3-dithiol-2-ylidene malonate (NKK-105) on acute toxicity of various drugs and heavy metals. J Toxicol Sci 7(2):123-34. *Chiba University; Hoshi College of Pharmacy; Showa University -- Japan*
Cadmium II chloride	88	170	140 - 206 (95% CL)	Thompson and Weil (1952); method of moving averages	albino rats; 18 weeks	female	oral; stomach tube	1 mL/200 g bw	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Cadmium II chloride	88	211	182 - 252 (95% CL)	Thompson and Weil (1952); method of moving averages	albino rats; 6 weeks	female	oral; stomach tube	1 mL/200 g bw; 6 dose levels in each group	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic, T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Cadmium II chloride	88	240	198 - 291 (95% CL)	Thompson and Weil; 1952; method of moving averages	albino rats; 3 weeks	male and female	oral; stomach tube	1 mL/200 g bw; 6 dose levels in each group	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic, T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Caffeine	192	192	+/- 18 (S.E.)	NA	albino rats	NA	oral	NA	NA	see Boyd 1959	NA	Boyd EM. 1965. Caffeine addiction and drug toxicity. The Journal of New Drugs 5:252. (secondary reference) *Queen's University, Canada* **(RTECS REFERENCE)**
Caffeine	192	192	+/- 18 (S.E.)	NA	albino rats; 203 +/-28 g; 3-6 months	female	oral; stomach tube	aqueous solution; 2 mL/kg dose; 0 mg/kg-20 rats; 160 mg/kg-8 rats; 180 mg/kg-16 rats; 200 mg/kg-8 rats; 220 mg/kg-8 rats	19 rats survived; 21 rats died; death time 300 +/- 96 hours after dosing; survivors: lack of curiosity, weak, tense, hyperreflexia, ataxic, cataleptic stances, swollen and inflammed eyelids,loose stools, tremors, anorexia, loss of body weight, fluctuation in body temperature; normal clinical appearance at 72 hours; dead rats: similar clinical signs as survivors, clinical deterioration progressive from 10th hour till death, didn't eat or drink, diarrhea, loss of body weight, anuric, drop in body temp.; two-thirds died of respiratory failure following tetanic convulsions; remainder died of cardiovascular collapse	fasted for 16 hours; 60 rats used	NA	Boyd EM. 1959. The acute oral toxicity of caffeine. Toxic Appl Pharmac 1: 250-257. *Queen's University, Ontario, Canada*
Caffeine	192	247	220 - 277 (95% CL; slope=7.7)	Cornfield and Mantel (1950)	Sprague-Dawley CD rats; mean wt. of 164 g; young adult	female	oral intubation	single dose	observed for 15 days; death usually 1-2 days after dosing; diarrhea, wt loss/gain; 40% of female rats died	15 rats per dose level; 16 hour fasting before dosing; 5 -6 dose levels; 75-90 rats	Schwarz/Mann - Becton Dickinson Co.	Palm PE, Arnold EP, Rachwall PC, Leyczeck JC, Teague KW, Kensler CJ. 1978. Evaluation of the teratogenic potential of fresh brewed coffee and caffeine in the rat. Toxic Appl Pharmac 44:1 - 16. *Arthur D. Little, Inc., Cambridge, MA*
Caffeine	192	264	+/- 10 (S.E.)		CBL Wistar albino rats; 150-200 g	female	intragastric	single dose; range of 200 - 350 mg/kg; dissolved in distilled water; 20 mL/kg volume to each rat	observed for 5 days	no overnight fasting; 50 rats used; groups of 10 rats	Merck Reagent	Boyd EM, Dolman M, Knight LM, Sheppard EP. 1965. The chronic oral toxicity of caffeine. Canad J Physiol Pharm 43:995 - 1007. *Queen's University, Ontario, Canada*
Caffeine	192	279	259 - 302 (95% CI)	Probit analysis	Crl-CD rats; Charles River Breeding lab; 220 -280 g; 60 days old	male	oral; intragastric intubation	0.5 - 3.9% suspension; dissolved/suspended in corn oil; single dose; 100, 200, 250, 300, 500 mg/kg doses	observed daily for 14 days; death within 2 days; toxic symptoms: staining of the face, wet perineal area, slight weight loss, lacrimation, lethargy, diarrhea	fasted 24 hours before dosing; 5 groups of 10; 50 rats used; 19 rats died	99+% pure; Aldrich Chemical Co.	Dashiell OL, Kennedy GL Jr. 1984. The effects of fasting on the acute oral toxicity of nine chemicals in the rat. J Appl Toxicol 4(6): 320-325. *E.I. Du Pont de Nemours & Co., Newark, DE*
Caffeine	192	288	+/- 6 (S.E.)	Linear regression. Boyd (1965)	Wistar albino rats; 125-200 g	male	oral; intragastric dosing	dissolved in distilled water; 20 mL/kg dose; 14 doses ranging from 162 to 354 mg/kg; each dose given to 6 - 10 rats	observations recorded hourly 1st day then at 24 hour intervals; ave time to death is 14 hours; 1 - 40 hours range; cause of early deaths: tonic-clonic convulsions followed by resipiratory failure; for delayed death, immediate cause was hypothermic coma and respiratory failure following loss of corneal reflexes, impaired respiration, pallor, cyanosis, anuria; drop in colonic temperature; hypothermia appeared within 2 hours, peaked at 8 - 24 hour at which time it was dose dependent; hypothermia associated with stupor, anorexia, oligodipsia, loss of body weight, oliguria, aciduria, proteinuria	fasted for 16 hours; 84 - 140 rats used; unanethetized rats	U.S.P. grade	Boyd EM, Liu SJ, Singh J. 1968. The toxicity of aspirin, phenacetin, and caffeine following rectal administration. Clin Toxicol 1:425 - 430. *Queen's University, Ontario, Canada*
Caffeine	192	300	+/- 29 (S.E.)	Linear regression. Boyd (1965)	Wistar albino rats; 125-200 g	male	oral; intragastric dosing	dissolved in distilled water; 20 mL/kg dose; 14 doses ranging from 162 to 354 mg/kg; each dose given to 6 - 10 rats	observations recorded hourly 1st day then at 24 hour intervals; ave time to death is 14 hours; 1 - 40 hours range; cause of early deaths: tonic-clonic convulsions followed by resipiratory failure; for delayed death, immediate cause was hypothermic coma and respiratory failure following loss of corneal reflexes, impaired respiration, pallor, cyanosis, anuria; drop in colonic temperature; hypothermia appeared within 2 hours, peaked at 8 - 24 hour at which time it was dose dependent; hypothermia associated with stupor, anorexia, oligodipsia, loss of body weight, oliguria, aciduria, proteinuria	fasted for 16 hours; 84 - 140 rats used; rats used; rats given thiopental before dosing (anesthetized rats before dosing)	U.S.P. grade	Boyd EM, Liu SJ, Singh J. 1968. The toxicity of aspirin, phenacetin, and caffeine following rectal administration. Clin Toxicol 1:425 - 430. *Queen's University, Ontario, Canada*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Caffeine	192	310	+/- 33	NA	rats; 220 +/- 40 g	NA	oral; intragastric	NA	NA	(source of information not provided)	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Caffeine	192	344	307 - 383 (95% CI)	Probit analysis	Sprague-Dawley rats; 190-300 g	male	oral gavage	geometric progression of 14 for dosing	observed for 14 days after dosing;	fasted 18 - 20 hours before dosing; conventional LD50 method; groups of 10; 40 rats used	NA	Bruce RD. 1987. A confirmatory study of the up-and-down method for acute oral toxicity testing. Fundam Appl Toxicol 8(1): 97-100. *The Proctor and Gamble Co., Cincinnati, OH*
Caffeine	192	355	312 - 403 (95% CL; slope=5.1)	Cornfield and Mantel (1950)	Sprague Dawley CD rats; mean wt. of 210 g; young adult	male	oral intubation	single dose; dose in water	observed for 15 days; death usually 1-2 days after dosing; diarrhea, wt loss/gain; 21% of male mice died	15 rats per dose level; 16 hour fasting before dosing; 5 -6 dose levels; 75-90 rats	Schwarz/Mann - Becton Dickinson Co.	Palm PE, Arnold EP, Rachwall PC, Leyczech JC, Teague KW, Kensler CJ. 1978. Evaluation of the teratogenic potential of fresh brewed coffee and caffeine in the rat. Toxic Appl Pharmac 44:1 - 16. *Arthur D. Little, Inc., Cambridge, MA*
Caffeine	192	421	320 - 553 (95% CI)	Probit analysis	Sprague-Dawley rats; 190-300 g	male	oral gavage	NA	observed for 7 days	fasted 18 - 20 hours before dosing; Up-and-down LD50 method; 9 rats used	NA	Bruce RD. 1987. A confirmatory study of the up-and-down method for acute oral toxicity testing. Fundam Appl Toxicol 8(1): 97-100. *The Proctor and Gamble Co., Cincinnati, OH*
Caffeine	192	483	433 -562 (95% CI)	Probit analysis	Crl-CD rats; Charles River Breeding lab; 220 -280 g; 60 days old	male	oral; intragastric intubation	0.5 - 3.9% suspens; dissolved or suspended in corn oil; single dose; 300, 400, 450, 650 mg/kg doses	observed daily for 14 days; death within 3 days; toxic symptoms: staining of the face, wet perineal area, slight weight loss, lacrimation, lethargy, diarrhea	non fasted; 4 groups of 10; 40 rats used; 15 rats died	99+% pure; Aldrich Chemical Co.	Dashiell OL, Kennedy GL Jr. 1984. The effects of fasting on the acute oral toxicity of nine chemicals in the rat. J Appl Toxicol 4(6): 320-325. *E.I. Du Pont de Nemours & Co., Newark, DE*
Carbamazepine	1957	1957	NA	NA	rats	NA	oral	NA	NA	reference in Japanese	NA	Japanese Kokai Tokyo Koho Patents. 54-163823 (U.S. Patent and Trademark Office. 79-163823) **(RTECS REFERENCE)**
Carbamazepine	1957	4025	NA	NA	rats; 120-140 g	female	oral	suspension in arabica gum	observed for 8 days	reference paper in German; 20 animals per dose	NA	Stenger Von EG, Roulet FC. 1964. Zur Toxikologie des Antiepilepticum Tegretol. Medicina Experimentalis 11:191-201.
Carbon tetrachloride	2350	1020	861 - 1211 (95% CL)	Weil (1952)	Wistar-derived Porton strain rats (SPF); 100 - 160 g	male	oral gastric intubation	1:1 (v/v) mixture in liquid paraffin; lightly anesthetized w/ether; geometric doses by factor of 12 or 144	deaths observed for 1 week	18 hour fasting before dosing; 20 - 25 rats used; groups of 5 rats; normal stock diet	NA	McLean AEM, McLean EK. 1966. The effect of diet and 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) on microsomal hydroxilating enzymes and on sensitivity of rats to carbon tetrachloride poisoning. Biochem J 100:564-571. *Royal Free Hospital, London, UK*
Carbon tetrachloride	2350	2343	2136 - 2566 (95% CL)	Weil (1952)	Wistar-derived Porton strain rats (SPF); 100 - 160 g	male	oral gastric intubation	1:1 (v/v) mixture in liquid paraffin; lightly anesthetized w/ether; geometric doses by factor of 1.2 or 1.44	deaths observed for 1 week	18 hour fasting before dosing; 20 - 25 rats used; groups of 5 rats; protein-free diet; rats fed protein-free diet 1 - 3 weeks before dosing; continued protein-free diet through out observation period	NA	McLean AEM, McLean EK. 1966. The effect of diet and 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) on microsomal hydroxilating enzymes and on sensitivity of rats to carbon tetrachloride poisoning. Biochem J 100:564-571. *Royal Free Hospital, London, UK*
Carbon tetrachloride	2350	2350	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	1500, 2000, 2800, 3900 mg/kg	1500 mg/kg: 0/1 dead; 2000 mg/kg: 0/1 dead; 2800 mg/kg: 1/1 dead; 3900 mg/kg: 1/1 dead; 2 of 4 rats dead; LD50 based on 4 rats used	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group; 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Carbon tetrachloride	2350	2500	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	1500, 2000, 2800, 3900 mg/kg	1500 mg/kg: 0/2 dead; 2000 mg/kg: 2/2 dead; 2800 mg/kg: 1/2 dead; 3900 mg/kg: 2/2 dead; 5 of 8 rats dead; LD50 based on 8 rats used	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group, 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Carbon tetrachloride	2350	2500	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	1500, 2000, 2800, 3900 mg/kg	1500 mg/kg: 0/5 dead; 2000 mg/kg: 3/5 dead; 2800 mg/kg: 3/5 dead; 3900 mg/kg: 5/5 dead; 11 of 20 rats dead; LD50 based on 20 rats used	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group, 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Carbon tetrachloride	2350	2500	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	1500, 2000, 2800, 3900 mg/kg	1500 mg/kg: 0/11 dead; 2000 mg/kg: 5/11 dead; 2800 mg/kg: 6/11 dead; 3900 mg/kg: 11/11 dead; 22 of 44 rats dead; LD50 based on same rats used for other Lorke (1983) values	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group; 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Archives of Toxicology. (Springer-Verlag, Heidelberger Pl. 3, D-1000 Berlin 33, Fed. Rep. Ger.) V.32- 1974. Lorke D. 1983. "A new approach to practical acute toxicity testing." Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany* **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Carbon tetrachloride	2350	2821 (1.77 mL/kg; sp. density is 1.594; convert LD50 to mg/kg	NA	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 10 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF, Weil CS, West JS, Carpenter CP. (1970). An exploration of joint toxic action:II. Equitoxic versus equivolume mixtures. Toxicol Appl Pharmacol. 17:498-503. (LD50 value) Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30:470-476. *Carnegie-Mellon University, Pittsburgh, PA* Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)
Carbon tetrachloride	2350	2850	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	1500, 2000, 2800, 3900 mg/kg	1500 mg/kg: 0/3 dead; 2000 mg/kg: 0/3 dead; 2800 mg/kg: 1/3 dead; 3900 mg/kg: 3/3 dead; 4 of 412 rats dead; LD50 based on 12 rats used	rats acclimated for 5 days; rats observed for 14 days; 4 groups of rats used for each dose (1, 2, 3, 5 rats per group; 11 rats per dose); 9 rats for initial range finding;10 mg/kg - 0/3 dead; 100 mg/kg - 0/3 dead; 1000 mg/kg - 2/3 dead	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Carbon tetrachloride	2350	2920	2450 - 3470 (95% CL)	NA	rats	male and female	oral; stomach intubation	10 dosage levels; suspended in corn oilk with acacia; single dose	190 rats used	NA	NA	McCollister DD, Hollingsworth RL, Oyen F, Rowe VK. 1955. Comparative inhalation toxicity of fumigant mixtures. Arch Ind Health pp.1 - 7. *Dow Chemical, Midland, MI*
Carbon tetrachloride	2350	2981 (1.87 mL/kg; sp. density is 1.594; convert LD50 to mg/kg)	slope = 1.62	Litchfield and Wilcoxon method (1949)	Scho:Wistar C rats; 150-180 g; 56 +/- 2 days	female	oral	single dose; 50 mg/kg bw carbon tetrachloride in 5 mL peanut oil/kg bw	48 hour observation; LD50 determined on rats monthly for a year and average reported for whole year	reference in German; year 4	NA	Von Schmidt P, Wolff DL, Burck D, Wilhelm M. 1979. Sensitivity of female Wistar rats to carbon tetrachloride, determined by the LD50, and the hexobarbital sleeping time after a single oral dose. Z Versuchstierkd. 21(3):153-162. *Zentralinstitut fur Arbeitsmedizin der DDR, Berlin, Germany*
Carbon tetrachloride	2350	3682 (2.31 mL/kg; sp. density is 1.594; convert LD50 to mg/kg)	slope = 1.83	Litchfield and Wilcoxon method (1949)	Scho:Wistar C rats; 150-180 g; 56 +/- 2 days	female	oral	single dose; 50 mg/kg bw carbon tetrachloride in 5 mL peanut oil/kg bw	48 hour observation; LD50 determined on rats monthly for a year and average reported for whole year	reference in German; year 3	NA	Von Schmidt P, Wolff DL, Burck D, Wilhelm M. 1979. Sensitivity of female Wistar rats to carbon tetrachloride, determined by the LD50, and the hexobarbital sleeping time after a single oral dose. Z Versuchstierkd. 21(3):153-162. *Zentralinstitut fur Arbeitsmedizin der DDR, Berlin, Germany*
Carbon tetrachloride	2350	4081 (2.56 mL/kg; sp. density is 1.594; convert LD50 to mg/kg)	slope = 1.60	Litchfield and Wilcoxon method (1949)	Zam:Wistar C rats; 150-180 g; 56 +/- 2 days	female	oral	single dose; 50 mg/kg bw carbon tetrachloride in 5 mL peanut oil/kg bw	48 hour observation; LD50 determined on rats monthly for a year and average reported for whole year	reference in German; year 4	NA	Von Schmidt P, Wolff DL, Burck D, Wilhelm M. 1979. Sensitivity of female Wistar rats to carbon tetrachloride, determined by the LD50, and the hexobarbital sleeping time after a single oral dose. Z Versuchstierkd. 21(3):153-162. *Zentralinstitut fur Arbeitsmedizin der DDR, Berlin, Germany*
Carbon tetrachloride	2350	4288 (2.69 ml/kg; sp.density is 1.594; converted LD50 to mg/kg	slope = 1.59	Litchfield and Wilcoxon method (1949)	Zam:Wistar C rats; 150-180 g; 56 +/- 2 days	female	oral	single dose; 50 mg/kg bw carbon tetrachloride in 5 mL peanut oil/kg bw	48 hour observation; LD50 determined on rats monthly for a year and average reported for whole year	reference in German; year 3	NA	Von Schmidt P, Wolff DL, Burck D, Wilhelm M. 1979. Sensitivity of female Wistar rats to carbon tetrachloride, determined by the LD50, and the hexobarbital sleeping time after a single oral dose. Z Versuchstierkd. 21(3):153-162. *Zentralinstitut fur Arbeitsmedizin der DDR, Berlin, Germany*
Carbon tetrachloride	2350	4336 (2.72 mL/kg; sp. density is 1.594; convert LD50 to mg/kg)	slope = 1.44	Litchfield and Wilcoxon method (1949)	Zam:Wistar C rats; 150-180 g; 56 +/- 2 days	female	oral	single dose; 50 mg/kg bw carbon tetrachloride in 5 mL peanut oil/kg bw	48 hour observation; LD50 determined on rats monthly for a year and average reported for whole year	reference in German; year 2	NA	Von Schmidt P, Wolff DL, Burck D, Wilhelm M. 1979. Sensitivity of female Wistar rats to carbon tetrachloride, determined by the LD50, and the hexobarbital sleeping time after a single oral dose. Z Versuchstierkd. 21(3):153-162. *Zentralinstitut fur Arbeitsmedizin der DDR, Berlin, Germany*
Carbon tetrachloride	2350	4670 (2.93 mL/kg; sp. density is 1.594; convert LD50 to mg/kg)	slope = 1.57	Litchfield and Wilcoxon method (1949)	Zam:Wistar C rats; 150-180 g; 56 +/- 2 days	female	oral	single dose; 50 mg/kg bw carbon tetrachloride in 5 mL peanut oil/kg bw	48 hour observation; LD50 determined on rats monthly for a year and average reported for whole year	reference in German; year 1	NA	Von Schmidt P, Wolff DL, Burck D, Wilhelm M. 1979. Sensitivity of female Wistar rats to carbon tetrachloride, determined by the LD50, and the hexobarbital sleeping time after a single oral dose. Z Versuchstierkd. 21(3):153-162. *Zentralinstitut fur Arbeitsmedizin der DDR, Berlin, Germany*
Carbon tetrachloride	2350	> 5000	NA	Dixon (1965) and Bruce (1985)	Fischer 344 rats; 77 days old at test	female	oral gavage	in deionized water; maximum volume dose 10 mL/kg; 5 dose levels: 0, 150, 500, 1500, 5000 mg/kg; single dose	7 day survival time	fasted overnight; initial dose levels = 100, 1000, and 5000 mg/kg; subsequent doses selected by up-and-down method (Bruce, 1985, 1987); 5 groups of 8 rats each; 40 rats used; 7 15 rats used in first LD50 estimate	analytical grad_; 99+% pure; Aldrich Chemical Co.	Berman E, Schlicht M, Moser VC, MacPhail RC. 1995. A multidisciplinary approach to toxicological screening: I. Systemic toxicity. J Toxicol Environ Health 45(2): 127-43. *Health Effects Res. Lab., U.S.EPA, Research Triangle Park, NC*
Carbon tetrachloride	2350	5453	4660 - 6404 (95% CI)	Probit analysis	Crl-CD rats from Charles River Breeding lab; 220-280 g; 60 days old	male	oral; intragastric intubation	15 - 45% solution dissolved or suspended in corn oil; single dose; 2500, 3000, 4000, 5000, 8000, 10000, 11000 mg/kg doses	observed daily for 14 days; death within 2 days; toxic symptoms: salivation, weakness, pallor, lethargy, diarrhea, weight loss	24 hour fast before dosing; 7 groups of 10; 70 rats used; 35 rats died; doses of 10000 mg/kg or greater administered in 2 portions at 15 minutes apart	99+% pure; E.I. Du Pont de Nemours	Dashiell OL, Kennedy GL Jr. 1984. The effects of fasting on the acute oral toxicity of nine chemicals in the rat. J Appl Toxicol 4(6): 320-325. *E.I. Du Pont de Nemours & Co., Newark, DE*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Carbon tetrachloride	2350	6200	5082 - 7564	NA	rats; 220 +/- 40 g		oral; intragastric	NA	NA	(source of information not provided)	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Carbon tetrachloride	2350	7540 (4.73 mL/kg; sp. density is 1.594; convert LD50 to mg/kg)	6631 - 8576 (95% CL)	Weil (1952)	Sprague-Dawley rats; 260-360 g; 12-16 weeks	male	oral; stomach tube	solution in 1.5 mL peanut oil; light anesthesia; doses (mL/kg)=3.6, 4.5, 5.4, 6.4	observed for 48 hour; doses (mL/kg), dead animals: 3.6 -- 0/4; 4.5 -- 1/4; 5.4 -- 3/4; 6.4 -- 4/4	16 rats used	British Drug Houses Ltd, Pool, Great Britain	Pound AW, Horn L, Lawson TA. 1973. Decreased toxicity of dimethylnitrosamine in rats after treatment with carbon tetrachloride. Pathology 5:233-242. *University of Queensland, Brisbane, Australia*
Carbon tetrachloride	2350	10054	8758 - 11009 (95% CI; slope = 9.2)	Finney (1971) Probit Analysis	Crl-CD rats from Charles River Breeding lab; 220-280 g; 60 days old	male	oral; intragastric intubation	0.5 - 3.9% suspension; dissolved or suspended in corn oil; single dose; 2000, 2700, 3500, 4500, 8000, 10000, 11000, 12000, 14000, 15000, 17000 mg/kg doses	observed daily for 14 days; death within 3 days; toxic symptoms: salivation, weakness, pallor, lethargy, diarrhea, weight loss	non fasted; 11 groups of 10; 110 rats used; 49 rats died; doses of 10000 mg/kg or greater were administered in 2 portions at 15 minutes apart	99+% pure; E.I. Du Pont de Nemours	Dashiell OL, Kennedy GL Jr. 1984. The effects of fasting on the acute oral toxicity of nine chemicals in the rat. J Appl Toxicol 4(6): 320-325. E.I. Du Pont de Nemours & Co., Newark, DE data from EPA TSCATS database; Oral LD50 test in rats with methane,tetrachloro-* with cover letter dated 081092;. (1981) EPA Document No. 88-920010018 Fiche No. OTS0571676; *E.I Dupont DeNemours & Co., Inc./Haskell Labs*
Chloral hydrate	479	285	+/- 21 (S.E.)	NA	Charles River Sprague-Dawley rats; 1-2 days	NA	oral	NA	NA	data is from Yeary et al.1966	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharmacology 18:185-207.
Chloral hydrate	479	479	+/- 42 (S.E.)	NA	Charles River Sprague-Dawley rats; adult	NA	oral	NA	NA	data is from Yeary et al.1966	NA	Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1 1959 Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185-207
Chloral hydrate	479	479	+/- 42 (S.E.)	Miller and Tainter (1944)	Charles River CD and Sprague-Dawley rat strains; > 100 g; adult	NA	oral intubation; up to 50 mL/kg	NA	rats observed for 7 days; observed up to 14 days when heavy metals or other compounds that produce latent death were investigated	fasted overnight	NA	Yeary RA, Benish RA, Finkelstein M. 1966. Acute Toxicity of Drugs in Newborn Animals. Journal of Pediatrics 69 (4):663-667. *Dept. of Veterinary Preventive Medicine, Ohio State University, Columbus, OH*
Chloral hydrate	479	500	NA	NA	NA	rat	oral	aqueous solution or suspension	produced degree of CNS depression	NA	NA	Finnegan JK, Larson PS, Haag HB, Page SG Jr. 1951. March. Sedative and toxic effects of several chloral derivatives. Federation Proceedings v. 10:294. *Medical College of Virginia, Richmond, VA*
Chloral hydrate	479	800	NA	graphically	white rats; 125-250 g	male and female	oral;stomach tube	single dose; 4% solutions in distilled water; dose is mg/kg, rats per dose: 700-25; 800-34; 900-22; 1000-32; 1100-24	acute toxicity same for male and female;	fasted for 16 hour; 137 rats used; first report for chloral hydrate LD50	NA	Adams WL. 1943. The comparative toxicity if chloral alcoholate and chloral hydrate. J Pharm Exp Ther 78:340-345. *Union University, Albany, NY*
Chloral hydrate	479	863	622.9 - 832.1	Bliss-Probit method	Sprague-Dawley rats; 5 weeks	male	oral gavage	dissolved in saline; range (mg/kg) of doses 417, 583, 816, 1143, 1600	rats observed at 6 hours after dosing and a once a day for 1 - 2 weeks; most rats found dead within 3 days; 29 of 50 rats died; toxic symptoms: sleep to coma	animals acclimated to environment for 1 week before testing; 5 groups of 10 rats each; fasted 16 hours before dosing; 100% mortality = 1600 mg/kg; 0% mortality = 417 mg/kg	Wako Pure Chemicals Co.	Kitagawa H, Saito H, Sugimoto T, Yanaura S, Kitagawa H, Hosokawa T, Sakamoto K. 1982. Effects of diiospropyl-1,3-dithiol-2-ylidene malonate (NKK-105) on acute toxicity of various drugs and heavy metals. J Toxicol Sci 7(2):123-34. *Chiba University; Hoshi College of Pharmacy; Showa University -- Japan*
Chloramphenicol	2500	692.9	-/+ 70 (SEM)	Bliss (1938)	Harlan rats; < 4 days; 6-9 g	NA	intragastric	cmpd suspended in 4% acacia saline solution; 2% solution doses at 400, 500, 620, 800 mg/kg	observed for 7 days; death within 24 h; 400 mg/kg-0/5, 500 mg/kg-0/5, 620 mg/kg-3/5, 800 mg/kg-3/5	NA	NA	Worth HM, Kachman C, Anderson RC. 1963. Inartistric injection for toxicity studies with newborn rats. Toxic Appl Pharmac 5:719-727. *Eli Lilly and Company, Indianapolis, IN*
Chloramphenicol	2500	1040	776 - 1394	NA	MJ rats; 1-2 days	NA	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology. 18. Pp. 185 -207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.*
Chloramphenicol	2500	2188	NA	Bliss (1938)	Harlan rats; 30-40 g; 21-25 days; weanling	NA	gavage	cmpd suspended in 4% acacia saline solution; 20% solution administ; 1800, 2500, 3300 mg/kg doses	observed for 7 days; death within 3 days; 1800 mg/kg-0/5, 2500 mg/kg-4/5, 3300 mg/kg-5/5	NA	NA	Worth HM, Kachman C, Anderson RC. 1963. Inartistric injection for toxicity studies with newborn rats. Toxic Appl Pharmac 5:719-727. *Eli Lilly and Company, Indianapolis, IN*
Chloramphenicol	2500	2500	NA	NA	albino rats	NA	oral	NA	NA	reference paper in Italian;. 1983/84 RTECS used the same reference but RC had a different LD50 and ZEBET did not provide the reference)	NA	Farmaco, Edizione Scientifica. (Casella Postale 227, 27100 Pavia, Italy) V.8-43 1953-88 ---- Almirante L, Caprio L, de Carneri I, Defranceschi A, Zamboni V. 1955. Studi sul cloroamfenicolo: (1) nuove sintesi della d-treo-2-dichlorometil-4-[(4'-nitrofenil)Ossimetil] Ossazolina (2) E dati sur potere antibiotico della stessa. Farmaco, Edizione Scientifica 10(1):3-13. **(RTECS REFERENCE)**
Chloramphenicol	2500	3400	2252 - 5139	NA	MJ rats; adult	NA	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185-207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.* This value used by RC (1977 RTECS).
Chloramphenicol	2500	5000	NA	NA	Harlan Wistar rats	NA	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185-207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Chloramphenicol	2500	> 5000	NA	Bliss (1938) method	Harlan rats; 150 g; adult	NA	gavage	cmpd suspended in 4% acacia saline solution; 30% solution dose at 5000 mg/kg	observed for either 7 or 14 days; 10 rats used; 2 dead; death on 1st day	NA	NA	Worth HM, Kachman C, Anderson RC. 1963. Inartistric injection for toxicity studies with newborn rats. Toxic Appl Pharmac 5:719-727. *Eli Lilly and Company, Indianapolis, IN*
Citric acid	3000	3000	NA	approximative	THOM (SPF) rats; 151-213 g; 48 days-males; 62 days-female	male and female	oral gavage	2500 - 5000mg/kg doses; cmpd in hydroxyethylcellulose	NA	32 male and 32 female rats; 64 rats used; performed under GLPs	NA	Schneider PM, Bauer A, Eckenfels C, Hohbach L, Lutzen H, Puschner R, Serbedija J, Wiegleb P, Lehmann H. 1992. Acute, subacute and chronic toxicity studies of pimobendan in laboratory animals. Oyo Yakuri/Pharmacometrics 43(6):561-578. **(RTECS REFERENCE)**
Citric acid	3000	11700	10080 - 13570 (95% CL)	Litchfield and Wilcoxon method	SD-JCL rats; 110-140 g; 5 weeks	male	oral	2 mL/100 g bw	observed for 7 days; stimulation within several minutes, then ataxia and prostration at 50 minutes; mydriasis, decreased heart rate and respiration; death at 12500 and 18000 mg/kg in 20-180 minutes by resp. failure; 1 rat at 10420 mg/kg died at 20 hours; autopsy showed hemorrage of gastric mucosa	6 rats/dose; number of doses not reported	TAKEDA-citric acid (refined product produced by yeast fermention of paraffins)	Yokotani H, Usui T, Nakaguchi T, Kanabayashi T, Tanda M, Aramaki Y. 1971. Acute and subacute toxicological studies of TAKEDA-citric acid in mice and rats. J Takeda Res Lab 30(1):25-31.
Colchicine	NA	5.886 (mouse)	3.901 - 7.508	NA	B6D2F1 (BDF1) mice	NA	Oral	in saline	NA	Mice fasted prior to dosing	NA	National Cancer Institute Screening Program Data Summary, Developmental Therapeutics Program. (Bethesda, MD 20205) JAN1986. **(RTECS REFERENCE)**
Colchicine	NA	18 (mouse)	NA	Lorke (1983)	MS/Ae mice from Hitachi Medical Laboratories (Sanwa, Japan); 317-346 g; 7 weeks	male	oral	1.0, 10.0, 14.0, 22.5, 37.5, 60.0, 100.0 mg/kg in physiological saline	Dose and Deaths: 1.0 - 0/3; 10.0 - 0/3; 14.0 - 0/1; 22.5 -1/1; 37.5 - 1/1; 60.0 - 1/1;100.0 - 3/3	13 mice used; acclimated for 1 week before test	Wako Pure Chemical Industries Ltd. (Osaka, Japan)	Asano N, Morita T, Watanabe Y. 1989. Micronucleus test with colchicine given by intraperitoneal injection and oral gavage. Mutat Res 223:391-394.
Colchicine	NA	29 (mouse)	NA	Lorke (1983)	CD-1 mice from Charles River Japan Inc (Hino, Japan); 312-382 g; 7 weeks	male	oral	1.0, 10.0, 14.0, 22.5, 37.5, 60.0, 100.0 mg/kg in physiological saline	Dose and Deaths: 1.0 - 0/3; 10.0 - 0/3; 14.0 - 0/1; 22.5 -0/1; 37.5 - 1/1; 60.0 - 1/1; 100.0 - 3/3	13 mice used; acclimated for 1 week before test	Wako Pure Chemical Industries Ltd. (Osaka, Japan)	Asano N, Morita T, Watanabe Y. 1989. Micronucleus test with colchicine given by intraperitoneal injection and oral gavage. Mutat Res 223:391-394.
Cupric sulfate pentahydrate	300	236.2	NA	NA	Sprague-Dawley rats	NA	oral	200, 500, 1000, 2000	NA	NA	T.C. copper sulfate powdered (50% in water)	U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; EPA Chem. Code: 024401; Core Grade/Tox Record No. 002705
Cupric sulfate pentahydrate	300	300	NA	NA	rats	NA	oral	NA	NA	value assumed to be from Lehman 1951	NA	Agricultural Chemicals. Thomson, W.T., 4 vols., Fresno, CA, Thomson Publications, 1976/77 revision **(RTECS REFERENCE)**
Cupric sulfate pentahydrate	300	300	NA	NA	rats	NA	oral; stomach tube	NA	violent retching, muscular spasms and collapse; onset within minutes	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletine (Association of Food and Drug Officials of the United States). v15:22 - 133. *U.S. FDA* **(RTECS SOURCE)**
Cupric sulfate pentahydrate	300	450	346 - 585 (95% CL)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley rats; 155-175 g	female	oral gavage	single dose; 9 dose levels from 100 - 5000mg/kg	animals observed daily and survivors killed 14 days post-dose; all deaths within first week of dosing; weight loss, lethargy and death; dose (mg/kg), no dead/no dosed: 100 - 0/5; 200 - 0/5; 300 - 3/10; 500 - 0/5; 625 - 0/10; 750 - 4/5; 5000 - 5/5	tested under GLPs; groups of rats (5/sex/dose group) were administered vehicle (10 mL/kg) or test article; 45 animals used	powder 99% pure	Deenihan MJ.1987; Fine 20 Copper Sulfate Pentahydrate - Acute Toxicology Testing: (A) Acute Oral Toxicity. Northview Pacific laboratories, Inc. U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No.433962-01A; EPA Chem. Code: 024401; Core Grade/Tox Record No. acceptable; 011521; Apr. 20, 1995
Cupric sulfate pentahydrate	300	472.5	NA	NA	rat	NA	oral	NA	NA	NA	copper sulfate (powder)	WARF Institute, Inc.; WARF No. 5032161; Jan. 1, 1975; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No.00058839; EPA Chem. Code: 024401; Core Grade/Tox Record No. supplementary 004457
Cupric sulfate pentahydrate	300	790	416 - 1501 (95% CL)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley rats; 225-250 g	male	oral gavage	single dose; 9 dose levels from 100 - 5000 mg/kg	animals observed daily and survivors killed 14 days post-dose; all deaths within first week of dosing; weight loss, lethargy and death; dose (mg/kg), no dead/no dosed: 100 - 0/5; 300 - 2/5; 750 - 1/5; 1000 - 3/5; 1250 - 2/5; 5000 - 5/5	tested under GLPs; groups of rats (5/sex/dose group) were administered vehicle (10 ml/kg) or test article; 30 animals used	powder 99% pure	Deenihan MJ.1987; Fine 20 Copper Sulfate Pentahydrate - Acute Toxicology Testing: (A) Acute Oral Toxicity. Northview Pacific laboratories, Inc. U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No.433962-01A; EPA Chem. Code: 024401; Core Grade/Tox Record No. acceptable; 011521; Apr. 20, 1995
Cupric sulfate pentahydrate	300	960	710 - 1300 (these limits are +/- 1.96 S.D.)	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 50 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30:470-476. *Carnegie-Mellon University, Pittsburgh, PA* (LD50 value) Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)
Cupric sulfate pentahydrate	300	1570	1030 - 2400	NA	rat	NA	oral	NA	NA	low purity (20%)	copper sulfate pentahydrate 20% (Odor inhibitor/bactericide)	Hazleton Laboratories America, Inc.; HLA B1100274; Feb 27, 1989; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 41043001; EPA Chem. Code: 024401; Core Grade/Tox Record No. Guideline 009092; Feb. 5, 1992
Cupric sulfate pentahydrate	300	2300	1150 - 3390	NA	rat	female	oral	NA	NA	low purity (11%)	copper sulfate 11%	BASF; 82/168; Aug. 11, 1986; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00149179; EPA Chem. Code: 024401; Core Grade/Tox Record No. Guideline 006197
Cupric sulfate pentahydrate	300	2530	2010 - 3170	NA	rat	male and female	oral	NA	NA	low purity (11%)	copper sulfate 11%	BASF; 82/168; Aug. 11, 1986; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00149179; EPA Chem. Code: 024401; Core Grade/Tox Record No. Guideline 006197
Cupric sulfate pentahydrate	300	2610	1890 - 4140	NA	rat	male	oral	NA	NA	low purity (11%)	copper sulfate 11%	BASF; 82/168; Aug. 11, 1986; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00149179; EPA Chem. Code: 024401; Core Grade/Tox Record No. Guideline 006197

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Cupric sulfate pentahydrate	300	> 0.5mL/kg < 2.0 mL/kg	NA	NA	Sprague-Dawley rats	male	oral	0.5, 2.0, 5.0 mL/kg	no toxic signs	NA	Cutrine (28% copper sulfate)	WARF Institute, Inc.; WARF No. 1052198; Mar. 20, 1978; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No.00157309; EPA Chem. Code: 024401; Core Grade/Tox Record No. supplementary 002707
Cycloheximide	2	1 (calculated by NICEATM)	NA	NA	rats	NA	oral; stomach tube	aqueous solutions or suspensions; 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 5.0, 7.5, 10, 15, 25, 50, 75, 100, 150, 200 mg/kg dose range	rats at higher doses had bloody urine and profuse watery feces	2 rats/dose; 32 rats used; 27/32 rats dead; 75-200 mg/kg: all dead within 5 hour; 10-50 mg/kg: all dead overnight; 7.5 mg/kg: 1 dead overnight, other at 26 hour; 5.0 mg/kg: 1 dead overnight, other at 24 hour; 2.5 mg/kg: all dead at 24 and 25 hour; 2.0 mg/kg: all dead overnight and 23 hour; 1.5 mg/kg: all dead at 25 hour; 1.0 mg/kg: 1 dead at 25 hour, 1 survived; 0.5 - 0.75 mg/kg: all survived	Upjohn Company	Traub R, DeWitt JB, Welch JF, Newman DJ. 1950. Toxicity and repellency to rats of actidione. J Am Pharm Assoc (Sci. Ed.) 39(10):552 - 555. *Army Medical Department Research and Graduate School, Washington, D.C.*
Cycloheximide	2	1.8	NA	NA	rats	NA	oral	NA	NA	NA	NA	Compounds Available for Fundamental Research, Volume II-6. Antibiotics. A Program of Upjohn Company Research Labo (Kalamazoo, MI 49001).1971. **(RTECS REFERENCE)**
Cycloheximide	2	2.5	NA	NA	rats	NA	oral	NA	excessive salivation, diarrhea, nervousness, depression	NA	Upjohn Company	Ford JH, Klomparens W. 1960. Cycloheximide (Acti-dione) and its non agricultural uses. Antibiotics and Chemotherapy 10:682 - 687. *The Upjohn Co., Kalamazoo, MI*
Dibutyl phthalate	7499	7499	7072 - 8006 (95% CL)	NA	rats	NA	oral	NA	NA	NA	NA	Weisheng Dulixue Zazhi. Journal of Health Toxicology. (Weisheng Dulixue Zazhi Bianjibu, Dongdaqiao, Chaoyang Menwai, Beijing, Peop. Rep. China) V.1- 1987, 1991. **(RTECS REFERENCE)**
Dibutyl phthalate	7499	8000	NA	NA	Sprague-Dawley rats; 60-75 g; 5-6 weeks	male	oral	single undiluted doses; 4000, 8000, 16000, 32000 mg/kg doses	7 day observation	4000 mg/kg - 0/3 dead; 8000 mg/kg - 4/9 dead; 16000 mg/kg - 6/6 dead; 32000 mg/kg - 6/6 dead; 24 rats used	NA	Smith CC. 1953. Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate, and methoxyyethyl oleate. Arch Ind Hyg 7:310-318.
Dibutyl phthalate	7499	8380	6860 - 10230	NA	Sherman strain rats; 120 g	NA	NA	dosage series when expressed in /kg constitutes the antilogarithms of 1.0, 1.1, 1.2, etc	NA	NA	NA	Smyth HF, Carpenter CP. 1948. Further experience with the range finding test in the industrial toxicology laboratory. J Ind Hyg Toxicol 30:63-68. *Melon Institute, Pittsburgh, PA*
Dibutyl phthalate	7499	12436 (11.9 mL/kg)	NA	Karber's method	white rats; 60-75 g; 6 weeks	NA	oral	NA	degenerative liver changes noted	reference is untranslated Russian with English abstract; NICEATM converted 11.9 mL/kg LD50 to mg/kg using provided density of 1.045 g/mL	NA	Homrowski S, Nikonorow M. 1959. Toksycznose ostra ftalanu dwubutylu oraz ftalanu dwu-2-etyloheksylu produkcji krajowej. Roczniki Panstwowego Zakladu Higieny 10:321-327.
Dichlorvos (DDVP)	17	17	NA	NA	rats	NA	oral	NA	NA	unknown primary reference	NA	Japan Pesticide Information. (Japan Plant Protection Assoc., 1-43-11, Komagome, Toshima-ku, Tokyo 170, Japan) No.1-61, 1969-92. 1972. **(RTECS REFERENCE)**
Dichlorvos (DDVP)	17	50	NA	Litchfield and Wilcoxon method (1949)	CFY strain rats; 120+ g; adult	female	oral	NA	NA	NA	93% pure; Ciba-Geigy, Switzerland	Desi I. 1983. Neurotoxicological investigaton of pesticides in animal experiments. Neurobehav Toxicol 5:503-515. *National Institute of Hygiene, Hungary*
Dichlorvos (DDVP)	17	54 (calculated from negative log in mol/kg [3.61])	24 - 111 (CL)	Litchfield and Wilcoxon method (1949)	Wistar rats; 150 g	female	intragastric-ally (metal tube)	ethanol: water 1:4 solution used as solvent; 2 mL/kg dosage;	observed for 72 hours; decreased body weight	30 rats tested (5 groups of 6 rats)	95% pure	Gajewski D, Katkiewicz M. 1981. Activity of certain enzymes and histomorphological changes in subacute intoxication of rats with selected organophosphates. Acta Physiol Pol 32(5):507-520. *Agricultural Academy (and others), Warsaw, Poland*
Dichlorvos (DDVP)	17	56	48 - 65 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min.wt.: female = 200 g; min.age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival = died within 1 hour	80 rats tested; LD50 value from Durham et al. 1957	technical grade	Gaines TB. 1960. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 2:88-99. *U.S. Dept. of Health, Education, and Welfare, Savannah, GA* Mattson AM, Spillane JT, Pearce GW. 1955. Dimethyl 2,2-dichlorvinyl phosphate (DDVP), an organic phosphorous compound highly toxic to insects. J Agr Food Chem 3:319-321. *Communicable Disease Center, Savannah, GA*
Dichlorvos (DDVP)	17	56	48 - 65 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman albino rats	female	oral; stomach tube	dissolved in peanut oil; dosage rate of 5uL/g; DDVP concentration varied	bulging eyes, excessive lacrimation, sialorrhea, generalized muscle fasciulations, tremors; killed rats dead within 1 hour; all survivors completely recovered within 24 hours	NA	technical grade, 90%DDVP	Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson MA, Hayes WJ. 1957. Studies on the toxicity of 0,0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). AMA Arch Ind Health 15:340-349. *U.S. Dept. of Health, Education and Welfare, Savannah, GA*
Dichlorvos (DDVP)	17	68	59 - 79 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman albino rats	female	oral; stomach tube	dissolved in peanut oil; dosage rate of 5uL/g; DDVP concentration varied	bulging eyes, excessive lacrimation, sialorrhea, generalized muscle fasciulations, tremors; killed rats dead within 1 hour; all survivors completely recovered within 24 hours	NA	99% pure DDVP	Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson MA, Hayes WJ. 1957. Studies on the toxicity of 0,0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). AMA Arch Ind Health 15:340-349. *U.S. Dept. of Health, Education and Welfare, Savannah, GA*
Dichlorvos (DDVP)	17	80	62 - 104 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min. wt.: male = 175 g; min. age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival = died within 1 hour	59 rats tested; LD50 value from reseach paper of Durham et al. 1957	technical grade	Gaines TB. 1960. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 2:88-99. *U.S. Dept. of Health, Education, and Welfare, Savannah, GA* Mattson AM, Spillane JT, Pearce GW. 1955. Dimethyl 2,2-dichlorvinyl phosphate (DDVP), an organic phosphorous compound highly toxic to insects. J Agr Food Chem 3:319-321. *Communicable Disease Center, Savannah, GA*
Dichlorvos (DDVP)	17	80	NA	Litchfield and Wilcoxon method (1949)	CFY strain rats; 120+ g; adult	male	oral	NA	NA	NA	93% pure; Ciba-Geigy, Switzerland	Desi I. 1983. Neurotoxicological investigaton of pesticides in animal experiments. Neurobehav Toxicol 5:503-515. *National Institute of Hygiene, Hungary*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Dichlorvos (DDVP)	17	80	62 - 104 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman albino rats	male	oral; stomach tube	dissolved in peanut oil; dosage rate of 5 ul/g; DDVP concentration varied	bulging eyes, excessive lacrimation, sialorrhea, generalized muscle fasiculations, tremors; killed rats dead within 1 hour; all survivors completely recovered within 24 hours	NA	technical grade, 90%DDVP	Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson MA, Hayes WJ. 1957. Studies on the toxicity of 0,0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). AMA Arch Ind Health 15:340-349. *U.S. Dept. of Health, Education and Welfare, Savannah, GA*
Dichlorvos (DDVP)	17	80	71 - 90 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman albino rats	female	oral; stomach tube	dissolved in peanut oil; dosage rate of 5 ul/g; DDVP concentration varied	bulging eyes, excessive lacrimation, sialorrhea, generalized muscle fasiculations, tremors; killed rats dead within 1 hour; all survivors completely recovered within 24 hours	NA	technical grade, 90%DDVP	Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson MA, Hayes WJ. 1957. Studies on the toxicity of 0,0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). AMA Arch Ind Health 15:340-349. *U.S. Dept. of Health, Education and Welfare, Savannah, GA*
Dichlorvos (DDVP)	17	97.5	88.6 - 107 (95% CL; slope = 1.24 [1.15 - 1.34])	Litchfield and Wilcoxon method (1949)	Fischer 344 rats; 7 weeks	male	oral gavage	dissolved in olive oil; 5 mL/kg dosing solution; 4 -5 dosages	24 hour observation; anti-cholinesterase signs of salivation, fasiculation, lacrimation, tremors, irregular respiration, prostration; all deaths observed between 2 -24 hours	aclimated for 1 week before dosing; 5 - 10 animals per each dosage	98.7% pure; Nippon Chemical Industrial Company, Ltd.	Ikeda T, Kojima T, Yoshida M, Takahashi H, Tsuda S, Shirasu Y. 1990. Pretreatment of rats with an organophosphorous insecticide, chlorfenvinphos, protects against subsequent challenge with the same compound. Fundam Appl Toxicol 14(3):560-567. *Mitsukaido Laboratories, Institute of Environmental Toxicology, Japan*
Diethyl phthalate	8600	> 5590 (reported as > 5.0 mL/kg; specific density = 1.118)	95% CL (where possible);	Litchfield and Wilcoxon method (1949)	Wistar albino rats; 139-164 g	male and female	oral; gavage	0.5, 1, 2, 5 mL/kg; single dose	observed at 1, 3, 6, and 24 hours after dosing; then observed daily for 14 days; 2 rats dead	8 groups of 10 rats (5M, 5F); 80 rats used; fasted overnight	NA	data from EPA TSCATS database; ORAL LD50 TEST IN RATS OF DIETHYL PHTHALATE WITH COVER LETTER DATED 05/09/94 (SANITIZED) (1978) EPA Document No. 86-940000887S Fiche No. OTS0557297; *Consumer Product Testing, Fairfield, NJ*
Diethyl phthalate	8600	8600	7840 - 9890	NA	rats	NA	oral	NA	NA	NA	NA	Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1-36, 1957-1992. 1980. Timofeevshaia LA, Ivanova NI, Balinina ES. 1980. Toxicology of O-phthalate acid esters and hygiene reglamentation. Gigiena Truda i Professional'nye Zabolevaniya 24(3):25-27.**(RTECS REFERENCE)**
Diethyl phthalate	8600	10100	8920 - 11280	NA	rats; 220 +/- 40 g	NA	oral; intragastric	NA	NA	(source of information not provided)	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Digoxin	28.3	28.27	24.85 - 32.17 (limits of error [P=0.95])	Probit method	rats; 250-310 g	male and female (equal numbers)	oral	NA	mortality rate computed 7 days after administration	3 or 4 groups of 10; 30 - 40 rats used; fasted overnight	NA	Archives Internationales de Pharmacodynamie et de Therapie. (Heymans Institute of Pharmacology, DePintelaan 185, B-9000 Ghent, Belgium) V.4- 1898, 1966. Georges A, Page J, Duvernay G. 1966. Cardiotonic properties of formiloxin: a semi-synthetic cardiac glycoside. Arch Int Pharmacodyn 164(1):47-55. Research Dept., A. Christianens, S.A., Brussels, Belgium **(RTECS REFERENCE)**
Dimethylformamide	2800	1425 (1.5 mL/kg; converted to mg/kg using density = 0.950)	855 - 2565 (95% CL; 0.9 - 2.7 mL/kg; converted to mg/kg using density = 0.950)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 16-50 g; 14 days	male and female	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; 6-12 rats of both sexes used for studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Dimethylformamide	2800	> 2000	NA	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	male and female	oral gavage	single dose	14 day observation; toxicity symptoms: Ptosis, posture, respiratory effects, lethargy, abnormal gait, tremors, convulsions, prostrate coma; time to onset of signs --; duration of signs -- no signs reported; 0 rats dead (average per test)	3 dose levels (5 male and 5 female each); 30 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Dimethylformamide	2800	2800	NA	NA	rats	NA	oral	NA	NA	NA	NA	Druckery H, Preussmann R, Ivankovic S, Schmahl D. 1966. Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an BD-Ratten. Zeitschrift fur Krebsforschung 69:103-201. **(RTECS REFERENCE)**
Dimethylformamide	2800	3990 (4.2 mL/kg; converted to mg/kg using density = 0.950)	2565 - 6270 (95% CL; 2.7 - 6.6 mL/kg; converted to mg/kg using density = 0.950)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 80-160 g; young adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Dimethylformamide	2800	5800	+/- 1200	NA	rats; 220 +/- 40 g	NA	oral; intragastric	NA	NA	(source of information not provided)	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Dimethylformamide	2800	6840 (7.2 mL/kg; sp. density = 0.950; convert LD50 to mg/kg)	5700 - 8170 (95% CL; 6.0 - 8.6 mL/kg; sp. density is 0.950; convert LD50 to mg/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 300-470 g; older adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*

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Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Dimethylformamide	2800	7000	NA	based on assumption that probit mortality vs log dose has same slope as similar chemical	Sherman rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; doses (in g/kg) differ by 1 log to bracket LD50, then refine LD50 with doses in a series of antilog 1.1, 1.3, 1.5, etc	LD50 based on mortalities during a 14 day period	6 rats/dose at doses that differ by 1 log to bracket LD50 (given 1 week apart); then refined LD50 with 10 rats/dose in a dose series of antilog 1.1, 1.3, 1.5, etc.; assumed to use materials/methods of Smyth & Carpenter (1944) except for reported changes	reagent grade	Smyth HF Jr, Carpenter CP. 1948. Further experience with the range finding test in the industrial toxicology laboratory. J Ind Hyg Toxicol 30: 63-68. **(LD50 value)** Smyth HF Jr, Carpenter CP. 1944. The place of the range-finding test in the industrial toxicology laborotory. J Ind Hyg Toxicol 26:269-273. (most materials/methods)
Dimethylformamide	2800	7182 (7.6 mL/kg; sp. density listed as 0.945; convert LD50 to mg/kg)	6804 - 7655 (95% CL; 7.2 - 8.1 mL/kg; sp. density listed as 0.945; convert LD50 to mg/kg; slope=1.11)	Finney (1962) Probit Analysis	Sprague-Dawley SPF rats; 170-230 g	male and female	oral; stomach tube	diluted in 0.9% saline; 20 - 30 mL/kg dose	observed up to 7 days after administration; all deaths occurred within 24 hour	10 animals per dose (5 male, 5 female)	pure DMF	Bartsch W, Sponer G, Dietmann K, Fuchs G. 1976. Acute toxicity of various solvents in the mouse and rat. LD50 of ethanol, diethylacetamide, dimethylformamide, dimethylsulfoxide, glycerine, N-methylpyrrolidone, polyethylene glycol 400, 1,2- propanediol and Tween 20. Arzneimittelforschung 26(8):1581-1583.
Diquat dibromide	231	231	NA	NA	rats	NA	oral	NA	NA	assumed to be the value from Clark & Hurst 1970	NA	Pesticide Manual. (The British Crop Protection Council, 20 Bridport Rd., Thornton Heath CR4 7QG, UK) V.1- 1968. 1991. **(RTECS REFERENCE)**
Diquat dibromide	231	121	108 - 136 (95% CL; slope = 12.2)	Litchfield and Wilcoxon method (1949)	Sherman strain rats (SPF); min. wt. = 200 g; min. age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed for at least 14 days after dosing or until recovered from signs of toxicity	40 rats used; min. of 10 animals per group tested	technical grade	Gaines TB, Linder RE. 1986. Acute toxicity of pesticides in adult and weanling rats. Fundam Appl Toxicol 7(2):299-308. *Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC*
Diquat dibromide	231	147	138 - 155 (95% CL; slope = 22.5)	Litchfield and Wilcoxon method (1949)	Sherman strain rats (SPF); min. wt. = 175 g; min. age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed for at least 14 days after dosing or until recovered from signs of toxicity	40 rats used; min. of 10 animals per group tested	technical grade	Gaines TB, Linder RE. 1986. Acute toxicity of pesticides in adult and weanling rats. Fundam Appl Toxicol 7(2):299-308. *Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC*
Diquat dibromide	231	231 (diquat ion per kg bw)	194 - 274 (95% CL)	Thompson (1947); moving average interpolation method	Alderly Park albino rats (SPF); 180-200 g; young, mature	female	oral; stomach tube	chemical dissolved in water or physiological saline	observed for 14 days; lethargy, weight loss, respiratory difficulty	NA	99% pure diquat dichloride or diquat dibromide	Clark DG, Hurst EW. 1970. The toxicity of diquat. Br J Ind Med Jan;27(1):51-55. *Imperial Chemical Industries Limited, Cheshire, UK*
Disulfoton	2.6	2.3	1.7 - 3.1 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min. wt. = 200 g; min. age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 3 days	50 rats tested	technical grade	Gaines TB. 1969. Acute toxicity of pesticides. Toxicol Appl Pharmacol 14(3): 515-34. *U.S. Dept. of Health, Education, and Welfare, Atlanta, GA*
Disulfoton	2.6	2.6	NA	estimated by the logarithm-probability method	Sprague-Dawley rats; 175 - 225 g	female	NA	dissolved in 10% ETOH, 90% propylene glycol; strength of solutions adjusted so that less than 0.3% bw was administered to the rats	animals observed for 10 days; death or complete recovery occurred within this time; acute toxic dose symptoms typical of those produced by cholinergic organic phosphates; single doses produced effects resembling those resulting from excessive stimulation of the central nervous system, the patasympathetic nervous system and somatic motor nerves; after lethal doses death usually occurred within 48 hour	25 rats used	Chemagro Corp., New York	Bombinski TJ, Dubois KP. 1958. Toxicity and mechanism of action of Di-syston. AMA Arch Ind Health 17:192-199.
Disulfoton	2.6	2.6	NA	NA	rats	female	oral	NA	NA	reference is a review article in Japanese; this LD50 value is assumed to be from Bombinski and Dubois 1958	NA	Yakkyoku. Pharmacy. (Nanzando, 4-1-11, Yushima, Bunkyo-ku, Tokyo, Japan) V.1- 1950. 1986. (see Bombinski and Dubois [1958]) **(RTECS REFERENCE)**
Disulfoton	2.6	3.2	3.0 - 3.3 (95% CL)	NA	Hindustan Antibiotics strain rats; adult	female	oral	1 - 10 mg/kg doses; 6 different dose levels	acute 24 hour LD50 determination; percent mortality given for different timepoints within the 24 hour period; pretreatment of rats reduced mortality in some cases	overnight fasted; rats pretreated with one of the following: saline, oil, phenobarbital, 3-methyl-cholanthourene, nickel chloride, cobalt chloride, cycloheximide or ethylmorphine; reference doesn't adequately define which rats received what and if all data were used in LD50 determinations	NA	Pawar SS, Fawade MM. 1978. Alterations in the toxicity of thiodemeton due to the pretreatment of inducers, substrate, and inhibitors of mixed function oxidase system. Bull Environ Contam Toxicol 20:805-810. *Marathwada University, India*
Disulfoton	2.6	6.8	5.9 - 7.8 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min. wt. = 175 g; min age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 2 days	69 rats tested	technical grade	Gaines TB. 1969. Acute toxicity of pesticides. Toxicol. Appl. Pharmacol. 14(3):515-34. *U.S. Dept. of Health, Education, and Welfare, Atlanta, GA*
Disulfoton	2.6	7.2	7.0 - 7.3 (95% CL)	NA	Hindustan Antibiotics strain rats; adult	male	oral	1 - 10 mg/kg doses; 6 different dose levels	acute 24 hour LD50 determination; percent mortality given for different timepoints within the 24 hour period; pretreatment of rats reduced mortality in some cases	overnight fasted; rats pretreated with one of the following: saline, oil, phenobarbital, 3-methyl-cholanthourene, nickel chloride, cobalt chloride, cycloheximide or ethylmorphine; reference doesn't define which rats received what and if all data were used in LD50 determinations	NA	Pawar SS, Fawade MM. 1978. Alterations in the toxicity of thiodemeton due to the pretreatment of inducers, substrate, and inhibitors of mixed function oxidase system. Bull Environ Contam Toxicol 20:805-810. *Marathwada University, India*

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Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Disulfoton	2.6	12.6	NA	estimated by the logarithm-probability method	Sprague-Dawley rats; 175-225 g	male	NA	dissolved in 10% ETOH, 90% propylene glycol; strength of solutions adjusted so that less than 0.3% bw was administered to the rats	animals observed for 10 days; death or complete recovery occurred within this time; acute toxic dose symptoms typical of those produced by cholinergic organic phosphates; single doses produced effects resembling those resulting from excessive stimulation of the central nervous system, the patasympathetic nervous system and somatic motor nerves; after lethal doses death usually occurred within 48 hour	39 rats used	Chemagro Corp., New York	Bombinski TJ, Dubois KP. 1958. Toxicity and mechanism of action of Di-syston. AMA Arch Ind Health 17:192-199.
Endosulfan	18	18	NA	NA	NA	NA	NA	NA	NA	assumed to be the values from Gaines 1969	NA	Agricultural Research Service, USDA Information Memorandum. (Beltsville, MD 20705): 20,9,1966. (see Gaines 1969) **(RTECS REFERENCE)**
Endosulfan	18	18	15 - 21 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min wt. = 200 g; min age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 2 days	60 rats tested	technical grade	Gaines TB. 1969. Acute toxicity of pesticides. Toxicol Appl Pharmacol 14(3):515-34. *U.S. Dept. of Health, Education, and Welfare, Atlanta, GA*
Endosulfan	18	43	41 - 46 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min wt. = 175 g; min age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 5 days	70 rats tested	technical grade	Gaines TB. 1969. Acute toxicity of pesticides. Toxicol Appl Pharmacol 14(3):515-34. *U.S. Dept. of Health, Education, and Welfare, Atlanta, GA*
Epinephrine bitartrate	4 (mouse - oral)	NA	+/- 1	NA	NA	NA	NA	NA	observed for 5 days	NA	NA	Acta Pharmacologica et Toxicologica. (Copenhagen, Denmark) V.1-59, 1945-86. 1972. **(RTECS REFERENCE)**
Ethanol	7060	6162 (7.8 mL/kg; converted to mg/kg using density of 0.790)	4977 - 7663 (95% CL; 6.3 - 9.7 mL/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; (16-50 g); 14 days	male and female	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; 6-12 rats of both sexes used for studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Ethanol	7060	7060	6670 - 7460 (95% CL)	moving average of Weil (1952) or Litchfield and Wilcoxon method (1949)	Wistar albino rats; old adult; 11-12 months	male	oral	dose interval 1.1; ethanol concentration of 40% w/v	acute (24 hour) toxicity; respiratory failure	fasted overnight; 6 - 8 grouped of 10 rats each	NA	Wiberg GS, Trenholm HL, Coldwell BB. 1970. Increased ethanol toxicity in old rats: changes in LD50, in vivo and in vitro metabolism, and liver alcohol dehydrogenase activity. Toxicol Appl Pharmacol May 16(3):718-727. *Dept. of National Health and Welfare, Ottawa, Canada* **(RTECS REFERENCE)**
Ethanol	7060	7400	NA	NA	rats; 150-250 g; 70- 100 days	male (predominately)	oral	NA	observed for 6 days	18 hour fasting before dosing	NA	Welch H, Slocum GG. 1943. Relation of length of carbon chain to the primary and functional toxicities of alcohols. J Lab Chem Med 28:1440-1445. *U.S. FDA, Washington, D.C.*
Ethanol	7060	10600	10000 - 11200 (95% CL)	Litchfield and Wilcoxon method (1949) or moving average of Weil (1952)	Wistar albino rats; young adult; 100 days	male	oral	dose interval 1.1; ethanol concentration of 40% w/v	acute (24 hour) toxicity; respiratory failure	fasted overnight; 6 - 8 grouped of 10 rats each	NA	Wiberg GS, Trenholm HL, Coldwell BB. 1970. Increased ethanol toxicity in old rats: changes in LD50, in vivo and in vitro metabolism, and liver alcohol dehydrogenase activity. Toxicol. Appl. Pharmacol. May. 16(3):718-727. *Dept. of National Health and Welfare, Ottawa, Canada*
Ethanol	7060	11290 - A 11204 - B (A = 14.31 mL/kg; B = 14.20 mL/kg; used density of 0.789 to convert to mg/kg)	NA	A: Behrens (1929) B: Bliss (1938)	rats	NA	oral	NA	NA	40 - 90 animals used; NICEATM used value B since authors stated it was more accurate	NA	Deichmann WB, Mergard EG. 1948. Comparative evaluation of methods employed to express the degree of toxicity of a compound. J Ind Hyg Toxicol 30:373-378. *Albany Medical College, Albany, NY; University of Cincinnati, Cincinnati, OH*
Ethanol	7060	11534 (14.6 mL/kg; used density of 0.790 to convert to mg/kg)	10112 - 13193 (95% CL; 12.8 - 16.7 mL/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 300-470 g; older adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Ethanol	7060	13660	11170 - 16710 (95% probability; +/- 1.96 S.D.; slope = 4.57)	probits (Bliss)	Wistar albino rats; 90-120 g	male	oral; stomach tube; single doses	50% concentration in water; largest dose given was 50 g/kg	most deaths occurred in 2 days; all deaths occurred in 14 days	groups of 10 animals; 10 animals per dose	purified commercial grade	Smyth HF Jr, Seaton J, Fischer, L. 1941. The single dose toxicity of some glycols and derivatives. J Ind Hyg Toxicol 23:259-268. *Mellon Institute, Pittsburgh, PA* (This was the value used by the RC [from 1977 RTECS]).
Ethanol	7060	15543 (19.7 mL/kg; used density of 0.789 to convert to mg/kg)		Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 10 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF, Weil CS, West JS, Carpenter CP. 1970. An exploration of joint toxic action:II. Equitoxic versus equivolume mixtures. Toxicol Appl Pharmacol 17:498-503. (LD50 value) Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., Striegel, JA. And Nycum, JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30:470-476. *Carnegie-Mellon University, Pittsburgh, PA* Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., and Striegel, JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)

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Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Ethanol	7060	17775 (22.5 mL/kg; used density of 0.790 to convert to mg/kg)	14852 - 21330 (95% CL; 18.8 - 27.0 mL/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 80-160 g); young adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Ethylene glycol	4700	4000	3100 - 5200 (95% CI; slope = 258)	Litchfield and Wilcoxon method	Fischer 344 (COB CD F/Crl BR) rats; 150-200 g; 12-14 weeks	female	oral intubation	0.1 log dosages with 5 rats per level	animals observed for mortality daily for 14 days	fasted overnight; no dosage exceeded 24 g/kg bw; LD50 and 95% confidence limits calculated at 24 hour post-treatment; no deaths beyond 72 hour post-treatment	Aldrich Chemical Co.; high purity; > 99% ethylene glycol	Clark CR, Marshall TC, Merickel BS, et al. 1979. Toxicological assessment of heat transfer fluids proposed for use in solar energy applications. Toxicol Appl Pharmacol 5(1):529-535. *Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental research Institute, Albuquerque, NM*
Ethylene glycol	4700	4700	NA		rats	NA	oral	NA	NA	reference in intranslated Russian; same reference was cited in 1983/84 RTECs, but this is not the LD50 used by RC (ZEBET did not provide the reference)	NA	Filatova VS, Smirkova ES. 1982. Derivation of the maximum permissable concentration of ethylen glycol in the air of worksites. Gigiena Truda i Professional'nye Zabolevaniya. 26(6):28-30. **(RTECS REFERENCE)**
Ethylene glycol	4700	>5000	NA	NA	Holzman Sprague-Dawley rats	male	oral gavage	50 mg/kg, 500 mg/kg, and 5000 mg/kg in corn oil	clinical observations included depression, labored breathing, emaciation, and alopecia	3 groups of 10 males; no mortalities were observed	NA	from EPA TSCATS database; Acute Toxicity Study in Rats Administered 10 Materials (final report) with Cover Letter dated 062669, (1969), EPA Doc. No. 40-6942188, Fiche No. OTS0519234; *FMC Corporation*
Ethylene glycol	4700	5890 (5.28 cc/kg; converted to mg/kg using density of 1.1155)	5053 - 7106 (95% probability; 4.53 - 6.37 cc/kg)	probits (Bliss)	rats from the same strain; 275 +/- 25 g; 3 months +/- 9 days	NA	oral; stomach tube; single doses	single doses; 3904 mg/kg--7028 mg/kg; log doses 0.544, 0.608, 0.672, 0.735, 0.799; diluted 1 + 3	most deaths occurred in 1 - 5 days; weakness and lack of muscular coordination; no deaths per dose: 3904 mg/kg -- 2/11; 4440 mg/kg -- 3/11; 5243 mg/kg -- 3/11; 6057 mg/kg -- 5/11; 7028 mg/kg -- 8/11	5 doses for 11 animals each dose; 55 rats used	NA	Laug EP, Calvery HO, Morris HJ, Woodard G. 1939. The toxicology of some glycols and derivatives. J Ind Hyg Toxicol 21:173-201. *Division of Pharmacology, Food and Drug Administration, U.S. Dept. of Agriculture, Washington, D.C.*
Ethylene glycol	4700	6135 (5.50 cc/kg; converted to mg/kg using density of 1.1155)	5578 - 6749 (95% probability; 5.00 - 6.05 cc/kg)	probits (Bliss)	rats from different sources; 175-325 g	male and female (-- equal)	oral; stomach tube; single doses	single doses; 3904 mg/kg -- 8366 mg/kg	most deaths occurred in 1 - 5 days; weakness and lack of muscular coordination; no deaths per dose: 3904 mg/kg - 0/7; 4462 mg/kg - 4/20; 5020 mg/kg - 3/10; 5578 mg/kg - 11/20; 6135 mg/kg - 15/20; 6693 mg/kg - 4/10; 6972 mg/kg - 7/10; 7251 mg/kg - 2/10; 7809 mg/kg - 13/20; 8366 mg/kg - 17/20	rats fasted for about 18 hours; 147 rats used; 76 died	NA	Laug EP, Calvery HO, Morris HJ, Woodard G. 1939. The toxicology of some glycols and derivatives. J Ind Hyg Toxicol 21:173-201. *Division of Pharmacology, Food and Drug Administration, U.S. Dept. of Agriculture, Washington, D.C.*
Ethylene glycol	4700	6500	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation;	single dose; geometric factor between dosage levels=2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Ethylene glycol	4700	6537 (5.86 cc/kg; converted to mg/kg using density of 1.1155)	5064 - 8455 (95% probability; 4.54 - 7.58 cc/kg)	probits (Bliss)	rats from the same strain; 275 +/- 25 g; 3 months +/- 9 days		oral; stomach tube; single doses	single doses; 3904 mg/kg -- 7028 mg/kg; log doses 0.544, 0.608, 0.672, 0.735, 0.799; undiluted	most deaths occurred in 1 - 5 days; weakness and lack of muscular coordination; no deaths per dose: 3904 mg/kg -- 2/11; 4440 mg/kg -- 2/11; 5243 mg/kg -- 4/11; 6057 mg/kg -- 5/11; 7028 mg/kg -- 6/11	5 doses for 11 animals each dose; 55 rats used	NA	Laug EP, Calvery HO, Morris HJ, Woodard G. 1939. The toxicology of some glycols and derivatives. J Ind Hyg Toxicol 21:173-201. *Division of Pharmacology, Food and Drug Administration, U.s. Dept. of Agriculture, Washington, D.C.*
Ethylene glycol	4700	6860	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Ethylene glycol	4700	7460	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Ethylene glycol	4700	7887 (7.07 mL/kg; converted to mg/kg using density of 1.1155)	NA	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 10 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF, Weil CS, West JS, Carpenter CP. 1970. An exploration of joint toxic action:II. Equitoxic versus equivolume mixtures. Toxicol Appl Pharmacol 17:498-503. (LD50 value) Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., Striegel, JA. And Nycum, JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30:470-476. *Carnegie-Mellon University, Pittsburgh, PA* Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., and Striegel, JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)
Ethylene glycol	4700	8000	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Ethylene glycol	4700	8120	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Ethylene glycol	4700	8480	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Ethylene glycol	4700	8540	7310 - 9990 (95% probability; +/- 1.96 S.D.; slope = 5.71)	probits (Bliss)	Wistar albino rats; 90-120 g	male	oral; stomach tube; single doses	50% concentration in water; largest dose given was 50 g/kg	most deaths occurred in 2 days; all deaths occurred in 14 days	groups of 10 animals; 10 animals per dose	commercial grade	Smyth HF Jr, Seaton J, Fischer L. 1941. The single dose toxicity of some glycols and derivatives. J Ind Hyg Toxicol 23:259-268. *Mellon Institute, Pittsburgh, PA.* (This is the value used by the RC [from 1981/82 RTECS]).
Ethylene glycol	4700	9058 (8.12 mL/kg; converted to mg/kg using density of 1.1155)	NA	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 10 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF, Weil CS, West JS, Carpenter CP. 1970. An exploration of joint toxic action:II. Equitoxic versus equivolume mixtures. Toxicol Appl Pharmacol 17:498-503. (LD50 value) Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., Striegel, JA. And Nycum, JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30:470-476. *Carnegie-Mellon University, Pittsburgh, PA* Smyth HF Jr., Carpenter CP., Weil CS., Pozzani, UC., and Striegel, JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)
Ethylene glycol	4700	9850	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats; 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Ethylene glycol	4700	9900	NA	Thompson (1947) and Weil (1952); moving average tables	Manor farms Wistar rats (SPF); 150-200 g	male	oral; stomach intubation	single dose; geometric factor between dosage levels = 2; undiluted	14 day observation	5 rats per dosage level; fasted overnight	NA	Weil CS, Wright GJ. 1967. Intra- and Interlaboratory Comparative Evaluation of Single Oral Test. Toxicology and Applied Pharmacology 11:378-388. *Mellon Institute, Pittsburgh, PA and The Dow Chemical Company, Midland, MI*
Ethylene glycol	4700	> 10000	NA	NA	Sprague-Dawley rats	female	oral; gavage	single dose; 1250, 2500, 5000, 10000 mg/kg doses	14 day observation; no rats died	ethylene glycol engine coolant; test material is 50/50 (vol.) ethylene glycol and water mix with 1.5 oz./gal of DCA inhibitor	NA	from EPA TSCATS database; Initial Submission: Acute Toxicological Properties & Handling Hazards With Ethylene Glycol Tested In Rats (Final Report) With Cover Letter Dated 051492; EPA Doc. No. 88-920003189 Fiche No.OTS0539777. *The Dow Chemical Co.*
Ethylene glycol	4700	17800	NA	Litchfield and Wilcoxon method	Holzman Sprague-Dawley rats; 243- 274 g	male	oral intubation	316 mg/kg, 1000 mg/kg, 3160 mg/kg, 10000 mg/kg, 31600 mg/kg in corn oil	clinical observations included depression, rapid respiration and hunching; 2 rats dead at highest dose	5 groups of 2 males; only mortalities were both rats at the 31600 mg/kg dose; fasted overnight	NA	from EPA TSCATS database; Acute Toxicity Study in Rats Administered One of 10 Materials (final report) with Cover Letter dated 090869, (1969), EPA Doc. No. 40-6942189, Fiche No. OTS0519235. *FMC Corporation*
Fenpropathrin	18	18 - 24	NA	NA	Charles River (?) rats	female	oral	5% solution in DMSO	mortalities recorded 10 days after dosing	15 male, 15 female rats used; 30 total rats; rats injected with 0.9% saline i.p. (1 mL/kg) 2 hour before dosing	NA	Crawford MJ, Hutson DH. 1977. The metabolism of the pyrthroid insecticide (+/-)-a-cyano-3-phenoxybenzyl 2,2,3,3-tetramethyl-cyclopropanecarboxylate, WL 41706, in the rat. Pestic Sci 8:579-599. *Shell Research Limited, Kent, UK* **(RTECS REFERENCE)**
Fenpropathrin	18	24 - 36	NA	NA	Charles River (?) rats	male	oral	5% solution in DMSO	mortalities recorded 10 days after dosing	15 male, 15 female rats used; 30 total rats; rats injected with 0.9% saline i.p. (1 mL/kg) 2 hour before dosing	NA	Crawford MJ, Hutson DH. 1977. The metabolism of the pyrthroid insecticide (+/-)-a-cyano-3-phenoxybenzyl 2,2,3,3-tetramethyl-cyclopropanecarboxylate, WL 41706, in the rat. Pestic Sci 8:579-599. *Shell Research Limited, Kent, UK*
Fenpropathrin	18	24 - 36	NA	NA	Charles River (?) rats	female	oral	5% solution in DMSO	mortalities recorded 10 days after dosing	12 male, 12 female rats used; 24 total rats; rats pretreated with corn oil 18 hour before dosing	NA	Crawford MJ, Hutson DH. 1977. The metabolism of the pyrthroid insecticide (+/-)-a-cyano-3-phenoxybenzyl 2,2,3,3-tetramethyl-cyclopropanecarboxylate, WL 41706, in the rat. Pestic Sci 8:579-599. *Shell Research Limited, Kent, UK*
Fenpropathrin	18	24 - 36	NA	NA	Charles River (?) rats	male	oral	5% solution in DMSO	mortalities recorded 10 days after dosing	12 male, 12 female rats used; 24 total rats; rats pretreated with corn oil 18 hour before dosing	NA	Crawford MJ, Hutson DH. 1977. The metabolism of the pyrthroid insecticide (+/-)-a-cyano-3-phenoxybenzyl 2,2,3,3-tetramethyl-cyclopropanecarboxylate, WL 41706, in the rat. Pestic Sci 8:579-599. *Shell Research Limited, Kent, UK*
Fenpropathrin	18	48.5	37.6 - 62.6 (CL)	NA	rats	female	oral gavage	single doses (mg/kg): 15, 20, 30, 50, 59, 77, 100, 120, 169; doses in corn oil	observed for 14 days; decrease of spontaneous motor activity, hypersensitivity, fibrillation, tremor, clonic convulsion, salivation, lacrimation, incontinence, hind limb ataxia; deaths resulted within 24 hour and signs of intoxication dissapeared in 24 - 48 hour; min. toxic dose was 20 mg/kg	8 groups of 10 rats; 80 rats used	Fenpropathrin 97% (S-3206 lot. No. 022018)	Sumitomo Chemical Co., Japan; FT-50-0018; Jan. 1, 1979; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00127343; EPA Chem. Code: 127901; Core Grade/Tox Record No. minimum 004567; EPA Accession No. 249937
Fenpropathrin	18	49	NA	NA	rats	female	oral	NA	NA	assumed to be same LD50 value as Sumitomo 1979	NA	Fujita Y. 1981. Meothrin (Fenpropathrin). Japan Plant Protection Assoc. Japan Pesticide Information 38:21 -25.
Fenpropathrin	18	54	43.5 - 67.0 (CL)	NA	rats	male	oral gavage	single doses (mg/kg): 15, 20, 30, 50, 59, 77, 100, 120, 169; doses in corn oil	observed for 14 days; decrease of spontaneous motor activity, hypersensitivity, fibrillation, tremor, clonic convulsion, salivation, lacrimation, incontinence, hind limb ataxia; deaths resulted within 24 hour and signs of intoxication dissapeared in 24 - 48 hour; min. toxic dose was 20 mg/kg	9 groups of 10 rats; 90 rats used	Fenpropathrin 97% (S-3206 lot. No. 022018)	Sumitomo Chemical Co., Japan; FT-50-0018; Jan. 1, 1979; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00127343; EPA Chem. Code: 127901; Core Grade/Tox Record No. minimum 004567; EPA Accession No. 249937
Fenpropathrin	18	54	NA	NA	rats	male	oral	NA	NA	assumed to be same LD50 value as Sumitomo 1979	NA	Fujita Y. 1981. Meothrin (Fenpropathrin). Japan Plant Protection Assoc. Japan Pesticide Information 38:21-25.

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Fenpropathrin	18	66.7	50.6 - 87.9 (CL)	NA	Sprague Dawley rats	female	oral gavage	single doses (mg/kg): 0, 10, 25, 50, 60, 72, 86, 104, 125; doses in corn oil	observed for 14 days; signs of intoxication with doses 25 mg/kg and above; muscular fibrillation, soft feces, diarrhea, tremor, decreased spontaneous activity, ataxia, limb paralysis, irregular respiration, slight salivation, urinary incontinence; signs developed an hour after dosing but rats recovered after 3 days; deaths resulted on day of dosing or day after dosing	rats fasted 20 hour before dosing; 9 groups of 10 rats; 90 rats used	Fenpropathrin 91.8% (S-3206 technical grade, lot. No. 2TC019)	Sumitomo Chemical Co., Japan; FT-30-0081; Jan. 17, 1983; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00127342; EPA Chem. Code: 127901; Core Grade/Tox Record No. guideline 004567; EPA Accession No. 249937
Fenpropathrin	18	70.6	53.7 - 92.7 (CL)	NA	Sprague Dawley rats	male	oral gavage	single doses (mg/kg): 0, 10, 25, 50, 60, 72, 86, 104, 125; doses in corn oil	observed for 14 days; signs of intoxication with doses 25 mg/kg and above; muscular fibrillation, soft feces, diarrhea, tremor, decreased spontaneous activity, ataxia, limb paralysis, irregular respiration, slight salivation, urinary incontinence; signs developed an hour after dosing but rats recovered after 3 days; deaths resulted on day of dosing or day after dosing	rats fasted 20 hour before dosing; 9 groups of 10 rats; 90 rats used	Fenpropathrin 91.8% (S-3206 technical grade, lot. No. 2TC019)	Sumitomo Chemical Co., Japan; FT-30-0081; Jan. 17, 1983; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00127342; EPA Chem. Code: 127901; Core Grade/Tox Record No. guideline 004567; EPA Accession No. 249937
Fenpropathrin	18	71.6	56.1 - 92.0	NA	rats	female	oral	NA	NA	NA	Danitol S-3206 (2.4 lb/GEC)	International Reseach & Development Corp.; 491-003; FT-11-0052; Oct. 26, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00128341; EPA Chem. Code: 127901; Core Grade/Tox Record No. guideline 003814
Fenpropathrin	18	72.1	53.0 - 82.5	NA	rats	male and female	oral	NA	NA	NA	Danitol S-3206 (2.4 lb/GEC)	International Reseach & Development Corp.; 491-003; FT-11-0052; Oct. 26, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00128341; EPA Chem. Code: 127901; Core Grade/Tox Record No. guideline 003814
Fenpropathrin	18	72.4	62.1 - 84.3	NA	rats	male	oral	NA	NA	NA	Danitol S-3206 (2.4 lb/GEC)	International Reseach & Development Corp.; 491-003; FT-11-0052; Oct. 26, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00128341; EPA Chem. Code: 127901; Core Grade/Tox Record No. guideline 003814
Fenpropathrin	18	107	69.8 - 164 (CL)	NA	Sprague Dawley rats	female	oral gavage	single doses (mg/kg): 0, 25, 50, 90, 120, 160, 220, 300	observed for 14 days; toxic signs noted at 50 mg/kg and above; muscular fibrillation, tremor, ataxia, limb paralysis, irregular respiration, lacrimation, salivation, urinary incontinence, diarrhea	8 groups of 10 rats; 80 rats used	Fenpropathrin 97.3% (S-3206 lot. No. T-1)	Sumitomo Chemical Co., Japan; FT-20-0076; Sept. 12, 1982; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00127344; EPA Chem. Code: 127901; Core Grade/Tox Record No. guideline 004567; EPA Accession No. 249937
Fenpropathrin	18	164	115 - 234 (CL)	NA	Sprague Dawley rats	male	oral gavage	single doses (mg/kg): 0, 25, 50, 90, 120, 160, 220, 300	observed for 14 days; toxic signs noted at 50 mg/kg and above; muscular fibrillation, tremor, ataxia, limb paralysis, irregular respiration, lacrimation, salivation, urinary incontinence, diarrhea	8 groups of 10 rats; 80 rats used	Fenpropathrin 97.3% (S-3206 lot. No. T-1)	Sumitomo Chemical Co., Japan; FT-20-0076; Sept. 12, 1982; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00127344; EPA Chem. Code: 127901; Core Grade/Tox Record No. guideline 004567; EPA Accession No. 249937
Gibberellic acid	6300	> 5000	NA	NA	rats	male and female	oral	NA	NA	NA	Gibberellins Tech. GA47A, 90%	Hazleton Laboratories, Inc.; HLA 80602323; Aug. 29, 1988; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 40873201; EPA Chem. Code: 043801; Core Grade/Tox Record No. Guideline 007756; FEB. 9, 1990
Gibberellic acid	6300	> 5000	NA	NA	rats	female	oral	NA	NA	NA	Pro Gibb 4% (gibberellic acid); Lot 28-T80-CF	Abbott Research Center; TA89-363; Feb. 20, 1990; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No.41558201; EPA Chem. Code: 043801; Core Grade/Tox Record No. Guideline 008645; Oct. 8, 1991
Gibberellic acid	6300	> 5000	NA	NA	rats	NA	oral	5000 mg/mL	NA	NA	cytokinin (as kinetin) 0.012%; Gibberellic acid 0.0007%	University of Utah Reearch Institute 03-80; TR 05-485-002A; Jan. 20, 1984; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00142864; EPA Chem. Code: 043801; Core Grade/Tox Record No. Guideline 006198
Gibberellic acid	6300	> 5000	NA	NA	rats	NA	oral	NA	NA	NA	Pro Gibb (gibberellic acid 10%);	Ricerca, Inc.; 90-0138; May 31, 1990; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 41560401; EPA Chem. Code: 043801; Core Grade/Tox Record No. supplementary 008876; Dec. 5, 1991
Gibberellic acid	6300	> 5000	NA	NA	rats	male and female	oral	NA	NA	NA	Gibberellic acid 7.5% a.i.	Ricerca, Inc.; 90-0138; May 31, 1990; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 41591103; EPA Chem. Code: 043801; Core Grade/Tox Record No. Guideline 008571; Sept. 11, 1991
Gibberellic acid	6300	> 5000	NA	NA	Charles River Crl CD; 271-293 g; young adult	male	oral	5000 mg/mL in corn oil; 10 mL/kg dose;	14 day observation; 0/5 animals dead; dyspnea	5 animals used; tan to white powder	Gibberellins Tech., 88.0%	Hazleton Laboratories, Inc.; HLA 90305639; June 22, 1989; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 41605801; EPA Chem. Code: 043801; Core Grade/Tox Record No. Guideline 008916; Dec. 17, 1991
Gibberellic acid	6300	> 5000	NA	NA	Charles River Crl CD; 245-271 g; young adult	female	oral	5000 mg/mL in corn oil	14 day observation; 0/5 animals dead; dyspnea	5 animals used; tan to white powder	Gibberellins Tech., 88.0%	Hazleton Laboratories, Inc.; HLA 90305639; June 22, 1989; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 41605801; EPA Chem. Code: 043801; Core Grade/Tox Record No. Guideline 008916; Dec. 17, 1991
Gibberellic acid	6300	5780	NA	NA	rats	male	oral	NA	NA	NA	Pro Gibb 4% (gibberellic acid); Lot 28-T80-CF	Abbott Research Center; TA89-363; Feb. 20, 1990; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No.41558201; EPA Chem. Code: 043801; Core Grade/Tox Record No. Guideline 008645; Oct. 8, 1991
Gibberellic acid	6300	6300	NA	NA	rats	NA	oral	NA	NA	NA	NA	Agricultural Chemicals. Thomson, W.T., 4 vols., Fresno, CA, Thomson Publications, 1976/77 revision **(RTECS REFERENCE)**
Glutethimide	600	600	NA	NA	rats	NA	oral	NA	NA	NA	NA	Psychotropic Drugs and Related Compounds," 2nd ed., Usdin, E., and D.H. Efron, Dept. of Health, Education and Welfare, Washington, DC, 1972. **(RTECS REFERENCE)**
Glycerol	12600	12600	NA	NA	rats	NA	oral	NA	NA	reference in Russian	NA	Farmatsevtichnii Zhurnal (Kiev). (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.3-1930. 1977. **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Glycerol	12600	15890 (12.6 cc/kg; used density of 1.261 for conversion)	NA	NA	rats	NA	oral	NA	NA	Reference provided by ZEBET as source of RC value (i.e., from 1983/84 RTECS), but mg/kg value calculated from cc/kg value is different from RC value (12691 vs 15890 mg/kg). Maybe ZEBET didn't use density? This is not a primary reference.	NA	Woodard G, Johnson VD, Nelson AA. 1945. Acute toxicity of 2-methyl, 2-4 pentanediol. Fed Proc 4:142-143. (Supposed 1983/84 RTECS reference)
Glycerol	12600	27500	23950 - 31610 (95% probability; +/- 1.96 S.D.; slope = 8.90)	probits (Bliss)	Wistar albino rats; 90-120 g	male	oral; stomach tube; single doses	50% concentration in water; largest dose given was 50 g/kg	most deaths occurred in 2 days; all deaths occurred in 14 days	groups of 10 animals; 10 animals per dose	purified commercial grade	Smyth HF Jr, Seaton J, Fischer L. 1941. The single dose toxicity of some glycols and derivatives. J Ind Hyg Toxicol 23:259-268. *Mellon Institute, Pittsburgh, PA*
Glycerol	12600	26730 - A 27650 - B (A = 21.2 mL/kg; B = 21.93 mL/kg; used density of 1.261 to convert to mg)	NA	A: Behrens (1929) B: Bliss (1938)	rats	NA	oral	NA	NA	40 - 90 animals used; NICEATM used value B since authors stated it was more accurate	NA	Deichmann WB, Mergard EG. 1948. Comparative evaluation of methods employed to express the degree of toxicity of a compound. J Ind Hyg Toxicol 30:373-378. *Albany Medical College, Albany, NY; University of Cincinnati, Cincinnati, OH*
Haloperidol	128	128	77 - 212	NA	rat	NA	oral	NA	NA	unknown primary source of information	NA	Niemegeers CJC, Janssen PAJ. 1974. Bromoperidol, a new potent neuroleptic of the butyrophenone series. Arzneimittel-Forschung Drug Research 24 (1):45-52. *Janssen Pharmaceutica, Belgium* **(RTECS REFERENCE)**
Haloperidol	128	165	NA	NA	CFN; newborn	NA	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185-207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.*
Haloperidol	128	850	617 - 1173	NA	Holtzman; adult	male	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185-207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.*
Hexachlorophene	56	9	2 (S.E.)	Miller and Tainter (1944)	Sprague-Dawley rats; 10 day	male and female	oral; stomach tube	1% carboxymethylcellulose	observed for 10 day	approximately equal numbers of males and females; 28 rats	NA	Nieminen L, Bjondahn K, Mottonen M. 1973. Effect of hexachlorophene on the rat brain during ontogenesis. Fd Cosmet Toxicol 11:635-639.
Hexachlorophene	56	42	5 (S.E.)	Miller and Tainter (1944)	Sprague-Dawley rats; 20 day	male and female	oral; stomach tube	1% carboxymethylcellulose	observed for 10 day	approximately equal numbers of males and females; 22 rats; values from graph	NA	Nieminen L, Bjondahn K, Mottonen M. 1973. Effect of hexachlorophene on the rat brain during ontogenesis. Fd Cosmet Toxicol 11:635-639.
Hexachlorophene	56	56	8 (S.E.)	Miller and Tainter (1944)	Sprague-Dawley rats; 300 day	male and female	oral; stomach tube	1% carboxymethylcellulose	observed for 10 day	approximately equal numbers of males and females; 14 rats; values from graph	NA	Nieminen L, Bjondahn K, Mottonen M. 1973. Effect of hexachlorophene on the rat brain during ontogenesis. Fd Cosmet Toxicol 11:635-639.
Hexachlorophene	56	56	51 - 62 (95% CI)	Litchfield and Wilcoxon method (1949)	Sherman strain rats (SPF); adult;	female	oral; stomach tube	peanut oil solution	died within 3 days; severe depression and diarrhea	5 or more groups of 10 rats each	USP	Gaines TB, Kimbrough RD, Linder RE. 1973. The oral and dermal toxicity of hexachlorophene. Toxicology and Applied Pharmacology 25:332-343. **(RTECS REFERENCE)**
Hexachlorophene	56	57	52 - 61 (95% CL; slope = 13.5)	Finney's maximum likelihood probit	Sherman strain rats (SPF); min wt. = 200 g; min age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005mL/g of bw	observed for at least 14 days after dosing or until recovered from signs of toxicity	At least 40 rats used; min. of 10 animals per group tested; min. of 4 doses; animals used are the same as Gaines 1973	technical grade	Gaines TB, Linder RE. 1986. Acute toxicity of pesticides in adult and weanling rats. Fundam Appl Toxicol 7(2):299-308. *NC*
Hexachlorophene	56	57.6	50.8 - 65.5 (95% CI)	Weil (1952) method	Wistar albino rats; 400 g; 17 weeks	male	oral	corn oil solution; geometric dose factor of 1.2	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*
Hexachlorophene	56	60	4 (S.E.)	Miller and Tainter (1944)	Sprague-Dawley; 70 day	male and female	oral; stomach tube	1% carboxymethylcellulose	observed for 10 day	approximately equal numbers of males and females; 84 rats; values from graph	NA	Nieminen L, Bjondahn K, Mottonen M. 1973. Effect of hexachlorophene on the rat brain during ontogenesis. Fd Cosmet Toxicol 11:635-639.
Hexachlorophene	56	60.3	55.0 - 66.0 (95% CI)	Weil (1952) method	Wistar albino rats; 100 g; 45 weeks	male	oral	corn oil solution; geometric dose factor of 1.2	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*
Hexachlorophene	56	63	55.5 - 71.8 (95% CI)	Weil (1952) method	Wistar albino rats; 300 g; 10 weeks	male	oral	corn oil solution; geometric dose factor of 1.2	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*
Hexachlorophene	56	63	45.9 - 87.2 (95% CI)	Weil (1952) method	Wistar albino rats; 200 g; 9 weeks	female	oral	corn oil solution; geometric dose factor of 1.2	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Hexachlorophene	56	66	59 - 75 95% CL; slope 10.6	Finney's maximum likelihood probit	Sherman strain rats (SPF); min wt. = 175 g; min age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed for at least 14 days after dosing or until recovered from signs of toxicity	At least 40 rats used; min. of 10 animals per group tested; min. of 4 doses; animals used are the same as Gaines 1973	technical grade	Gaines TB, Linder RE.(1986) Acute toxicity of pesticides in adult and weanling rats. Fundam Appl Toxicol 7(2):299-308. *Health Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC*
Hexachlorophene	56	66	57 - 75 (95% CI)	Litchfield and Wilcoxon method (1949)	Sherman strain rats (SPF); adult	male	oral; stomach tube	peanut oil solution	died within 12 days; severe depression and diarrhea	5 or more groups of 10 rats each;	NA	Gaines TB, Kimbrough RD, Linder RE. 1973. The oral and dermal toxicity of hexachlorophene. Toxicology and Applied Pharmacology 25:332 -343. *Environmental Protection Agency, Chamblee, GA*
Hexachlorophene	56	69.1	64.6 - 94.2 (95% CI)	Weil (1952) method	Wistar albino rats; 100 g; 5 weeks	female	oral	corn oil solution; geometric dose factor of 1.2	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*
Hexachlorophene	56	69.2	55.5 - 86.2 (95% CI)	Weil (1952) method	Wistar albino rats; 200 g; 7 weeks	male	oral	corn oil solution; geometric dose factor of 1.2	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*
Hexachlorophene	56	83	6 (S.E.)	Miller and Tainter (1944)	Sprague-Dawley rats; 25 day	male and female	oral; stomach tube	1% carboxymethylcellulose	observed for 10 day	approximately equal numbers of males and females; 12 rats; values from graph	NA	Nieminen L, Bjondahn K, Mottonen M. 1973. Effect of hexachlorophene on the rat brain during ontogenesis. Fd Cosmet Toxicol 11:635-639.
Hexachlorophene	56	84	8 (S.E.)	Miller and Tainter (1944)	Sprague-Dawley rats; 50 day	male and female	oral; stomach tube	1% carboxymethylcellulose	observed for 10 day	approximately equal numbers of males and females; 16 rats; values from graph	NA	Nieminen L, Bjondahn K, Mottonen M. 1973. Effect of hexachlorophene on the rat brain during ontogenesis. Fd Cosmet Toxicol 11:635-639.
Hexachlorophene	56	87	79.2 - 95.5 (95% CI)	Weil (1952) method	Wistar albino rats; 67 g; 4 weeks	male	oral	corn oil solution; geometric dose factor of 12	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*
Hexachlorophene	56	87	79.5 - 95.0 (95% CI)	Weil (1952) method	Wistar albino rats; 68 g; 4 weeks	female	oral	corn oil solution; geometric dose factor of 12	preliminary observations over a 1 - 2 week period after dosing; no significant mortalities occurred after 5 days; toxicity signs: lethargy, posterior paralysis, increased rate of respiration, hyperthermia, and diarrhea	16 rats at 4 dosage levels; fasted overnight	U.S.P. grade; Givaudan Corp., Clifton, NJ	Nakaue HS, Dost FN, Buhler DR. 1973. Studies On The Toxicity Of Hexachlorophene In Rats. Toxicol Appl Pharmacol 24:239-49.A19 *Oregon State University, Corvallis, OR*
Hexachlorophene	56	104.03	84.45 - 128.20 (95% fiducial limit)	Bliss method	normal white rats; 150-250 g	NA	NA	40, 80, 120, 160, 200 mg/kg	25 rats used; 12 dead within 40 hours	5 groups of 5 rats each	NA	Chung HL., 1963. Hexachlorophene (G-11) as a new specific drug against Clonorchiasis Sinensis. Chinese Medical Journal. 82. No. 11. November. *Peking Sino-Soviet Friendship Hospital, Peking, China*
Hexachlorophene	56	111	12 (S.E.)	Miller and Tainter (1944)	Sprague-Dawley rats; 32 day	male and female	oral; stomach tube	1% carboxymethylcellulose	observed for 10 days	approximately equal numbers of males and females; 66 rats	NA	Nieminen L, Bjondahn K, Mottonen M. 1973. Effect of hexachlorophene on the rat brain during ontogenesis. Fd Cosmet Toxicol 11:635-639.
Hexachlorophene	56	120	110 - 131 (95% CI)	Litchfield and Wilcoxin 1949	Sherman strain rats (SPF); weanling	female	oral; stomach tube	peanut oil solution	died within 5 days; depression and posterior paralysis	5 or more groups of 10 rats each	NA	Gaines TB, Kimbrough RD, Linder RE. 1973. The oral and dermal toxicity of hexachlorophene. Toxicology and Applied Pharmacology 25:332 -343. *Environmental Protection Agency, Chamblee, GA*
Hexachlorophene	56	121	112 - 133 95% CL; slope 14.8	Finney's maximum likelihood probit	Sherman strain rats (SPF); 4-6 weeks	female	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed for at least 14 days after dosing or until recovered from signs of toxicity	At least 40 rats used; min. of 10 animals per group tested; min. of 4 doses; animals used are the same as Gaines 1973	technical grade	Gaines TB, Linder RE.(1986) Acute toxicity of pesticides in adult and weanling rats. Fundam Appl Toxicol 7(2):299-308. *Health Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC*
Hexachlorophene	56	165	149 - 179 (95% CI)	Probit analysis	Crl-CD rats from Charles River Breeding lab; 220 -280 g; 60 days	male	oral; intragastric intubation	0.5 - 3.9% suspens; dissolved or suspended in corn oil; single dose; 100, 140, 175, 200 mg/kg doses	observed daily for 14 days; death within 6 days; toxic symptoms: staining of the face and perineal area, weakness, diarrhea, weight loss	non fasted; 4 groups of 10; 40 rats used; 17 rats died	99+% pure; Givaudan Corp., Clifton, NJ	Dashiell OL, Kennedy GL Jr. 1984. The effects of fasting on the acute oral toxicity of nine chemicals in the rat. J Appl Toxicol 4(6): 320-325. *E.I. Du Pont de Nemours & Co., Newark, DE*
Hexachlorophene	56	215	191 - 237 (95% CI)	Probit analysis	Crl-CD rats from Charles River Breeding lab; 220 -280 g; 60 days	male	oral; intragastric intubation	0.26 - 1.4% suspens dissolved or suspended in corn oil; single dose; 50, 100, 170, 225, 275 mg/kg doses	observed daily for 14 days; death within 6 days; toxic symptoms: staining of the face and perineal area, weakness, diarrhea, weight loss	fasted 24 hours before dosing; 5 groups of 10; 50 rats used; 16 rats died	99+% pure; Givaudan Corp., Clifton, NJ	Dashiell OL, Kennedy GL Jr. 1984. The effects of fasting on the acute oral toxicity of nine chemicals in the rat. J Appl Toxicol 4(6): 320-325. *E.I. Du Pont de Nemours & Co., Newark, DE*
Lactic acid	3543	3543	NA	NA	NA	NA	NA	NA	NA	NA	NA	Farm Chemicals Handbook. (Meister Pub., 37841 Euclid Ave., Willoughy, OH 44094). 1991. **(RTECS REFERENCE)**
Lactic acid	3543	3730	3020 - 4610 (95% probability; +/- 1.96 S.D. slope = 4.04)	probits (Bliss)	Wistar albino rats; 90-120 g	male	oral; stomach tube; single doses	concentration in water; largest dose given was 50 g/kg	most deaths occurred in 2 days; all deaths occurred in 14 days	groups of 10 animals; 10 animals per dose	purified commercial grade	Smyth HF Jr, Seaton J, Fischer L. 1941. The single dose toxicity of some glycols and derivatives. J Ind Hyg Toxicol 23:259-268. *Mellon Institute, Pittsburgh, PA*
Lindane	76	76 - 200	NA	NA	rats	NA	oral	NA	NA	secondary source; unknown primary source	NA	Special Publication of the Entomological Society of America. (4603 Calvert Rd., College Park, MD 20740). 1978. Kenaga EE, Morgan RW. 1978. Commercial and Experimental Organic Insecticides. 1978 Revision. Special Publication 78-1:1-76. *The Dow Chemical Company, Midland, MI* **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Lindane	76	88	76 - 101 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min. wt. = 175 g; min. age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 8 days; 14 days observation	89 rats tested; not fasted	technical grade	Gaines TB. 1960. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 2:88-99 *U.S. Dept. of Health, Education, and Welfare, Savannah, GA*
Lindane	76	91	83 - 100 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min. wt. = 200 g; min. age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 7 days; 14 days observation	69 rats tested; not fasted	technical grade	Gaines TB. 1960. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 2:88-99 *U.S. Dept. of Health, Education, and Welfare, Savannah, GA*
Lindane	76	100	NA	Litchfield and Wilcoxon method (1949)	CFY strain rats; 120+ g; adult	female	oral	NA	NA	NA	99.5% pure; Budapest Chemical Works	Desi I. 1983. Neurotoxicological investigaton of pesticides in animal experiments. Neurobehav Toxicol 5:503-515. *National Institute of Hygiene, Hungary*
Lindane	76	125	NA	NA	rats	NA	oral; stomach tube	NA	hypersensitivity and convulsions	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletine (Association of Food and Drug Officials of the United States). Vol. 15:122-133. *U.S. FDA*
Lithium I carbonate	525 553	525	460-598 (95% CI)	Litchfield and Wilcoxon method	Wistar rats; 180 g (ave)	female	oral	in solution; 347, 417, 500, 600, 720, 864 mg/kg	7 days observation; deaths/dose (mg/kg): 347-0/10, 417-1/10, 500-3/10, 600-5/10, 720-8/10, 864-10/10; 14 deaths on day 1, 12 deaths on day 2 , 1 death on day 3; all rats at highest dose dead by day 2	Used 10 rats/dose; RTECS reference: in Japanese	reagent grade	Nakasawa M, et al. 1973. Lithium carbonate toxicity tests, rat and mouse acute toxicity. Kiso to Rinsho Clinical Report 7:1273-1277. **(RTECS REFERENCE)**
Lithium I carbonate	525 553	553	NA	NA	rats	NA	oral	NA	NA	RTECS reference that provides summary data only. LD50 value is unreferenced and unsupported	reagent grade	Filov VA, Ivin BA, Bandman AL (eds).1993. Harmful Chemical substances. Volume 1: Elements in Groups I-IV of the Periodic Table and their Inorganic Compounds. Ellis Horwood Limited (publisher). First published in Russian asVrednye khimicheskIye veehestra. Neorganicheskiye soyedineniga elementor I-IV grup. VA Filov, ed. Khimiya, St. Petersburg. 1988.
Lithium I carbonate	525 553	590	505-691 (95% CI)	Litchfield and Wilcoxon method	Wistar rats; 220 g (ave)	male	oral	in solution; 347, 417, 500, 600, 720, 864 mg/kg	7 d observation; deaths/dose (mg/kg): 347- 0/10, 417- 2/10, 500- 3/10, 600- 5/10, 720 - 8/10, 864- 10/10; most deaths on day 2; 3 deaths on day 1 at highest dose; 3 deaths at lower doses on day 3	Used 10 rats/dose; RTECS reference, in Japanese	reagent grade	Nakasawa M, et al. 1973. Lithium carbonate toxicity tests, rat and mouse acute toxicity. Kiso to Rinsho Clinical Report 7:1273-1277.
Lithium I carbonate	525 553	710	NA	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 200 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period;	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30:470-476. *Carnegie-Mellon University, Pittsburgh, PA* (LD50 value). Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)
Meprobamate	794	486	+/- 24 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 21 days	female	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Meprobamate	794	794 (outlier)	584 - 1080 (95% CL)	Litchfield and Wilcoxon method (1949)	rats; 117-180 g; adult	female	oral	suspension; 2.3 - 23.2 mg/kg dose levels	hypothermia, prostration, bradypnea, ptosis, sluggish corneal reflex	5 rats per dose level; 20 rats used	NA	Franko BV, Ward JW, Gilbert DL, Woodard G. 1971. Toxicologic studies of glycopyrralate in combination with other drugs. Toxicology and Appled Pharmacology 19:93-102. *Woodard Research Corporation, Herndon, VA* **(RTECS REFERENCE)**
Meprobamate	794	1286	+/- 81 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 100 days	male	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Meprobamate	794	1290	+/- 104 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 63 days	male	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Meprobamate	794	1346	+/- 82 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 21 days	male	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Meprobamate	794	1361	+/- 76 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 100 days	female	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Meprobamate	794	1410	+/- 83 (S.E.)	Miller and Tainter (1944)	FDRL-strain rats; 63 days	female	oral	NA	observed for 7 days post-treatment	NA	NA	Weinberg MS, Goldhamer RE, Carson S. 1966. Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxic Appl Pharmac 9:234-239. *Food and Drug Research Laboratories, Inc., Maspeth, NY*
Meprobamate	794	1470		Litchfield and Wilcoxon method (1949)	rats; 117-180 g; adult	male	oral	suspension; 2.3 - 23.2 mg/kg dose levels	hypothermia, prostration, bradypnea, ptosis, sluggish corneal reflex	5 rats per dose level; 20 rats used	NA	Franko BV, Ward JW, Gilbert DL, Woodard G. 1971. Toxicologic studies of glycopyrralate in combination with other drugs. Toxicology and Appled Pharmacology 19:93-102. *Woodard Research Corporation, Herndon, VA*
Meprobamate	794	1522	+/- 16 (S.E.)	Miller and Tainter (1944)	Charles River CD and Sprague-Dawley strains; > 100 g; adult	NA	oral intubation	up to 50 mL/kg	rats observed for 7 days; observed up to 14 days when heavy metals or other compounds that produce latent death were investigated	fasted overnight	NA	Yeary RA, Benish RA, Finkelstein M. 1966. Acute Toxicity of Drugs in Newborn Animals. Journal of Pediatrics 69 (4):663-667. *Dept. of Veterinary Preventive Medicine, Ohio State University, Columbus, OH*
Mercury II chloride	1	1 - 5	NA	NA	rats	NA	oral	NA	NA	lists LD50 range as 1 - 5 mg/kg	NA	Pesticide Manual. (The British Crop Protection Council, 20 Bridport Rd., Thornton Heath CR4 7QG, UK) V.1- 1968. 1991. **(RTECS REFERENCE)**
Mercury II chloride	1	12	9 - 17 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats; 190-300 g	female	oral gavage	single dose	14 day observation; toxicity symptoms: motor activity decrease, respiratory effects, tremors, blanching, piloerection, diarrhea, chouromodaceryorrhoea; time to onset of signs < 1day; duration of signs 11 days; animals fasted 16 -20 hours before administration	UDP Test	NA	Yam J, Reer PJ, Bruce RD. 1991. Comparison of the up-and-down method and the fixed-dose procedure for acute oral toxicity testing. Food Chem Toxicol 29(4):259-264. *The Procter and Gamble Co., Cincinnati, OH*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Mercury II chloride	1	24	17.9 - 32.2	Bliss-Probit method	Sprague-Dawley rats; 5 weeks	male	oral gavage	dissolved in saline; range (mg/kg) of doses 10.6, 13.8, 17.9, 23.3, 30.3, 39.7	observed at 6 hours after dosing and a once a day for 1-2 weeks; most dead within 3 days; 25/60 died; toxic symptoms: piloerection, drooling, hypothermia, abdominal posture, tremor, and diarrhea; dose (mg/kg), dead rats per dose: 10.6-0/10; 13.8-1/10; 17.9-1/10; 23.3-4/10; 30.3-9/10; 39.7-10/10	animals acclimated to environment for 1 week before testing; 6 groups of10 rats each; fasted 16 hours before dosing; 100% lethal dose = 39.7 mg/kg; 0% lethal dose = 10.6 mg/kg	Kishida Chemical Co., Ltd.	Kitagawa H, Saito H, Sugimoto T, Yanaura S, Kitagawa H, Hosokawa T, Sakamoto K. 1982. Effects of diospropyl-1,3-dithiol-2-ylidene malonate (NKK-105) on acute toxicity of various drugs and heavy metals. J Toxicol Sci 7(2):123-34. *Chiba University; Hoshi College of Pharmacy; Showa University -- Japan*
Mercury II chloride	1	32	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	10, 15, 25, 40, 60, 100 mg/kg	15 mg/kg: 0/3 dead; 25mg/kg: 0/3 dead; 40 mg/kg: 3/3 dead; 60 mg/kg: 3/3 dead; 6/12 rats dead; LD50 from 12 rats used; LD50 recalculated using US EPA Benchmark Dose soft-ware; Lorke used data from 10 and 100 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 32 mg/kg	acclimated for 5 days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose; range finder: 10 mg/kg - 0/3 dead; 100 mg/kg - 3/3 dead; 1000 mg/kg - 3/3 dead; 9 rats in range finder	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Mercury II chloride	1	39	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	10, 15, 25, 40, 60, 100 mg/kg	15 mg/kg: 1/11 dead; 25mg/kg: 1/11 dead; 40 mg/kg: 7/11 dead; 60 mg/kg: 10/11 dead; 19/44 rats dead; LD50 from 44 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 10 and 100 mg/kg in range finder for all animal groups; omitted this data in recalculation; Orginial LD50 from Lorke = 37 mg/kg; this value based on accumulated data from 4 different test groups	acclimated for five days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose; range finder showed: 10 mg/kg - 0/3 dead; 100 mg/kg - 3/3 dead; 1000 mg/kg - 3/3 dead; 9 rats in range finder	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Mercury II chloride	1	40	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	10, 15, 25, 40, 60, 100 mg/kg	15 mg/kg: 1/5 dead; 25mg/kg: 1/5 dead; 40 mg/kg: 3/5 dead; 60 mg/kg: 5/5 dead; 10/20 rats dead; LD50 based on 20 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 10 and 100 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 32 mg/kg	acclimated for five days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose; range finder showed: 10 mg/kg - 0/3 dead; 100 mg/kg - 3/3 dead; 1000 mg/kg - 3/3 dead; 9 rats in range finder	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Mercury II chloride	1	49	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	10, 15, 25, 40, 60, 100 mg/kg	15 mg/kg: 0/1 dead; 25mg/kg: 0/1 dead; 40 mg/kg: 0/1 dead; 60 mg/kg: 1/1 dead; 1/4 rats dead; LD50 from 4 rats used; T306	acclimated for five days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose; range finder showed: 10 mg/kg - 0/3 dead; 100 mg/kg - 3/3 dead; 1000 mg/kg - 3/3 dead; 9 rats in range finder	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Mercury II chloride	1	50	40 - 63	Thompson and Weil; 1952; method of moving averages	albino rats; 18 weeks	female	oral; stomach tube	1 mL/200 g bw	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic, T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-8. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Mercury II chloride	1	50	43 - 59	Thompson and Weil; 1952; method of moving averages	albino rats; 54 weeks	female	oral; stomach tube	1 mL/200 g bw	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic, T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Mercury II chloride	1	51	39 - 66 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	male	oral gavage	single dose	14 day observation; toxicity symptoms: posture, respiratory effects, lethargy, abnormal gait, prostrate coma, salivation; time to onset of signs < 1 day; duration of signs 5 days	3 dose levels (5 male each); 15 rats used; OECD TG401 (1981) followed for experimental procedures; 8 rats dead (average per test)	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Mercury II chloride	1	52	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	10, 15, 25, 40, 60, 100 mg/kg	15 mg/kg: 0/2 dead; 25mg/kg: 0/2 dead; 40 mg/kg: 1/2 dead; 60 mg/kg: 1/2 dead; 2/8 rats dead; LD50 based on 8 rats used; LD50 recalculated using US EPA Benchmark Dose software; Lorke used data from 10 and 100 mg/kg in range finder for all animal groups; omitted this data in recalculation; orginial LD50 from Lorke = 50 mg/kg	acclimated for five days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose; range finder showed: 10 mg/kg - 0/3 dead; 100 mg/kg - 3/3 dead; 1000 mg/kg - 3/3 dead; 9 rats in range finder	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Mercury II chloride	1	92	77 - 108	Thompson and Weil; 1952; method of moving averages	albino rats; 6 weeks	female	oral; stomach tube	1 mL/200 g bw; 6 dose levels in each group	observed after 8 days after single oral administration	6 dose levels per group, 6 rats per group; 36 rats used	NA	Kostial K, Kello D, Jugo S, Rabar I, Maljkovic, T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86. *Yugoslav Academy of Sciences and Art, Zagreb, Yugoslavia*
Mercury II chloride	1	160 (outlier)	119 - 235 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	female	oral gavage	single dose	14 day observation; toxicity symptoms: posture, respiratory effects, lethargy, abnormal gait, prostrate coma, salivation; time to onset of signs < 1 day; duration of signs 5 days	3 dose levels (5 female each); 15 rats used; OECD TG401 (1981) followed for experimental procedures; 8 rats dead (average per test)	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Methanol	5628	5628	4613 - 6866	NA	rats	NA	oral	NA	NA	reference in Russian; was also cited in 1983/84 RTECS but value was different from that used by RC and reference was not provided by ZEBET	NA	Lazinov AG, Broitman AT. 1975. On the combined action of 2, 6-dimethylphenol and methanol. Gigiena Truda i Professional'nye Zabolevaniya 19(11):27-30. **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Methanol	5628	5890 (7.4 mL/kg; used density of 0.796 to convert to mg/kg)	4776 - 7244 (95% CL; 6.0 - 9.1 mL/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 16-50 g; 14 days	male and female	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; 6-12 rats of both sexes used for studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Methanol	5628	7005 (8.8 mL/kg; used density of 0.796 to convert to mg/kg)	5731 - 8597 (95% CL; 7.2 - 10.8 mL/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 300-470 g; older adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Methanol	5628	7400	NA	NA	rats; 150-250 g; 70- 100 days	male (predominately)	oral	NA	observed for 6 days	18 hour fasting before dosing	NA	Welch, H, Slocum GG. 1943. Relation of length of carbon chain to the primary and functional toxicities of alcohols. J Lab Chem Med 28:1440-1445. *U.S. FDA, Washington, D.C.*
Methanol	5628	10348 (13.0 mL/kg; used density of 0.796 to convert to mg/kg)	9472 - 11303 (95% CL; 11.9 - 14.2 mL/kg)	Litchfield and Wilcoxon method and probit analysis	Sprague-Dawley rats; 80-160 g; young adult	male	oral	solvent used in undiluted form	animals observed for a week after medication	nonfasted rats; groups of 6 rats used for the studies; solvent used in undiluted form	analytical grade meeting A.C.S. specifications	Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704. *Abbott Laboratories, Chicago, IL*
Methanol	5628	12086 - A 11303 - B (A = 15.28 mL/kg; B = 14.29 mL/kg; used density of 0.791 for conversion to mg/kg)	NA	A= Behrens (1929) B = Bliss (1938)	rats	NA	oral	NA	NA	40 - 90 animals used; NICEATM used value B since authors stated it was more accurate	NA	Deichmann WB, Mergard EG. 1948. Comparative evaluation of methods employed to express the degree of toxicity of a compound. J Ind Hyg Toxicol 30:373-378 *Albany Medical College, Albany, NY; University of Cincinnati, Cincinnati, OH*
Methanol	5628	12880	11440 - 14460 (95% probability; +/- 1.96 S.D. slope = 8.53)	probits (Bliss)	Wistar albino rats; 90-120 g	male	oral; stomach tube; single doses	50% concentration in water; largest dose given was 50 g/kg	most deaths occurred in 2 days; all deaths occurred in 14 days	groups of 10 animals; 10 animals per dose	purified commercial grade	Smyth HF Jr, Seaton J, Fischer, L. 1941. The single dose toxicity of some glycols and derivatives. J Ind Hyg Toxicol 23:259-268. *Mellon Institute, Pittsburgh, PA* (This was the value used by the RC [from 1977 RTECS]).
Nicotine	50	50 - 60	NA	NA	rats	NA	oral	NA	NA	reference is secondary; assumed to be values from Lehman (1951)	NA	Farm Chemicals Handbook. (Meister Pub., 37841 Euclid Ave., Willoughy, OH 44094). 1991. **(RTECS REFERENCE)**
Nicotine	50	50 - 60	NA	NA	rats	NA	oral; stomach tube	NA	clonic convulsions; onset within minutes; paralysis of respiratory muscles and death	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletine (Association of Food and Drug Officials of the United States). Vol.15:122-133. *U.S. FDA*
Nicotine	50	68	41 -129 (95% CL; slope = 3.0 [S.E. 0.8])	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	male	oral gavage	single dose	14 day observation; toxicity symptoms: Ptosis, posture, respiratory effects, lethargy, abnormal gait, tremors, convulsions, prostrate coma; time to onset of signs < 1day; duration of signs 3 days; 13 rats dead (average per test)	3 dose levels (5 male each); 15 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Nicotine	50	70	49 - 109 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	male and female	oral gavage	single dose	14 day observation; toxicity symptoms: Ptosis, posture, respiratory effects, lethargy, abnormal gait, tremors, convulsions, prostrate coma; time to onset of signs < 1day; duration of signs 3 days; 13 rats dead (average per test)	3 dose levels (5 male each and 5 female); 30 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Nicotine	50	70	51 - 96 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats; 190-300 g	female	oral gavage	single dose	14 day observation; toxicity symptoms: motor activity decrease, respiratory effects, tremors, blanching, piloerection, ataxia, convulsions, extension of the limbs; time to onset of signs < 1day; duration of signs 5 days; animals fasted 16 -20 hours before administration	UDP Test	NA	Yam J, Reer PJ, Bruce RD. 1991. Comparison of the up-and-down method and the fixed-dose procedure for acute oral toxicity testing. Food Chem Toxicol 29(4):259-264. *The Procter and Gamble Co., Cincinnati, OH*
Nicotine	50	71	42 - 128 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	female	oral gavage	single dose	14 day observation; toxicity symptoms: Ptosis, posture, respiratory effects, lethargy, abnormal gait, tremors, convulsions, prostrate coma; time to onset of signs < 1day; duration of signs 3 days; 13 rats dead (average per test)	3 dose levels (5 female each); 15 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Paraquat	57	57	NA	NA	rats	NA	oral	NA	NA	NA	NA	*Residue Reviews. (Springer-Verlag New York, Inc., Service Center, 44 Hartz Way, Secaucus, NJ 07094) V.1- 1962. 1965.* Bailey GW, White JL. 1965. Herbicides: a compilation of their physical, chemical, and biological properties. Journal paper no. 2413. Purdue University Agricultural Experiment Station. Residue Reviews 10:7-122. **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Paraquat	57	95	79-114 (95 % CL)	Litchfield and Wilcoxon method (1949)	Wistar rats; 292 +/- 13 g	male	oral intubation	single dose	observe several times daily and at least once on weekends for 30 days; most of the rats that died did so within 5 days of administration; weight loss, diarrhea, piloerection and red drainage around mouth, eyes, and nose	used 29 paraquat-dichloride	Ortho Chemical Co.	Sharp CW, Ottolenghi A, Posner HS. 1972. Correlation of paraquat toxicity woth tissue concentrations and weight loss of the rat. Toxicology and Appied Pharmacology 22:241-251. *NIEHS, RTP, NC USA*
Paraquat	57	100	85 - 117 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min. wt. = 175 g; min. age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 14 days	50 rats tested	technical grade	Gaines TB. 1969. Acute toxicity of pesticides. Toxicol Appl Pharmacol 14(3):515-34. *U.S. Dept. of Health, Education, and Welfare, Atlanta, GA*
Paraquat	57	110	90 - 134 (95% CL)	Litchfield and Wilcoxon method (1949)	Sherman strain rats; min. wt. = 200 g; min. age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 13 days	50 rats tested	technical grade	Gaines TB. 1969. Acute toxicity of pesticides. Toxicol Appl Pharmacol 14(3):515-34. *U.S. Dept. of Health, Education, and Welfare, Atlanta, GA*
Paraquat	57	112 (paraquat ion)	104-122 (95% CL)	Thompson (1947); moving average interpolation method	rats; 130-160 g	male and female	oral; in food	single dose; mixed salt of paraquat in food with 20% malt extract and fed to rats	fasted overnight; observed up to 12 days	6 rats per group	99.9% pure paraquat dichloride	Clark DG, McElligott TF, Hurst EW. 1966. The toxicity of paraquat. Br J Ind Med 23:126-132. *Imperial Chemical Industries Limited, Cheshire, UK*
Paraquat	57	115	90-150 (95% CL)	Litchfield and Wilcoxin method (1949)	Sprague Dawley rat; 290 +/- 37 g	male	oral intubation	single dose	observe several times daily and at least once on weekends for 30 days; most of the rats that died did so within 5 days of administration; weight loss, diarrhea, piloerection and red drainage around mouth, eyes, and nose	used 29 paraquat-dichloride	Ortho Chemical Co.	Sharp CW, Ottolenghi A, Posner HS. 1972. Correlation of paraquat toxicity woth tissue concentrations and weight loss of the rat. Toxicology and Appied Pharmacology 22:241-251. *NIEHS, RTP, NC USA*
Paraquat	57	141 (paraquat ion)	140-142 (95% CL)	Thompson (1947); moving average interpolation method	rats; 130-160 g	male and female	oral; in food	single dose; mixed salt of paraquat in food with 20% malt extract and fed to rats	fasted overnight; observed up to 12 days	6 rats per group	99.9% pure paraquat dimetho-sulfate	Clark DG, McElligott TF, Hurst EW. 1966. The toxicity of paraquat. Br J Ind Med 23:126-132. *Imperial Chemical Industries Limited, Cheshire, UK*
Paraquat	57	150 (paraquat ion)	139-162 (95% CL)	Thompson (1947); moving average interpolation method	rats; 150-205 g	male and female	oral; in food	single dose; mixed salt of paraquat in food with 20% malt extract and fed to rats	fasted overnight; observed up to 12 days	10 rats per group	99.9% pure paraquat dichloride	Clark DG, McElligott TF, Hurst EW. 1966. The toxicity of paraquat. Br J Ind Med 23:126-132. *Imperial Chemical Industries Limited, Cheshire, UK*
Parathion	2	1.8 (actual value)	1.26 - 2.57 (95% CL; slope = 1.5 [1.0 - 2.25 95% CL])	Litchfield and Wilcoxin method (1949)	Osborne-Mendel (?) rats	female	oral	5 dose levels; constant vol. dose of solvent of 5 mL/kg; single dose; aqueous solution (sodium carboxymethyl-cellulose, 0.5%; NaCl, 0.9%; benzyl alcohol, 0.2% v/v; Tween 80, 0.4%)	observed for 24 hours; deaths infrequent after 24 hour; onset of anticholinesterase poisoning syptoms slower with corn oil than DMSO or aqueous	fasted for 20 hours	NA	Weis LR, Orzel RA. 1967. Some comparative toxicologic and pharmacologic effects of dimethyl sulfoxide as a pesticide solvent. Toxicology and Applied Pharmacology 11:546-557. *U.S. FDA, Washington, D.C.* **(RTECS REFERENCE)**
Parathion	2	2.1	1.72 - 2.56 (95% CL; slope = 1.25 [1.01 - 1.55 95% CL])	Litchfield and Wilcoxon method (1949)	Osborne-Mendel (?) rats	female	oral	5 dose levels; constant vol. dose of solvent of 5 mL/kg; single dose; cmpd dissolved in DMSO (industrial grade, 99% pure)	observed for 24 hours; deaths infrequent after 24 hour; onset of anticholinesterase poisoning syptoms slower with corn oil than DMSO or aqueous	fasted for 20 hours	NA	Weis LR, Orzel RA. 1967. Some comparative toxicologic and pharmacologic effects of dimethyl sulfoxide as a pesticide solvent. Toxicology and Applied Pharmacology 11:546-557. *U.S. FDA, Washington, D.C.*
Parathion	2	3	NA	NA	rats	NA	oral; stomach tube	NA	generalized fibrillary tremors, salivation, lacrimation, diarrhea, and convulsions; onset within 1 hour	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals; LD50 value is from research by Frawley et al. 1952	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletine (Association of Food and Drug Officials of the United States). Vol.15:122-133. *U.S. FDA*
Parathion	2	3	+/- 0.25 (S.E.)	Litchfield and Fertig (1941)	Osborne-Mendel strain rats; 180-200 g	female	oral; stomach tube	cmpd in corn oil	toxicity symptoms: muscle fibrillation, red colored lacrimation, diarrhea, dyspnea, convulsions; respiratory paralysis, anoxia, terminal convulsion	rats fasted for 24 hours; LD50 value was used in Lehman 1951	NA	Frawley JP, Hagan EC, Fitzhugh OG. 1952. A comparative pharmacological and toxicological study of organic phosphate-anticholinesterase compounnds. J Pharmacol Exp Ther 152:156-165. *U.S. FDA, Washington, D.C.*
Parathion	2	3.6	3.2 - 4.0 (95% CL)	Litchfield and Wilcoxin method (1949)	Sherman strain rats; min. wt. = 200 g; min. age of 90 days	female	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 3 days	70 rats tested	technical grade	Gaines TB. 1960. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 2:88-99. *U.S. Dept. of Health, Education, and Welfare, Savannah, GA* Mattson AM, Spillane JT, Pearce GW. 1955. Dimethyl 2,2-dichlorvinyl phosphate (DDVP), an organic phosphorous compound highly toxic to insects. J Agr Food Chem 3:319-321. *Communicable Disease Center, Savannah, GA*
Parathion	2	3.6	3.2 - 4.0 (95% CL)	Litchfield and Wilcoxin method (1949)	Sherman albino rats	female	oral; stomach tube	NA	NA	LD50 value from research in Gaines 1960	NA	Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson MA, Hayes WJ. 1957. Studies on the toxicity of 0,0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). AMA Arch Ind Health 15:340-349. *U.S. Dept. of Health, Education and Welfare, Savannah, GA*
Parathion	2	4.7	3.98 - 5.55 (95% CL; slope = 1.21 [0.98 - 1.50 95% CL])	Litchfield and Wilcoxin method (1949)	Osborne-Mendel (?) rats	female	oral	5 dose levels; constant vol. dose of solvent of 5 mL/kg; single dose; cmpd dissolved in corn oil mixture (90% corn oil, 10% N, N-dimethyl formamide)	observed for 24 hours; deaths infrequent after 24 hour; onset of anticholinesterase poisoning syptoms slower with corn oil than DMSO or aqueous	fasted for 20 hours	NA	Weis LR, Orzel RA. 1967. Some comparative toxicologic and pharmacologic effects of dimethyl sulfoxide as a pesticide solvent. Toxicology and Applied Pharmacology 11:546-557. *U.S. FDA, Washington, D.C.*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Parathion	2	6	4.6 - 7.8 (95% CL)	Litchfield and Wilcoxin method (1949)	CD (COBS) rats Charles River, France; 120-200 g	female	oral gavage	cmpd dissolved in 1 mL methylene chloride; emulsified in 10% arabic gum solution with Tween 80; dose 5 mL/kg	LD50 determined after 10 days of observation	5 dose levels; 10 female per dose; 50 rats used	95+% pure	Pasquet J, Mazuret A, et al. 1976. Acute oral and percutaneous toxicity of phosalone in the rat, in comparison with azinphosmethyl and parathion. Toxicol Appl Pharmacol 37(1):85-92. *Rhone-Poulenc, France*
Parathion	2	10	8 - 13 (95% CL)	Litchfield and Wilcoxin method (1949)	CD (COBS) rats Charles River, France; 120-200 g	male and female	oral gavage	cmpds dissolved in 1 mL methylene chloride and emulsified in 10% arabic gum solution with Tween 80; dose 5mL/kg	LD50 determined after 10 days of observation	5 dose levels; 10 male and 10 female per dose; 100 rats used	95+% pure	Pasquet J, Mazuret A, et al. 1976. Acute oral and percutaneous toxicity of phosalone in the rat, in comparison with azinphosmethyl and parathion. Toxicol Appl Pharmacol 37(1):85-92. *Rhone-Poulenc, France*
Parathion	2	13	10 - 17 (95% CL)	Litchfield and Wilcoxin method (1949)	Sherman strain rats; min. wt. = 175 g; min. age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival 3 days	50 rats tested	technical grade	Gaines TB. 1960. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 2:88-99. *U.S. Dept. of Health, Education, and Welfare, Savannah, GA* Mattson AM, Spillane JT, Pearce GW. 1955. Dimethyl 2,2-dichlorvinyl phosphate (DDVP), an organic phosphorous compound highly toxic to insects. J Agr Food Chem 3:319-321. *Communicable Disease Center, Savannah, GA*
Parathion	2	15	10.2 - 16.5 (95% CL)	Litchfield and Wilcoxin method (1949)	Sherman albino rats	male	oral; stomach tube	NA	NA	LD50 value from research in Gaines 1960	NA	Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson MA, Hayes WJ. 1957. Studies on the toxicity of 0,0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). AMA Arch Ind Health 15:340-349. *U.S. Dept. of Health, Education and Welfare, Savannah, GA*
Parathion	2	16	13 - 20 (95% CL)	Litchfield and Wilcoxin method (1949)	CD (COBS) rats Charles River, France; 120-200 g	male	oral gavage	cmpds dissolved in 1 mL methylene chloride and emulsified in 10% arabic gum solution with Tween 80; dose 5 mL/kg	LD50 determined after 10 days of observation	5 dose levels; 10 male per dose; 50 rats used	95+% pure	Pasquet J, Mazuret A, et al. 1976. Acute oral and percutaneous toxicity of phosalone in the rat, in comparison with azinphosmethyl and parathion. Toxicol Appl Pharmacol 37(1):85-92. *Rhone-Poulenc, France*
Parathion	2	30	+/- 3.6 (S.E.)	Litchfield and Fertig (1941)	Osborne-Mendel strain rats; 180 - 200 g	male	oral; stomach tube	cmpd in corn oil	toxicity symptoms: muscle fibrillation, red colored lacrimation, diarrhea, dyspnea, convulsions; respiratory paralysis, anoxia, terminal convulsion	rats fasted for 24 hours;	NA	Frawley JP, Hagan EC, Fitzhugh OG. 1952. A comparative pharmacological and toxicological study of organic phosphate-anticholinesterase compounnds. J Pharmacol Exp Ther 152:156-165. *U.S. FDA, Washington, D.C.*
Phenobarbital	162	162	+/- 14	NA	Wistar rats; adult	NA	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185-207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.* **(RTECS REFERENCE)**
Phenobarbital	162	220	NA	NA	MJ rats; 80 - 100 days	NA	oral	NA	NA	information from: drug applications from pharmaceutical manufacturers, the literature, and FDA labs	NA	Goldenthal EI. 1971. A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharamacology 18:185-207. *Bureau of Drugs, Food and Drug Administration, Dept. of Health, Education, and Welfare, Rockville, MD.*
Phenobarbital	162	318	+/- 23 (S.E.)	Miller and Tainter (1944)	Charles River CD and Sprague-Dawley rat strains; > 100 g; adult	NA	oral intubation; up to 50 mL/kg	NA	rats observed for 7 days; observed up to 14 days when heavy metals or other cmpds that produce latent death were investigated	fasted overnight	NA	Yeary RA, Benish RA, Finkelstein M. 1966. Acute Toxicity of Drugs in Newborn Animals. Journal of Pediatrics 69 (4):663-667. *Dept. of Veterinary Preventive Medicine, Ohio State University, Columbus, OH*
Phenol	317	317 (0.30 cc/kg of drug lethal to 50% of rats; density = 1.055)	NA	graphically	white rats	NA	oral; stomach tube	5% ethylene glycol added to phenol to liquify it so that it would pass through the stomach tube	most rats died within 2 - 6 hour; practically all dead within 8 - 12 hour; convulsions began several minutes after dosing and continued for several hours	NA	NA	*Gigiena i Sanitariya. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1- 1936. 1976.*---- Brown HW, Lamson PD. 1935. Oral Toxicity of Ortho-n-alkylphenols to White Rats. Proc Soc Exp Biol Med 32:592-594. **(RTECS REFERENCE)**
Phenol	317	340	NA	NA	Wistar rats; 100- 200 g	male and female	oral	20% aqueous emulsion 0.3, 0.4, 0.5 g/kg doses	45 rats used; 30 dead; death within 1 hour; twitching, weak pulse and respiration, salivation, dyspnea	45 rats used (equal numbers of male and female used)	Merck reagent quality	Deichmann WB, Witherup S. 1944. Phenol Studies VI: the acute and comparative toxicity of phenol and o-, m-, and p-cresols for experimental animals. J of Pharmacol and Exp Therapeutics 80:233-240. *College of Medicine, University of Cincinnati, Cincinnati, OH.*
Phenol	317	400	297 - 539 (95% CL)	Dixon (1965) and Bruce (1985)	Fischer 344 rats; 77 days old at test	female	oral gavage	in deionized water; maximum volume dose 10mL/kg; 5 dose levels: 0, 12, 40, 120, 224 mg/kg; single dose	7 day survival time	fasted overnight; initial dose levels were 100, 1000, and 5000 mg/kg; subsequent doses selected by up-and-down method (Bruce, 1985, 1987); 5 groups of 8 rats each; 40 rats used; 7 15 rats used in first LD50 estimate	analytical grad_; 99+% pure; Aldrich Chemical Co.	Berman E, Schlicht M, Moser VC, MacPhail RC. 1995. A multidisciplinary approach to toxicological screening: I. Systemic toxicity. J Toxicol Environ Health 45(2): 127-43. *Health Effects Res. Lab., U.S.EPA, Research Triangle Park, NC*
Phenol	317	445	NA	Probit method	Sprague-Dawley rats; 190-200 g	female	oral	geometric progression of 14 for dosing; in water or neat	9 dead; observed for 14 days	non-fasted; 4 groups of 5 female; 20 rats used	Polysciences, Inc. Warrington, PA	Thompson ED, Gibson DP. 1984. A method for determining the maximum tolerated dose for acute in vivo cytogenetic studies. Food Chem Toxicol 22(8):665-76. *The Procter and Gamble Co., Cincinnati, OH*
Phenol	317	512	455 - 568	NA	rats; 220 +/- 40 g	NA	oral; intragastric	NA	NA	(source of information not provided)	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Phenol	317	520	NA	Probit method	Sprague-Dawley rats; 190-200 g	male	oral	geometric progression of 14 for dosing; in water or neat	10 dead; observed for 14 days	non-fasted; 3 groups of 5 male; 1 group of 10 male; 25 rats used	Polysciences, Inc. Warrington, PA	Thompson ED, Gibson DP. 1984. A method for determining the maximum tolerated dose for acute in vivo cytogenetic studies. Food Chem Toxicol 22(8):665-76. *The Procter and Gamble Co., Cincinnati, OH*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Phenol	317	530	NA	NA	Wistar rats; 100- 200 g	male and female	oral	2% aqueous solution; 0.4, 0.5, 0.6, 0.7, 0.8 g/kg doses	45 rats used; 32 dead; death within 3 hours; twitching, weak pulse and respiration, salivation, dyspnea	45 rats used (equal numbers of male and female used)	Merck reagent quality	Deichmann WB, Witherup S. 1944. Phenol Studies VI: the acute and comparative toxicity of phenol and o-, m-, and p-cresols for experimental animals. J of Pharmacol and Exp Therapeutics 80:233-240. *College of Medicine, University of Cincinnati, Cincinnati, OH.*
Phenol	317	530	NA	NA	Wistar rats; 100- 200 g	male and female	oral	5% aqueous solution; 0.4, 0.5, 0.6, 0.7 g/kg doses	45 rats used; 27 dead; death within 80 minutes twitching, weak pulse and respiration, salivation, dyspnea	45 rats used (equal numbers of male and female used)	Merck reagent quality	Deichmann WB, Witherup S. 1944. Phenol Studies VI: the acute and comparative toxicity of phenol and o-, m-, and p-cresols for experimental animals. J of Pharmacol and Exp Therapeutics 80:233-240. *College of Medicine, University of Cincinnati, Cincinnati, OH.*
Phenol	317	540	NA	NA	Wistar rats; 100- 200 g	male and female	oral	10% aqueous emulsion 0.5, 0.6, 0.7, 0.8 g/kg doses	40 rats used; 28 dead; death within 120 minutes; twitching, weak pulse and respiration, salivation, dyspnea	40 rats used (equal numbers of male and female used)	Merck reagent quality	Deichmann WB, Witherup S. 1944. Phenol Studies VI: the acute and comparative toxicity of phenol and o-, m-, and p-cresols for experimental animals. J of Pharmacol and Exp Therapeutics 80:233-240. *College of Medicine, University of Cincinnati, Cincinnati, OH.*
Phenol	317	550 - A 530 - B	NA	A= Behrens (1929) B = Bliss (1938)	rats	NA	oral	2% aqueous solution	NA	41 - 90 animals used; NICEATM used value B since authors stated it was more accurate	NA	Deichmann WB, Mergard EG. 1948. Comparative evaluation of methods employed to express the degree of toxicity of a cmpd. J Ind Hyg Toxicol 30:373-378. *Albany Medical College, Albany, NY; University of Cincinnati, Cincinnati, OH*
Phenol	317	580 - A 540 - B	NA	A= Behrens (1929) B = Bliss (1938)	rats	NA	oral	10% aqueous solution	NA	42 - 90 animals used; NICEATM used value B since authors stated it was more accurate	NA	Deichmann WB, Mergard EG. 1948. Comparative evaluation of methods employed to express the degree of toxicity of a compound. J Ind Hyg Toxicol 30:373-378. *Albany Medical College, Albany, NY; University of Cincinnati, Cincinnati, OH*
Phenol	317	550 - 650	NA	NA	Normal albino rats	male and female	oral	single doses in mg/kg: 400, 450, 500, 550, 600, 650, 700; phenol as 5% aqueous solution	dose (mg/kg), percent mortality, minutes till death: 400, 10%, 20; 450, 20%, 10 to 80; 500, 30%, 10 to 30; 500, 30%, 10 to 30; 550, 50%, 5 to 90; 600, 60%, 3 to 8; 650, 60%, 4 to 60; 700, 90%, 4 to 50; 500 mg/kg repeated in reference paper	rats divided into 5 test groups and 1 control; 10 rats per group; 80 rats used	NA	Deichmann W, Oesper P. 1940. Ingestion of phenol: effects on the albino rat. Industr Med 9:296-298.
Phenol	317	650	490 - 860 (95% CL)	NA	albino rats	male	oral; stomach intubation	4 doses: 200, 398, 795, 1580 mg/kg; single dose	observed for 14 days; 9 of 20 rats dead; dose (mg/kg), rats dead: 200 - 0/5; 398 - 0/5; 795 - 4/5 (dead within 1 day after dosing); 1580 - 5/5 (dead < 2 hour after dosing)	4 groups of 5 rats; 20 rats used; test procedures outlined in the Federal Hazardous Substances Act (FSHA) in the Federeal Register 8/12/61, pages 7333-7341, entitled "Part 191 - Hazardous Sub-stances: Definitions and Procedural and Interpretive Regulations, Final Order"	Fisher Scientific Co.	Flickinger CW. 1976. The benzenediols: catechol, resorcinol and hydroquinone -- a review of the industrial toxicoloogy and current industrial exposure limits. Am Ind Hyg Assoc J 37:596-606. *Koppers Company, Inc., Monroeville, PA*
Phenol	317	1030	940 - 1120	NA	albino rats; 90-120 g	male	oral; stomach tube	5% phenol solution in water; single dose	observed for 14 days; 10 rats dead	non-fasted; 4 groups of 10 rats	rwagent grade	from EPA TSCATS database; Acute Toxicity of Phenol (1949), EPA Document No. 86-870001405 Fiche No. OTS0515567 *Mellon Institute of Industrial research, Univ. of Pittsburgh, Pittsburgh, PA*
Phenol	317	1460 - A 1500 - B	NA	A= Behrens (1929) B = Bliss (1938)	rats	NA	oral	10% solution in olive oil	NA	40 - 90 animals used; NICEATM used value B since authors stated it was more accurate	NA	Deichmann WB, Mergard EG. 1948. Comparative evaluation of methods employed to express the degree of toxicity of a cmpd. J Ind Hyg Toxicol 30:373-378. *Albany Medical College, Albany, NY; University of Cincinnati, Cincinnati, OH*
Phenylthiourea	3	3.1	NA	NA	rats		oral	NA	NA	value cited from unknown reference	NA	Scheline RR, Smith RL, Williams RT. 1961. The metabolism of arylthioureas -- II. The metabolism of ^{14}C- and ^{35}S-labelled 1-phenyl-2-thiourea and its derivatives. Journal of Medicinal and Pharmaceutical Chemistry 4(1):109-134. *University of London, UK* **(RTECS REFERENCE)**
Phenylthiourea	3	< 21.5	NA	NA	Fischer rats; 6 weeks	male and female	oral intubation	NA	observed up to 14 days	NA	NA	Carcinogenesis bioassay of environmental chemicals annual progress report NIH-NCI-E-C-72-3252. 5/13/71 -- 8/6/73 and Final report NIH-NCI-E-71-2146. Submitted to The National Cancer Institute, National Institutes of Health, Bethesda, MD. 8/15/73 (revised 8/10/73). *Litton Bionetics, Inc. Bethesda, MD.*
Physostigmine (Eserine)	4.5	4.5	NA	NA	rat	NA	oral	NA	NA	NA	NA	Alisi MA, Brufani M, Cesta MC, Filocamo L, Gostoli G, Lappa S, et al. 1994. U.S. Patent 5,302,593. Aminoalkylcarbamic esters of eseroline suitable for use as cholinesterase activity inhibitors (April 12, 1994). **(RTECS REFERENCE)**
Potassium I chloride	2600	2600	2330 - 2900	Bliss method	Wistar rats; 110- 140 g	male	oral gavage	approximately 5 doses; in water or oil solution	14 day observation period;	reference in Czechoslovakian; intro to reference in English; generally 10 animals per dose; up to 50 rats used	NA	Sbornik Vysledku Toxixologickeho Vysetreni Latek A Pripravku. Marhold, J.V., Institut Pro Vychovu Vedoucicn Pracovniku Chemickeho Prumyclu Praha, Czechoslovakia, 1972. **(RTECS REFERENCE)**
Potassium I chloride	2600	3020	+/- 140 (S.E.)	Croxton (1953) Least squares linear regression.	Wistar albino rats; adult	female	oral; stomach tube	in distilled water: 0, 2.1, 2.4, 2.7, 3.3, 3.6, and 3.9 g/kg bw doses; volume of 20 mL/kg bw	respiratory failure, convulsions, gastroenteritis, anorexia, polydipsia, polyurea, fever; 14 day observation; death occurred in about half the rats	109 female rats used; fasted for 16 hours	NA	Boyd EM, Shanas MN. 1961. The Acute Oral Toxicity of Potassium Chloride. Arch Int Pharmacodyn 133.275. *Queen's University, Kingston, Ontario, Canada*
Potassium cyanide	5	5	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	2, 4, 9, 14 mg/kg	2 mg/kg: 0/11 dead; 4 mg/kg:2/11 dead; 9 mg/kg:10/11 dead; 14 mg/kg:11/11 dead; 23 of 44 rats dead; LD50 based on groups containing 3 and 5 rats	acclimated for 5 days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose); 9 rats used for initial range finding	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany* **(RTECS REFERENCE)**

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Potassium cyanide	5	5	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	2, 4, 9, 14 mg/kg	2 mg/kg: 0/3 dead; 4 mg/kg: 1/3 dead; 9 mg/kg: 3/3 dead; 14 mg/kg: 3/3 dead; 7 of 12 rats dead; LD50 based on 12 rats used; used same rats as experiments using 44 or 20 rats	acclimated for 5 days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose); 9 rats used for initial range finding	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Potassium cyanide	5	5	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	2, 4, 9, 14 mg/kg	2 mg/kg: 0/5 dead; 4 mg/kg: 1/5 dead; 9 mg/kg: 5/5 dead; 14 mg/kg: 5/5 dead; 11 of 20 rats dead; LD50 based on 20 rats used	acclimated for 5 days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose); 9 rats used for initial range finding	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Potassium cyanide	5	6	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	2, 4, 9, 14 mg/kg	2 mg/kg: 0/1 dead; 4 mg/kg: 0/1 dead; 9 mg/kg:1/1 dead; 14 mg/kg:1/1 dead; 2 of 4 rats dead; LD50 based on 4 rats used; used same rats as experiments using 44 rats	acclimated for 5 days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose); 9 rats used for initial range finding	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Potassium cyanide	5	6	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	2, 4, 9, 14 mg/kg	2 mg/kg: 0/11 dead; 4 mg/kg:2/11 dead; 9 mg/kg:10/11 dead; 14 mg/kg:11/11 dead; 23 of 44 rats dead; LD50 based on all rats used (44); summary data from four tests; Test 1 = 4 rats; test 2 = 8 rats; test 3 = 12 rats; test 4 = 20 rats	acclimated for 5 days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose); 9 rats used for initial range finding	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Potassium cyanide	5	7.26	6.50 - 8.09	Bliss-Probit method	Sprague-Dawley rats; 5 weeks	male	oral gavage	dissolved in saline; range (mg/kg) of doses 4.9, 5.8, 7.0, 8.4, 10.1, 12.1	rats observed at 6 hours after dosing and a once a day for 1-2 weeks; most dead within 3 days; 33/60 rats died; toxic symptoms: decrease in spontaneous movement, abdominal posture, apsychia and hyperventilation within seconds or minutes of all rats dosed with 84 mg/kg or greater; in all dead rats, convulsion due to asphyxia; dose (mg/kg), dead rats per dose: 49-0/10; 58-3/10; 70-5/10; 84-7/10; 101-8/10; 121-10/10	animals acclimated to environment for 1 week before testing; 6 groups of 10 rats each; fasted 16 hours before dosing; 100% mortality = 12.1 mg/kg; 0% mortality = 4.9 mg/kg	Wako Pure Chemicals Co.	Kitagawa H, Saito H, Sugimoto T, Yanaura S, Kitagawa H, Hosokawa T, Sakamoto K. 1982. Effects of diospropyl-1,3-dithiol-2-ylidene malonate (NKK-105) on acute toxicity of various drugs and heavy metals. J Toxicol Sci 7(2):123-34. *Chiba University; Hoshi College of Pharmacy; Showa University -- Japan*
Potassium cyanide	5	9	NA	Rosiello (1979) and Bliss (1938)	rats	male	oral	2, 4, 9, 14 mg/kg	2 mg/kg: 0/2 dead; 4 mg/kg: 0/2 dead; 9 mg/kg: 1/2 dead; 14 mg/kg: 2/2 dead; 3 of 8 rats dead; LD50 based on 8 rats used	acclimated for 5 days; observed for 14 days; 4 groups used for each dose (1, 2, 3, 5 animals per group; total of 11 rats per dose); 9 rats used for initial range finding	NA	Lorke D. 1983. A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275-288. *Institut fur Toxikologie, Wuppertal, Federal Republic of Germany*
Potassium cyanide	5	10	8.7 - 11.5 (95% CL)	Litchfield and Wilcoxin method (1949)	Sherman strain rats; min. wt. = 175 g; min. age of 90 days	male	oral; stomach tube	chemical in peanut oil; 0.005 mL/g of bw	observed hourly on first day of dosage and twice a day thereafter until time of death; max survival = died within 1 hour	50 rats tested	technical grade	Gaines TB. 1969. Acute toxicity of pesticides. Toxicol Appl Pharmacol 14(3):515-34. *U.S. Dept. of Health, Education, and Welfare, Atlanta, GA*
Potassium cyanide	5	10	9 - 12 (95% CL; slope = 14.5)	Finney (1971)	Crl: CD rats; ave bw = 243-251 g; young adult	male	oral; intragastric intubation	single dose as suspension in corn oil (0.1% susp.); 5, 8, 10, 15 mg/kg dose; dose = 126-377 mL	observed for 14 days; 16 rats dead; all deaths occurred within 1 hour; convulsions, tremors, fascilations, gasping, lethargy, weakness, hyperemia, weight loss	4 groups of 10 rats	NA	from EPA TSCATS database; INITIAL SUBMISSION: ORAL LD50 TEST OF POTASSIUM CYANIDE IN RATS WITH COVER LETTER DATED 08/10/92; EPA Document No. 88-920009041 Fiche No. OTS0555358; *E.I Dupont DeNemours & Co., Inc./Haskell Labs*
Procainamide	1950	1950	NA	NA	rats	NA	oral	NA	NA	no source given for LD50 value	NA	Protiva M, Valenta V, Trcka V, Hladovec J, Nemec J. 1977. Basic amided of 3,4,5-trimethoxyphenoxyacetic acid; synthesis and phaarmacology of trimethoxamide and analogues. Collection of Czechoslovak Chemical Communications 42:3628-3642. *Research Institute for Pharmacy and Biochemistry, Prague, Czechoslovakia* **(RTECS REFERENCE)**
Procainamide	1950	> 2000	NA	Litchfield and Wilcoxon method or Thompson method	Wistar rats	male	oral	single dose	NA	20 rats used	NA	Turba C, Sanna GP, Bianchi C. 1968. 1: Acute toxicity and general pharmacologic properties of 1,5-dimorpholino-3-(1-naphthyl)-pentane: DA 1686. Arzneimittelforschung Sep. 18(9):1127-1132. *LABORATORI RICERCHE ISTITUTO DE ANGLELI, MILANO, ITALY*
Propranolol HCl	466	466	NA	Litchfield and Wilcoxon method	Sprague-Dawley rats; 2 months	male	gastric intubation; single high oral doses	NA	determined at 10 days by administering po to groups of 5 animals for each dose a series of doses increasing serially by a factor of 2	fasted 12 hour before dosing	pharmaceu-tical grade	Maura A, Carlo P, et al. 1985. Absence of DNA damage in mice and rats given high doses of five beta adrenergic blocking agents. Arzneimittelforschung 35(8):1236-1238. *University of Genova, Italy* **(RTECS REFERENCE)**
Propylparaben	6332 (mouse oral)	6332 (mouse)	5740 - 6984 (S.E.)	NA	dd strain mice	NA	oral	NA	NA	NA	NA	Sado I. 1973. Synergistic toxicity of officially permitted food preservatives. Nippon Eiseigaku Zasshi 28(5):463-476. **(RTECS REFERENCE)**
Propylparaben	6332 (mouse oral)	> 8000 (mouse)	NA	Miller and Tainter (1944)	uniform strain of albino mice from a single source	NA	oral	suspended in 3% starch, proplene glycol, or olive oil	rapid onset of ataxia, deep depression resembling anesthesia; deaths usually occurred within 1 hour; recovery from nonfatal doses seldom lasted > 30 minutes	fasted 12 hour prior to dosing	NA	Matthews C, Davidson J, Bauer E, Morrison JL, Richardson AP. 1956. p- Hydroxybenzoic acide esters as preservatives II. Acute and chronic toxicity in dogs, rats, and mice. J Am Pharmaceut Assoc 45:260-267.
Sodium arsenite	41	36	27 - 52 (95% CL; slope = 7.6 [S.E. 2.7])	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	male	oral gavage	single dose	14 day observation; toxicity symptoms: diarrhea, diuresis, posture, respiratory effects, lethargy, abnormal gait; time to onset of signs < 1day; duration of signs 3 days; 9 rats dead (average per test)	3 dose levels (5 male each); 15 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Sodium arsenite	41	41	31 - 53 (these limits are +/- 1.96 S.D.)	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 10 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum, JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30: 470-476. *Carnegie-Mellon University, Pittsburgh, PA (LD50 value)* **(RTECS REFERENCE)** Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA (experimental parameters)*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Sodium arsenite	41	42	35 - 50 (95% CL)	Litchfield and Wilcoxon method	Holtsman rats; 300- 500 g; 100-300 days (13 - 41 weeks)	male and female	oral, gelatin capsules	20, 50, 100, 200 (all in mg/kg)	death occurred within 4 days	approximately 40 rats used; 24 hour fasting before dosing; rats dosed under light anesthesia	Baker Analyzed Reagent with 0.02% impurities	Done AK, Peart AJ. 1971. Acute Toxicities of Arsenical Herbicides. Cinical Toxicology, 4(3):343-355. *University of Utah, Salt Lake City, UT*
Sodium arsenite	41	42	35 - 58 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	male and female	oral gavage	single dose	14 day observation; toxicity symptoms: diarrhea, diuresis, posture, respiratory effects, lethargy, abnormal gait; time to onset of signs < 1 day; duration of signs 3 days; 9 rats dead (average per test)	3 dose levels (5 male each and 5 female); 30 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Sodium arsenite	41	48	37 - 76 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats	female	oral gavage	single dose	14 day observation; toxicity symptoms: diarrhea, diuresis, posture, respiratory effects, lethargy, abnormal gait; time to onset of signs < 1 day; duration of signs 3 days; 9 rats dead (average per test)	3 dose levels (5 female each); 15 rats used; OECD TG401 (1981) followed for experimental procedures	NA	Vandenheuvel MJ, Clark DG, Fielder RJ, Koundakjian PP, Oliver GJA, Pelling D, Tomlinson NJ, Walker AP. 1990. Jul. The International Validation Of A Fixed-Dose Procedure As An Alternative To The Classical LD50 Test Food And Chemical Toxicology 28(7):469-482.
Sodium arsenite	41	53	39 - 74 (95% CL)	acceptable methods (e.g., Bliss, Litchfield and Wilcoxon, Weil, Thompson, etc.)	Sprague-Dawley rats; 190-300 g	female	oral gavage	single dose	14 day observation; toxicity symptoms: motor activity decrease, respiratory effects, blanching, piloerection, salivation, diarrhea; time to onset of signs < 1 day; duration of signs 3 days; animals fasted 16 -20 hours before administration	UDP Test	NA	Yam J, Reer PJ, Bruce RD. 1991. Comparison of the up-and-down method and the fixed-dose procedure for acute oral toxicity testing. Food Chem Toxicol 29(4):259-264. *The Procter and Gamble Co., Cincinnati, OH*
Sodium chloride	3000	3000	NA	NA	rats	NA	oral	NA	NA	No information/reference provided.	NA	Tucker RK, Haegel MA. 1971. Compararive acute oral toxicity of pesticides to six species of birds. Toxicology and Applied Pharmacology 20:57-65. **(RTECS REFERENCE)**
Sodium chloride	3000	3620	+/-300 (S.E.)	Croxton (1953) and Waugh (1952)	Wistar albino rats; female: 167+/-27 g; young adult	female	oral; intragastric tube	doses = 0, 0.8, 3, 3.2, 3.5, 3.8, 4, 5, 10, 16 g/kg in water; 20 mL/kg dose; 2 largest doses in larger volumes	convulsive movements, diarrhea, muscular rigidity, prostration, respiratory failure; death within 14 hours	fasted for 16 hours; 84 rats used; 12 - 44 rats per dose	NA	Boyd EM, Shanas MN. 1963. The acute oral toxicity of sodium chloride. Arch Internat Pharmacodyn 144:86-96. *Quebecs' University, Kingston, Ontario, Canada*
Sodium chloride	3000	3750	+/-430 (S.E.)	Croxton (1953) and Waugh (1952)	Wistar albino rats; male: 202+/-42 g; female: 167+/-27 g; young adult	male and female (equal numbers)	oral; intragastric tube	doses = 0, 0.8, 3, 3.2, 3.5, 3.8, 4, 5, 10, 16 g/kg in water; 20 mL/kg dose; 2 largest doses in larger volumes	convulsive movements, diarrhea, muscular rigidity, prostration, respiratory failure; death within 14 hours	fasted for 16 hours; 168 rats used; equal numbers of male and female; 12-44 rats per dose; this LD50 is determined from the data used to determine LD50 of 3620 mg/kg (female) and 3890 mg/kg (male) also reported in this reference [Boyd and Shanas 1963]	NA	Boyd EM, Shanas MN. 1963. The acute oral toxicity of sodium chloride. Arch Internat Pharmacodyn 144:86-96. *Quebecs' University, Kingston, Ontario, Canada*
Sodium chloride	3000	3890	+/-300 (S.E.)	Croxton (1953) and Waugh (1952)	Wistar albino rats; male: 202+/-42 g; young adult	male	oral; intragastric tube	doses = 0, 0.8, 3, 3.2, 3.5, 3.8, 4, 5, 10, 16 g/kg in water; 20 mL/kg dose; 2 largest doses in larger volumes	convulsive movements, diarrhea, muscular rigidity, prostration, respiratory failure; death within 14 hours	fasted for 16 hours; 84 rats used; 12 - 44 rats per dose	NA	Boyd EM, Shanas MN. 1963. The acute oral toxicity of sodium chloride. Arch Internat Pharmacodyn 144:86-96. *Quebecs' University, Kingston, Ontario, Canada*
Sodium chloride	3000	4200	3980 - 4430 (95% CL)	Litchfield and Wilcoxon method (1949)	rats	NA	oral	NA	NA	reference in Italian	NA	Scognamiglio WP, Amorico L, Gatti GL. 1972. Esperienze di tossicita e di tolleranza al monosioglutammato con un saggio di condizionamento di salvaguardia. Il Farmaco Edizone Pratica 27:19-27.
Sodium chloride	3000	6140	+/-310 (S.E.)	NA	CBL Wistar albino rats; 150-200 g	male	oral; intragastric tube	single dose; 5000 - 7500 mg/kg dose range; cmpd dissolved in distilled water; 20 mL/kg dosage	observed for 5 days; premortal diarrhea; convulsive movements	5 rats per dose; 30 rats used; rats not fasted	Merck Reagent	Boyd EM, Abel MM, Knight LM. 1966. The chronic oral toxicity of sodium chloride at the range of the LD50 (0.1L). Canad J Physiol Pharmacol 44:157-172. *Queen's University, Ontario, Canada*
Sodium dichromate (Sodium bichromate VI)	50	34.17	+/- 20.95 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	female	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1,0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 10% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals used	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	38.55	+/- 7.79 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	female	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1,0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 5% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals/dose	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	39.02	+/- 13.54 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	female	oral gavage	single dose: 40, 50, 60, 80, 100 mg/kg; dosing solution 50% (w/v); 0.8-2.0 mL/kg dosing volume; doses in distilled water	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 male and 5 female rats per dose; 10 rats/dose; 5 female rats/dose for this value	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Sodium dichromate (Sodium bichromate VI)	50	48.98	+/- 10.50 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	male	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1.0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 10% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals/dose	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	50	NA	NA	rats	NA	NA	NA	NA	reference in Russian	NA	Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1-36, 1957-1992. 1978. **(RTECS REFERENCE)**
Sodium dichromate (Sodium bichromate VI)	50	51.1	+/- 5.93 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	male and female	oral gavage	single dose: 40, 50, 60, 80, 100 mg/kg; dosing solution 50% (w/v); 0.8-20 mL/kg dosing volume; doses in distilled water	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 male and 5 female rats per dose; 10 rats/dose; this LD50 is determined from the data used to determine LD50 of 39.02 mg/kg (female) and 58.84 mg/kg (male) also reported in this reference	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	55.75	+/- 15.98 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	male	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1.0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 5% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals/dose	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	57.13	+/- 8.81 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	female	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1.0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 0.5% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals/dose	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	58.84	+/- 5.78 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	male	oral gavage	single dose: 40, 50, 60, 80, 100 mg/kg; dosing solution 50% (w/v); 0.8-20 mL/kg dosing volume; doses in distilled water	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 male and 5 female rats per dose; 10 rats/dose; 5 male rats/dose for this value	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	59.84	+/- 7.74 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	male	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1.0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 0.5% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals/dose	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium dichromate (Sodium bichromate VI)	50	59.84	+/- 7.74 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	male	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1.0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 1% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals/dose	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium Dichromate (Sodium Bichromate VI)	50	64.5	+/- 10.18 (S.D.)	Gad and Weil (1982) Probit analysis	Fischer 344 rats (Harlen Sprague Dawley)	female	oral gavage	single dose: 40,60,80 mg/kg; dosing solution: 10,5,1.0.5% (w/v); dosing vol: 0.4-8.0 mL/kg (40 mg/kg); 0.6-12 mL/kg (60 mg/kl); 0.8-16 mL/kg (80 mg/kg); doses in distilled water; 1% dose	observed first 6 hours then day 1, 7 and 14; hypoactivity, lacrimation, mydriasis, diarrhea, change in body weight; LD50 increased as the concentration of the dosing solution increased	animals acclimated for 2 weeks before dosing; animals fasted overnight; 5 animals/dose	member companies of the Industrial Health Foundation	Gad SC, Powers WJ, Dunn BJ, Hoffman GM, Siino KM, Walsh RD. 1986. Acute toxicity of four chromate salts. Proceedings of the Chromium Symposium, pp. 43-58. *G.D. Searle and Co., Skokie, IL*
Sodium hypochlorite	8910 (from HSDB)	8200	NA	NA	NA	NA	NA	NA	NA	12.5% hypochlorite solution	NA	Sodium Hypochlorite Toxicity Profile. 1990. British Industrial Biological Research Association (BIBRA).
Sodium hypochlorite	8910 (from HSDB)	9360 - 11700	NA	NA	NA	NA	NA	NA	NA	12.5% hypochlorite solution	NA	Colgate-Palmolive. 1990. Internal Report: Investigation of the properties of the wash water in connection with washing using "Klorin" bleach. Unpublished.
Sodium hypochlorite	8910 (from HSDB)	>11800	NA	NA	NA	NA	NA	NA	NA	3.6% hypochlorite solution	NA	Colgate-Palmolive. 1990. Internal Report: Investigation of the properties of the wash water in connection with washing using "Klorin" bleach. Unpublished.

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Sodium hypochlorite	8910 (from HSDB)	13000	NA	NA	NA	NA	NA	NA	NA	5.25% hypochlorite solution	NA	MSDS Chlorine Institute 1982
Sodium I fluoride	115	64 (29 mg F/kg; converted to mg NaF/kg)	60 - 69 (95% CI)	Litchfield and Wilcoxon method (1949); Bliss (1938)	rats; mean bw = 169 g; 3 months	female	oral	5 mL/kg	22 rats died within 3 hour; 15 rats died after 3 hour; observed for 7 days; signs of toxicity appeared from 5-15 minutes after administration of NaF: muscle weakness, salivation, diarrhea, lacrimation, tremor, convulsion, hypopnea, cynosis, urinary incontinence; most animals died within 24 hour after dosing	reference paprer in Japanese; English summary and table/graph headers; see paper for information about regression coefficient of log dose-NED mortality curve	NA	Sakama H. 1980. Toxicological studies of fluorine compounds. I. Acute toxicity of sodium fluoride to rats and mice in relation to age, sex, animal genus, and administration route. Shika Gakuho. Journal of Dentistry. 80: 1519. *Tokyo Dental College, Japan.*
Sodium I fluoride	115	69 (31 mg F/kg; converted to mg NaF/kg)	55 - 84 (CL)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley rats; mean bw and ranges 250 g (200-359 g); 90 days	female	stomach tube	NaF in aqueous solution (0.2 - 1.6 mL/dose)	mortality confined to 24 hour; when doses equal to or greater than the LD50 were administered, half of the 250 g rats died within 3 hours	fasted 24 hour before dosing; at least seven dose levels used for each population; groups of 8 -15 rats	NA	DeLopez OH, Smith FA, Hodge HC. 1976. Plasma fluoride concentrations in rats acutely poisoned with sodium fluoride. Toxicol Appl Pharmacol 37:75-83. *Univ. of Rochester School of Med. And Dent., Rochester, NY*
Sodium I fluoride	115	73 (33 mg F/kg; converted to mg NaF/kg)	66 - 80 (95% CI)	Litchfield and Wilcoxon method (1949); Bliss (1938)	rats; mean bw = 295 g; 3 months	male	oral	3 mL/kg	6 rats died within 3 hour; 35 rats died after 3 hour; observed for 7 days; signs of toxicity appeared from 5-15 minutes after administration of NaF: muscle weakness, salivation, diarrhea, lacrimation, tremor, convulsion, hypopnea, cynosis, urinary incontinence; most animals died within 24 hour after dosing	reference paprer in Japanese; English summary and table/graph headers; see paper for information about regression coefficient of log dose-NED mortality curve	NA	Sakama H. 1980. Toxicological studies of fluorine compounds. I. Acute toxicity of sodium fluoride to rats and mice in relation to age, sex, animal genus, and administration route. Shika Gakuho. Journal of Dentistry. 80: 1519. *Tokyo Dental College, Japan.*
Sodium I fluoride	115	80	+/- 5 (S.E.)	Winthrop logarithmic probit graph paper; Miller and Tainter (1944)	Albino rats; 200- 300 g	NA	oral; stomach tube	single dose; 25% solution; 22 - 288 mg/kg doses;	percentage mortality observed in 24 hour calculated, then LD50 determined	98 rats used	NA	Shourie KL, Hein JW, Hodge HC. 1950. Preliminary studies of the caries inhibiting potential and acute toxicity of sodium monofluorophosphate. J Dent Res 29:529-533. *Univeristy of Rochester, School of Medicine and Denistry, Rochester, NY.*
Sodium I fluoride	115	84 (38 mg F/kg; converted to mg NaF/kg)	77 - 93 (95% CI)	Litchfield and Wilcoxon method (1949); Bliss (1938)	rats; mean bw = 60 g; 3 weeks	female	oral	5 mL/kg	16 rats died within 3 hour; 32 rats died after 3 hour; observed for 7 days; signs of toxicity appeared from 5-15 minutes after administration of NaF: muscle weakness, salivation, diarrhea, lacrimation, tremor, convulsion, hypopnea, cynosis, urinary incontinence; most animals died within 24 hour after dosing	reference paprer in Japanese; English summary and table/graph headers; see paper for information about regression coefficient of log dose-NED mortality curve.	NA	Sakama H. 1980. Toxicological studies of fluorine compounds. I. Acute toxicity of sodium fluoride to rats and mice in relation to age, sex, animal genus, and administration route. Shika Gakuho. Journal of Dentistry. 80: 1519. *Tokyo Dental College, Japan.*
Sodium I fluoride	115	107 (46 mg F/kg; converted to mg NaF/kg)	95 - 110 (95% CI)	Litchfield and Wilcoxon method (1949); Bliss (1938)	rats; mean bw = 58 g; 3 weeks	male	oral	5 mL/kg	2 rats died within 3 hour; 32 rats died after 3 hour; observed for 7 days; signs of toxicity appeared from 5-15 minutes after administration of NaF: muscle weakness, salivation, diarrhea, lacrimation, tremor convulsion, hypopnea, cynosis, urinary incontinence; most animals died within 24 hour after dosing	reference paprer in Japanese; English summary and table/graph headers; see paper for information about regression coefficient of log dose-NED mortality curve.	NA	Sakama H. 1980. Toxicological studies of fluorine compounds. I. Acute toxicity of sodium fluoride to rats and mice in relation to age, sex, animal genus, and administration route. Shika Gakuho. Journal of Dentistry. 80: 1519. *Tokyo Dental College, Japan.*
Sodium I fluoride	115	115 (52 mg F/kg; converted to mg NaF/kg)	106 - 126 (slope = 1.23 [1.06 - 1.43]; 95% CL)	Litchfield and Wilcoxin method (1949)	Sprague-Dawley rats; mean bw and ranges 150 g (112-184 g); 30-45 days	female	stomach tube	NaF in aqueous solution (0.2 - 1.6 mL/dose); 30 - 100 mg F/kg doses;	mortality confined to 24 hour; when doses > the LD50 were administered, one-third of the 150 g rats died within 7 hours; dose in mg F/kg and 24 hour mortality: 75-2/2 dead; 70-9/10 dead; 65-7/9 dead; 62-6/8 dead; 58-4/10 dead; 55-9/15 dead; 50-8/12 dead; 45-3/10 dead; 42-2/10 dead; 40-0/2 dead; 35-0/2 dead; salivation, diarrhea, thirst, lethargy	fasted 24 hour before dosing; 11 dose levels used; groups of 2 -15 rats; 90 rats used; 50 dead; detailed information from RTECS reference (master thesis for de Lopez 1970)	NA	DeLopez OH, Smith FA, Hodge HC. 1976. Plasma fluoride concentrations in rats acutely poisoned with sodium fluoride. Toxicol Appl Pharmacol 37:75-83. *Univ. of Rochester School of Med. And Dent., Rochester, NY*
Sodium I fluoride	115	115 (52 mg F/kg; converted to mg NaF/kg)	108 - 119 (slope = 1.28 [1.0 - 1.6]; 95% CL)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley rats; mean bw and ranges 80 g (50-108 g); 30-45 days	female	stomach tube	NaF in aqueous solution (0.2 - 1.6 mL/dose); 30 - 100 mg F/kg doses;	mortality confined to 24 hour; when doses equal to or greater than the LD50 were administered, half of the 80 g rats died within 6 hours; dose in mg F/kg and 24 hour mortality: 100-9/12 dead; 75-8/9 dead; 70-8/10 dead; 60-8/10 dead; 50-2/10 dead; 40-2/10 dead; 30-0/2 dead; salivation, diarrhea, thirst, lethargy	fasted 24 hour before dosing; at least seven dose levels used for each population; groups of 2 -12 rats; 63 rats used; 36 dead; detailed information from RTECS reference (master thesis for de Lopez 1970)	NA	DeLopez OH. 1970. Acute fluoride toxicity: plasma fluoride concentrations following acute oral doses of sodium fluoride in the rat. Master of Science thesis. *Univ. of Rochester School of Med. And Dent., Rochester, NY* (see DeLopez 1976) **(RTECS REFERENCE)**
Sodium I fluoride	115	119 (54 mg F/kg; converted to mg NaF/kg)	108 - 119 (slope = 1.28 [1.0 - 1.6]; 95% CL)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley rats; mean bw and ranges 80 g (50-108 g); 30-45 days	female	stomach tube	NaF in aqueous solution (0.2 - 1.6 mL/dose); 30 - 100 mg F/kg doses	mortality confined to 24 hour; when doses equal to or greater than the LD50 were administered, half of the 80 g rats died within 6 hours; dose in mg F/kg and 24 hour mortality: 100-9/12 dead; 75-8/9 dead; 70-8/10 dead; 60-8/10 dead; 50-2/10 dead; 40-2/10 dead; 30-0/2 dead; salivation, diarrhea, thirst, lethargy	fasted 24 hour before dosing; at least seven dose levels used for each population; groups of 2 -12 rats; 63 rats used; 36 dead; detailed information from RTECS reference (master thesis for de Lopez 1970)	NA	DeLopez OH, Smith FA, Hodge HC. 1976. Plasma fluoride concentrations in rats acutely poisoned with sodium fluoride. Toxicol Appl Pharmacol 37:75-83. *Univ. of Rochester School of Med. And Dent., Rochester, NY*
Sodium I fluoride	115	180	120 - 260 (these limits are +/- 1.96 S.D.)	Thompson method; Weil tables	Carworth-Wistar rats; 90-120 g; 4-5 weeks	male	oral gastric intubation	in aqueous solution; concentration intubated = 5 mg/mL; dosages arranged in a logarithmic series differing by a factor of 2	LD50 based on mortalities during a 14 day period	non-fasted; groups of 5 rats; single oral dose toxicity	reagent grade	Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum, JS. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30: 470-476. *Carnegie-Mellon University, Pittsburgh, PA* (LD50 value) Smyth HF Jr, Carpenter CP, Weil CS, Pozzani UC, Striegel JA. 1962. Range-finding toxicity data: List VI. Am Ind Hyg Assoc J 23:95-107. *Mellon Institute of Industrial Research, Pittsburg, PA* (experimental parameters)
Sodium I fluoride	115	189 (85.5 mg F/kg; converted to mg NaF/kg)	#2: 170 -209 (95%CI)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley rats; 152-202 g	male	oral; intragastric	50 to 220 mg F/kg (111 - 486 mg NaF/kg) in water	number of deaths determined at 1, 2, 4, 8, 24 hour and daily thereafter; 20 rats dead at 24 hour; 26 rats dead at 14 days; monitored for 2 weeks but no deaths after 4 days; deaths/dose (mg/kg): 111-0/10, 122-0/10, 134-1/10, 147-0/10, 162-0/10, 166-4/10, 183-4/10, 201-3/10, 221-6/10, 243-8/10	fasted 18 hour before dosing; 10 day acclimatization before dosing; 8 rats in each dosage group; 80 rats used	>99.5% purity	Whitford GM, Birdsong-Whitford NL, et al. 1990. Acute oral toxicity of sodium fluoride and monofluorophosphate separately or in combination in rats. Caries Res 24(2):121-126. *Medical College of Georgia, Augusta, GA; Dept. of Odonto-Stomatologie, Laboratoires Goupil SA, Cachan, France.*
Sodium I fluoride	115	200	NA	NA	rats	NA	oral; stomach tube	NA	abdominal distress, diarrhea, cyanosis, dyspnea, fibrillation of skeletal muscles; onset within 6 hours	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletine (Association of Food and Drug Officials of the United States). Vol.15:122-133. *U.S. FDA*
Sodium I fluoride	115	223	NA	Probit analysis	Sprague-Dawley rats; 190-315 g	male	oral gavage	0.101 - 0.500 g NaF/kg bw	animals observed for mortality frequently during first 4 hour after dosing; observed daily thereafter for 14 days	fasted 18 - 20 hour before dosing; 8 rats per group; 48 total rats used; mortality confined to 24 hour after dosing except 3 animals died on day 2, 3, and 5	J.T. Baker Chemical Co.	Skare JA, Schrotei KR, Nixon GA. 1986. Lack of DNA-strand breaks in rat testicular cells after in vivo treatment with sodium fluoride. Mutat Res 170:85-92. *The Proctor and Gamble Company, Cincinnati, OH*

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Sodium I fluoride	115	279 (126.3 mg F/kg; converted to mg NaF/kg)	#1: 218 - 358 (95%CI)	Litchfield and Wilcoxon method (1949)	Sprague-Dawley rats; 152-202 g	male	oral; intragastric	50 to 220 mg F/kg (111 - 486 mg NaF/kg) in water	number of deaths determined at 1, 2, 4, 8, 24 hour after dose and each day thereafter; 32% rats dead during 1st day; 23 rats dead at 14 days; monitored for 2 weeks but no deaths after 4 days; deaths/dose (mg/kg): 160- 1/10, 207- 4/10, 254-5/10, 330-6/10, 428-7/10	fasted 18 hour before dosing; 10 day acclimatization before dosing; 10 rats in each dosage group; 50 rats used	>99.5% purity	Whitford GM, Birdsong-Whitford NL, et al. 1990. Acute oral toxicity of sodium fluoride and monofluorophosphate separately or in combination in rats. Caries Res 24(2):121-126. *Medical College of Georgia, Augusta, GA; Dept. of Odonto-Stomatologie, Laboratoires Goupil SA, Cachan, France.*
Sodium oxalate	11160	11160	NA	NA	rat	NA	oral	NA	NA	Value derived from 7500 mg/kg from RTECS for oxalic acid, which is a typo. Original reference (Vernot et al 1977) has 7.5 ml/kg)	NA	EHP, Environmental Health Perspectives. (U.S. Government Printing Office, Supt of Documents, Washington, DC 20402) No.1-1971. 106(Suppl). **(RTECS REFERENCE)**
Sodium oxalate	11160	558.13 (converted from 7.5 mL/kg 5% oxalic acid)	372 - 819	moving average (Thompson & Weil)	Sprague-Dawley; 200-300 g	female	oral gastric intubation	5% aqueous solution; doses arranged in a logarithmic series differing by a factor of 2 (assumed from Smyth et al. 1962)	LD50 based on mortalities during a 14 day period (assumed from Smyth et al. 1962)	non-fasted; groups of 5 rats; single oral dose toxicity (assumed from Smyth et al 1962); reported as 7.5 ml/kg of 5% oxalic acid	NA	Vernot EH, MacEwen JD, Haun CC, Kinkead ER. 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicology and Applied Pharmacology 42:417-423. *(Indicates methods of Smyth et al. 1962 were used.)*
Sodium oxalate	11160	706.96 (converted from 9.5 mL/kg 5% oxalic acid)	402 - 915	moving average (Thompson & Weil)	Sprague-Dawley; 200-300 g	male	oral gastric intubation	5% aqueous solution; doses arranged in a logarithmic series differing by a factor of 2 (assumed from Smyth et al. 1962)	LD50 based on mortalities during a 14 day period (assumed from Smyth et al. 1962)	non-fasted; groups of 5 rats; single oral dose toxicity (assumed from Smyth et al 1962); reported as 9.5 ml/kg of 5% oxalic acid	NA	Vernot EH, MacEwen JD, Haun CC, Kinkead ER. 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicology and Applied Pharmacology 42:417-423. *(Indicates methods of Smyth et al. 1962 were used.)*
Sodium selenate	1.6	1.6	NA	NA	rats	NA	oral	NA	NA	reference in Russian	NA	Novikov JV, Plitman SE, et al. 1984. Selenium in water and its effect on the human body. Gigiena i Sanitariya 49(9):66-68. **(RTECS REFERENCE)**
Sodium selenate	1.6	5.98	NA	NA	rats	NA	oral; stomach tube	NA	violent gastroenteritis, diarrhea, rice water stools,garlic breath, nervousness, CNS depression; onset within 15 min.	information from the laboratories of Division of Pharmacology, U.S. FDA.; fasted animals	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletin (Association of Food and Drug Officials of the United States). Vol.15:122-133. *U.S. FDA*
Strychnine	2.35	2.35	NA	mortality curves	adult white rats	female	oral, stomach tube; single dose	2.25 - 15 mg/kg dose; single dose; cmpd mixed in gum acacia and water; 1 mg/mL dose solution	15, 10, 7.5, 6, 5mg/kg dose killed 90 rats (100% mortality); 4 mg/kg, 17/18 rats dead (95%); 3 mg/kg, 20/27 rats dead (74%); 2.5 mg/kg 19/27 rats dead (70%); 2.25 mg/kg, 7/18 rats dead (39%); 7.3 - 14.1 minutes average time to death	180 rats used	U.S.P IX Strychnine alkaloid	Ward JC, Crabtree DG. 1942. Strychnine X. Comparative accuracies of stomach tube and intraperitoneal injection methods of bioassay. Journal of the American Pharmaceutical Association, Scientific Edition 31:113-115. *U.S. Fish and Wildlife Service, Denver, CO* **(RTECS REFERENCE)**
Strychnine	2.35	6.5	NA	mortality curves	adult white rats	male	oral, stomach tube; single dose	5 - 15 mg/kg dose; single dose; cmpd mixed in gum acacia and water; 1 mg/mL dose solution	15 mg/kg, 16/18 rats dead (89% mortality); 10 mg/kg, 15/18 rats dead (83%); 7.5 mg/kg, 16/18 rats dead (89%); 6 mg/kg 6/18 rats dead (33%); 5 mg/kg, 4/18 rats dead (39%); 10.8 - 19.5 minutes average time to death	90 rats used	U.S.P IX Strychnine alkaloid	Ward JC, CrabtreeDG. 1942. Strychnine X. Comparative accuracies of stomach tube and intraperitoneal injection methods of bioassay. Journal of the American Pharmaceutical Association, Scientific Edition 31:113-115. *U.S. Fish and Wildlife Service, Denver, CO*
Strychnine	2.35	16.2	NA	NA	rats	NA	oral, stomach tube; single dose	NA	tonic convulsions; deaths from medullary paralysis and exhaustion and usually occur within a 12 hour period	NA	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletin (Association of Food and Drug Officials of the United States). Vol.15:122-133. *U.S. FDA*
Strychnine	2.35	25	NA	statistical formula based on mortality rates	wild Norway rats	NA	oral, stomach tube; single dose	a number of individual doses of a cmpd, each dose at a different concentration level, are given to an equal number of test animals	convulsions	NA	NA	Peardon DL, Kilbourn E, et al. 1972. New selective rodenticides. Soap Cosmet Chem Spec 48(12):6. *Rohm and Haas Company, Philadelphia, PA*
Thallium I sulfate	16	15.8	+/- 0.9 (S.E.)	Litchfield and Fetig (1941)	wild Norway rats (trapped in Baltimore, MD); 134-579 g (ave = 298 g), adult	male and female	oral gavage	chemical suspended in 10% acacia solution; received appropriate doses in 1 mL per 100 g bw	rats survived from 6 - 72 hours	37 rats used (approx. equal number of male/female); overnight fasting before dosing; assays performed in winter, repeated in summer; LD50 values from combined information; final LD50 was higher than winter LD50; attributed to not having enough rats in winter.	GIBCO brand; 99.0% pure	Dieke SH, Richter CP. 1946. Comparative assays of rodenticides on wild Norway rats. I. Toxicity. Publ Health Rep 61:672-679. *Johns Hopkins Hospital, Baltimore, MD*
Thallium I sulfate	16	16	NA	NA	rats	NA	oral	NA	NA	reference is a review article in Japanese; this LD50 value assumed to be from Peardon et al. 1972.	NA	Gekkan Yakuji. Pharmaceuticals Monthly. (Yakugyo Jihosha, Inaoka Bldg., 2-36 Jinbo-cho, Kanda, Chiyoda-ku, Tokyo 101, Japan) V.1- 1959. 1980. **(RTECS REFERENCE)**
Thallium I sulfate	16	16	NA	statistical formula based on mortality rates	wild Norway rats	NA	oral, stomach tube; single dose	a number of individual doses of a cmpd; each dose at a different conc level given to equal number of test animals	respiratory failure	NA	NA	Peardon DL, Kilbourn E, et al. 1972. New selective rodenticides. Soap Cosmet Chem Spec 48(12):6. *Rohm and Haas Company, Philadelphia, PA*
Thallium I sulfate	16	25	NA	NA	rats	NA	oral, stomach tube; single dose	NA	72 hour observation; most rats dead within this period	fasted animals	NA	Lehman AJ. 1951. Chemicals in Foods: a report to the association of food and drug officials on current developments. Part II. Pesticides. Quarterly Bulletin (Association of Food and Drug Officials of the United States). Vol.15:122-133. *U.S. FDA*
Trichloroacetic acid	NA	400	NA	NA	rats	NA	oral	NA	NA	(source of information not provided)	NA	Worthing CR, Walker SB, eds. 1987. Pesticide Manual. 8th edition. 765-766.
Trichloroacetic acid	NA	3320	3160 - 3480 (95% certainty; slope = 20.97)	Bliss	rats (raised in the laboratory); 150- 250 g; 70-100 days	male and female (mostly male)	oral intubation	single dose; acid adjusted with sodium hydroxide to pH range of 6 -7; 10 mL/kg dose volume	observed for 6 days; passed into narcosis to seminarcosis and died or recovered within 36 hours; dose in g/kg versus mortality: 2.594-0/5; 3.000-3/10; 3.153 - 1/5; 3.400-5/10; 3.800-9/10; 3991-5/5; 4.200-10/10; 4.600-10/10	fasted 18 hours before dosing; 65 rats used; 43 of 65 dead	NA	Woodard G, Lange SW, Nelson KW, Calvery HO. 1941. The acute oral toxicity of acetic, chloroacetic, dichloroacetic, and trichloroacetic acids. J Ind Hyg Toxicol 23(2):78-82.

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Trichloroacetic acid	NA	5000			rats	male	oral	NA	NA	NA	NA	Farm Chemicals Handbook. 1992. Meister Pub., 37841 Euclid Ave., Willoughy, OH. p. C326.
Trichloroacetic acid	NA	5060			rats	female	oral	NA	NA	NA	NA	Farm Chemicals Handbook. 1992. Meister Pub., 37841 Euclid Ave., Willoughy, OH. p. C326.
Trichloroacetic acid	NA	8900	7000 - 9900	NA	rats; 220 +/- 40 g	NA	oral; intragastric	NA	NA	(source of information not provided)	NA	Izmerov NF, Sanotsky IV, Sidorov KK. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. International Register of Potentially Toxic Chemicals (IRPTC). United Nations Environment Programme (UNEP). Centre of International Projects, GKNT. Moscow, Russia.
Triethylenemelamine	13	1	NA	NA	rats	NA	oral	NA	NA	Reference offers neither experimental details nor the primary reference for LD50. Value reported as "ca. 1"	NA	Hayes WJ Jr. 1964. The toxicology of chemosterilants. Bulletin of the World Health Organization. 31:721-736. *(RC's reference from 1983/84 RTECS.)*
Triethylenemelamine	13	4	NA	Probit method	Sprague-Dawley rats; 190-200 g	female	oral	geometric progression of 14 for dosing; in water or neat	20 rats used; 11 dead; observed for 14 days	non-fasted; 4 groups of 5 female; 20 rats used	Polysciences, Inc. Warrington, PA	Thompson ED, Gibson DP. 1984. A method for determining the maximum tolerated dose for acute in vivo cytogenetic studies. Food Chem Toxicol 22(8):665-76. *The Procter and Gamble Co., Cincinnati, OH*
Triethylenemelamine	13	6.9	NA	Probit method	Sprague-Dawley rats; 190-200 g	male	oral	geometric progression of 14 for dosing; in water or neat	20 rats used; 9 dead; observed for 14 days	non-fasted; 4 groups of 5 male; 20 rats used	Polysciences, Inc. Warrington, PA	Thompson ED, Gibson DP. 1984. A method for determining the maximum tolerated dose for acute in vivo cytogenetic studies. Food Chem Toxicol 22(8):665-76. *The Procter and Gamble Co., Cincinnati, OH*
Triethylenemelamine	13	13	8 - 20 (95% CL; slope = 2.1)	Litchfield and Wilcoxin (1949)	Wistar rats; 150- 350 g	male and female	oral; stomach tube	dissolved in isotonic saline within 30 minutes of dosing; less than 5% weight of insoluble matter filtered out; highest dose 500 mg/kg	14 observation period; absence of acute toxicity signs	information not grouped according to sex since differences not evident; 6 rats per dose; animals fasted overnight	NA	Philips FS, Thiersch JB. 1950. The nitrogen mustard-like actions of 2,4,6-tris(ethylenimino)-s-triazine and other bis(ethylenimines). Journal of Pharmacology and Experimental Therapeutics 100:398-407. *Sloan Kettering Institute for Cancer Research, New York, NY* **(RTECS REFERENCE)**
Triphenyltin hydroxide	46	46.4	NA	NA	Fischer rats; 6 weeks	male and female	oral intubation	single dose followed by daily doses up to 14 days	observed up to 14 days	NA	NA	Carcinogenesis bioassay of environmental chemicals annual progress report NIH-NCI-E-C-72-3252. 5/13/71 -- 8/6/73 and Final report NIH-NCI-E-71-2146. Submitted to The National Cancer Institute. National Institutes of Health, Bethesda, MD. 8/15/73 (revised 8/10/73). FM Garner (princ. investigat.) *Litton Bionetics, Inc., Bethesda, MD.* **(RTECS REFERENCE)**
Triphenyltin hydroxide	46	156	115 - 208 (CL)	NA	rats	female	oral	single dose; 80, 160, 315, or 630 mg/kg doses	observed for 19 days; toxicity develops slowly; toxic signs 2 days after dose; deaths 5 - 9 days after initial dose; dose (mg/kg), number dead: 80 - 1/10; 160 - 4/10; 315 - 10/10; 630 - 10/10; toxic signs included squatting, ataxy, bristled hair, blood-crusted adherent margins of the eyelid, decreased respiratory rate and poor general condition	fasted animals; 4 groups of 10 female rats each; each received one dose; 35 of 40 died	triphenyltin hydroxide 96%	Pharma Forschung Toxikologie; Report 183/81; A 21593; Apr. 22, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00124210 and 00139030; Hoechst Aktiengesellschaft; EPA Acc. No. 071364; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum 005275
Triphenyltin hydroxide	46	160	NA	NA	rats	NA	oral	NA	NA	NA	triphenyltin hydroxide 80.0%	Products Safety Labs; T-1399; May 8, 1992; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 42265507; EPA Chem. Code: 083601; Core Grade/Tox Record No. Guideline 009941; Jan. 5, 1993;
Triphenyltin hydroxide	46	165	113 - 230 (CL)	NA	rats	male	oral	single dose; 80, 160, 315, or 630 mg/kg doses	observed for 19 days; toxic signs 2 days after dose; toxicity develops slowly; deaths 5 - 13 days after initial dose; dose (mg/kg), number dead: 160 - 6/10; 315 - 10/10; 630 - 9/10; toxic signs included squatting, ataxy, bristled hair, blood-crusted adherent margins of the eyelid, decreased respiratory rate, dischourage of mucous feces, and poor general condition	fasted animals; 4 groups of 10 male rats each; each received one dose; 25 of 40 died	triphenyltin hydroxide 96%	Pharma Forschung Toxikologie; Report 182/81; A 21353; Apr. 22, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00124209; Hoechst Aktiengesellschaft; EPA Acc. No. 071364; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum 005275, minimum 003116
Triphenyltin hydroxide	46	240	NA	NA	rats	male	oral	NA	NA	NA	triphenyltin hydroxide tech	U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; EPA Chem. Code: 083601; Core Grade/Tox Record No. 001493
Triphenyltin hydroxide	46	313	232 - 422	NA	rats	male	oral	NA	NA	NA	triphenyltin hydroxide tech	Cannon Laboratories, Inc.; Jan. 31, 1978; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 099049; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum 001492
Triphenyltin hydroxide	46	345	138 - 862	NA	rats	female	oral	NA	NA	NA	triphenyltin hydroxide tech	Cannon Laboratories, Inc.; Jan. 31, 1978; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 099049; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum 001492
Triphenyltin hydroxide	46	360	NA	NA	rats	female	oral	NA	NA	NA	triphenyltin hydroxide tech	U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; EPA Chem. Code: 083601; Core Grade/Tox Record No. 001493
Triphenyltin hydroxide	46	375	280 - 502	NA	rats	male	oral	NA	NA	NA	Duter WP (TPTH 47%)	Cannon Laboratories, Inc.; Feb. 23, 1978; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 099049; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum 001492
Triphenyltin hydroxide	46	375		NA	rats	male and female	oral	NA	NA	NA	50% WP (Reg. No. 148-1195	U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 099049; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum
Triphenyltin hydroxide	46	380	288 - 502	NA	rats	female	oral	NA	NA	NA	Duter WP (TPTH 47%)	Cannon Laboratories, Inc.; Feb. 23, 1978; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 099049; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum 001492

Rat and Mouse Oral LD50 Database

Reference Substance	Rat Oral LD_{50}[1] mg/kg	Rat Oral LD_{50}[2] mg/kg Primary Reference	LD_{50}[3] mg/kg (range) Primary Reference	LD_{50} Calculation Method[4] Primary Reference	Animal Information (stock, weight, age)	Gender	Route and/or Method of Exposure	Dose	Observations	Notes	Reference Substance Source	Primary Reference
Triphenyltin hydroxide	46	720	520 - 920	NA	rats	female	oral	NA	NA	NA	Kansai Robamame soin B A/F 1000B (Red Point)	Bio/dynamics, Inc.; 6584-81; Sept. 30, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00086072; EPA Chem. Code: 083601; Core Grade/Tox Record No. Guideline 001881
Triphenyltin hydroxide	46	830	580 - 1080	NA	rats	male and female	oral	NA	NA	NA	Kansai Robamame soin B A/F 1000B (Red Point)	Bio/dynamics, Inc.; 6584-81; Sept. 30, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00086072; EPA Chem. Code: 083601; Core Grade/Tox Record No. Guideline 001881
Triphenyltin hydroxide	46	840	512 - 1378	NA	rats	unknown	oral	NA	NA	NA	Duter Flowable 30 (TPTH 19.7%)	Cannon Laboratories, Inc.; 9E-6359; Nov. 13, 1979; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00086591; EPA Chem. Code: 083601; Core Grade/Tox Record No. minimum 001496
Triphenyltin hydroxide	46	1200	600 - 1800	NA	rats	male	oral	NA	NA	NA	Kansai Robamame soln B A/F 1000B (Red Point)	Bio/dynamics, Inc.; 6584-81; Sept. 30, 1981; U.S. EPA, Office of Pesticide Programs; Health Effects Division; Tox Oneliners; MRID No. 00086072; EPA Chem. Code: 083601; Core Grade/Tox Record No. Guideline 001881
Valproic acid	670	670	598 - 750 (95% CL; slope = 1.2 [1.0 - 1.4; 95% CL])	Litchfield and Wilcoxon method (1949)	Osborne-Mendel rats; young adult	male and female	oral intubation	2% in water	usual observaton time of 2 weeks; depression, scrawny appearance, diarrhea; dead within 2 hour - 2 days	18 hours fasting; groups of 10 rats; evenly divided between male and female	commercially available material	Jenner PM, Hagan EC, Taylor JM, Cook EL, Fitzhugh OG. 1964. Food flavorings and compounds of related structure I. Acute Oral Toxicity. Fd Cosmet Toxicol 2:327-334. *U.S. Food and Drug Administration, Washington, D.C.* **(RTECS REFERENCE)**
Valproic acid	670	1480	NA	NA	rats	male and female	oral	NA	NA	reference in French	NA	Deboeck AM. Valproic acid salt, its preparation and utilization. European Patent Office, Publication No. EP 0078785A1. Application date 11/03/82.
Verapamil HCl	108	108	NA	NA	rats	NA	oral	NA	NA	NA	NA	Drugs in Japan (Ethical Drugs). (Yakugyo Jiho Co., Ltd., Tokyo, Japan). 1982. **(RTECS REFERENCE)**
Verapamil HCl	108	114	97 - 135	Litchfield and Wilcoxin (1949)	rats	NA	oral	NA	NA	reference in German	NA	Haas VH, Hartfelder G. 1962. A-Isopropyl-a-[(N-methyl-N-homoveratryl-g-amino-propyl]-3,4-dimethoxyphenylacetonitrile, eine Substanz mit coronargefaferweiternden Eigenschaften 12:549-558.
Xylene	4300	1537	1294 - 1781 (95% CL; slope = 9.6)	Finney (1971) Probit Analysis	ChR-CD; ave bw for each group = 253, 251, and 256 g; young adults	male	oral; intragastric intubation	single dose in aqueous solution (25%); doses = 1200, 1600, 2000 mg/kg; dose = 1.2 - 2.0 mL	16 dead; observed over 14-day recovery period; 1200 dose: lacrimation and wet perineal area (1/10 dead); 1600 dose: tremors, salivation, prostration, piloerection, lacrimation, wet perineal area, ataxia (7/10 dead; death within 15 hours after dosing); 2000 dose: tremors, severe fascicutations, ataxia, lacrimation, prostration, piloerection, lethargy, wet and stained perineal area, weakness (8/10 dead)	3 groups of 10 rats each; date of test is 1979	NA	from EPA TSCATS database; Oral LD50 test (1979), EPA Document No. 878221390 Fiche No. OTS0215213; *E.I Dupont DeNemours & Co., Inc./Haskell Labs*
Xylene	4300	4300	NA	NA	white rats; Wistar; 175- 250 g	male	oral; stomach tube	single dose in either olive oil or corn oil solution emulsified with aqueous solution of acacia; or undiluted; no more than 7 cc administered	all surviving rats observed up to 2 weeks; 20 rats used	percent of isomers: o = 19; p = 24; m = 52	NA	Wolfe MA, Rowe VK, McCollister DD, Hollingsworth RL, Oyen F. 1956. Toxicological studies of certain alkylated benzenes and benzene: experiments on laboratory animals. AMA Archives of Industrial Health. 14:387-397. *The Dow Chemical Co. Midland, MI.* **(RTECS REFERENCE)**
Xylene	4300	8314	7716 - 8803 (95% CL)	Finney (1971) Probit Analysis	ChR-CD; ave bw each group = 276, 258, 286, 262, 256 g; young adults	male	oral; intragastric intubation	single dose in corn oil (50% solution); doses = 7500, 8000, 9000, and 9500 mg/kg; dose = 3.93-5.25 mL	16 dead; observed over 14-day recovery period; 7500 dose: (3/10 dead); 8000 dose: (3/10 dead); 9000 dose: (6/10 dead); 9500 dose (10/10 dead); salivation, lethargy, ruffled fur, diarrhea, respiratory congestion, wet/bloody perineal areas	4 groups of 10 rats each; date of test is 1975	NA	from EPA TSCATS database; Oral LD50 test (1975), EPA Document No. 878221390 Fiche No. OTS0215213; *E.I Dupont DeNemours & Co., Inc./Haskell Labs*
Xylene	4300	8620 (10 mL/kg; density = 0.862)	6465 - 11465 (CL; reported as 7.5 - 13.3 mL/kg)	Litchfield and Wilcoxon method (1949)	Long-Evans rats; 150-300 g	male	oral; intragastric intubation	single dose; graded doses up to 25 mL/kg; undiluted samples	observed for 14 days; mortality values based on the number of animals which died during this time; 6 rats per dose	ortho, meta, and para xylene; ethyl benzene	aromatic concentrated from commercial source by an absorption technique; 98% aromatic.	Hine CH, Zuidema HH. 1970. The toxicological properties of hydrocarbon solvents. Industrial Medicine. 39(5):39-44.

Abbreviations: NA=Not available; CL=Confidence limit; CI=Confidence interval; SE=Standard error; UDP=Up-and-Down Procedure; TSCATS=Toxic Substances Control Act Test Submissions; RTECS=Registry of Toxic Effects of Chemical Substances; min=Minimum; HSDB=Hazardous Substances Data Bank (NLM 2001).

Gray cells highlight the rationale for exclusion of reference value.

[1] RTECS® database value at the time of database search by NICEATM (2002). If rat oral LD_{50} was unavailable, rat oral LD_{50} from HSDB was used, or mouse oral LD_{50} from RTECS was used.

[2] Value reported in the reference publication.

[3] Range (if provided in the reference publication).

[4] Method reported from the reference publication.

Appendix H2

Evaluation of the Candidate Reference Oral LD_{50} Data

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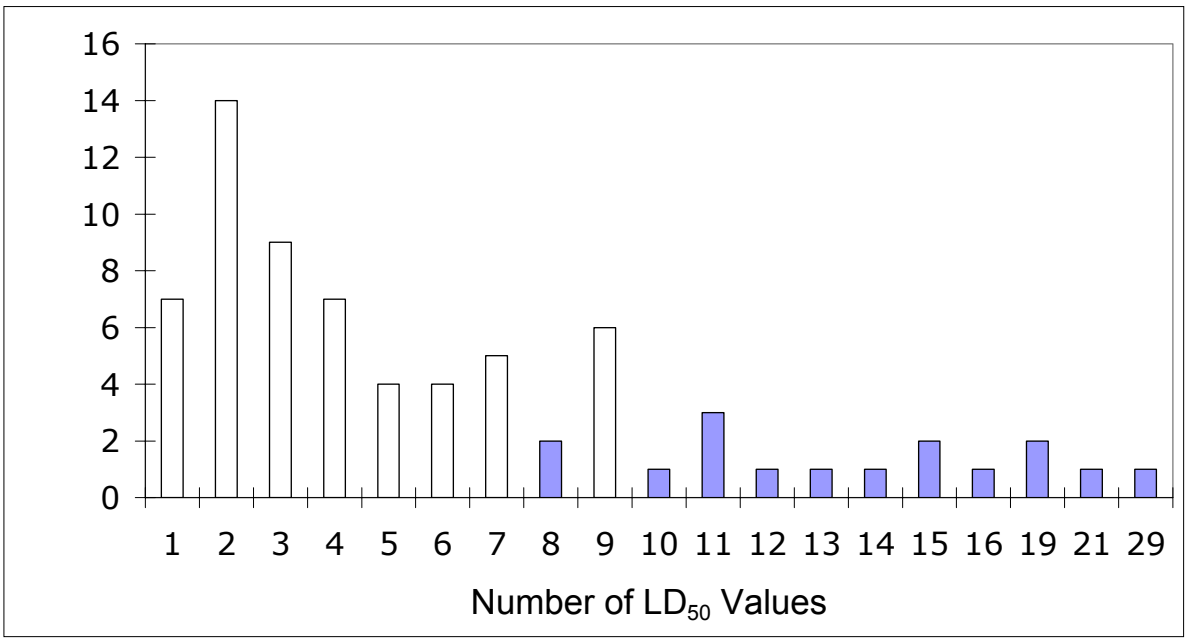
H.2 Evaluation of the Candidate Reference Oral LD_{50} Data

The 491 LD_{50} values identified by the literature search consisted of 485 rat oral LD_{50} values and six mouse oral LD_{50} values. Mouse oral LD_{50} values were used to determine reference values for colchicine, epinephrine bitartrate, and propylparaben since rat oral LD_{50} values for these three reference substances could not be located. Thirty rat oral LD_{50} values were believed to be duplicates of other reported values because the LD_{50} values and the experimental information matched exactly those cited by other publications from the same author(s) or because the same animal data were used to calculate multiple LD_{50} values (e.g., to evaluate various methods of calculation).

Two rat oral LD_{50} values provided by RTECS® were incorrect, possibly due to typographical errors. For the value of 200 mg/kg for acetylsalicylic acid, RTECS® cited a review by Diechmann (1969) that referred to a paper by Coldwell and Boyd (1966). Coldwell and Boyd (1966), however, actually reported an LD_{50} of 920 mg/kg. For sodium oxalate, RTECS® cited a review paper by Walum (1998) for an LD_{50} value of 11160 mg/kg. Although Walum (1998) provided no source, the LD_{50} is the same as that used in the MEIC study (Ekwall et al. 1998b). That LD_{50} was calculated from the LD_{50} for oxalic acid (Ekwall et al. 1998b) which is 7500 mg/kg according to RTECS®. The source for this figure, however, provides a value of 7.5 mL/kg of 5% oxalic acid (Vernot et al. 1977). Extrapolating this to sodium oxalate (MW=134.0 g/mole vs 90.04 g/mole for oxalic acid) yields an LD_{50} of 558 mg/kg.

After exclusion of the 30 duplicate values and the two erroneous values for acetylsalicylic acid and sodium oxalate, 459 records remained for further evaluation. **Figure H2-1** shows the frequency of the number of LD_{50} values retrieved for the 72 reference substances. The number of LD_{50} values identified for any one reference substance ranged from one to 29. The highest frequency was two LD_{50} values per reference substance (14 reference substances). The highest number of LD_{50} values retrieved for an individual reference substance (acetonitrile) was 29. A large number of LD_{50} values were also identified for hexachlorophene (21), ethylene glycol (19), and carbon tetrachloride (19). Only one LD_{50} value was identified for seven reference substances: aminopterin, digoxin, epinephrine bitartrate, glutethimide, physostigmine, and propranolol HCl.

Figure H2-1 Distribution of the Number of LD_{50} Values Per Reference substance



Bars show number of reference substances with the noted number of LD_{50} values for the 459 oral LD_{50} values remaining after the exclusion of 30 duplicate values and two erroneous values.

H.2.1 Protocols Used for the Candidate Reference Data

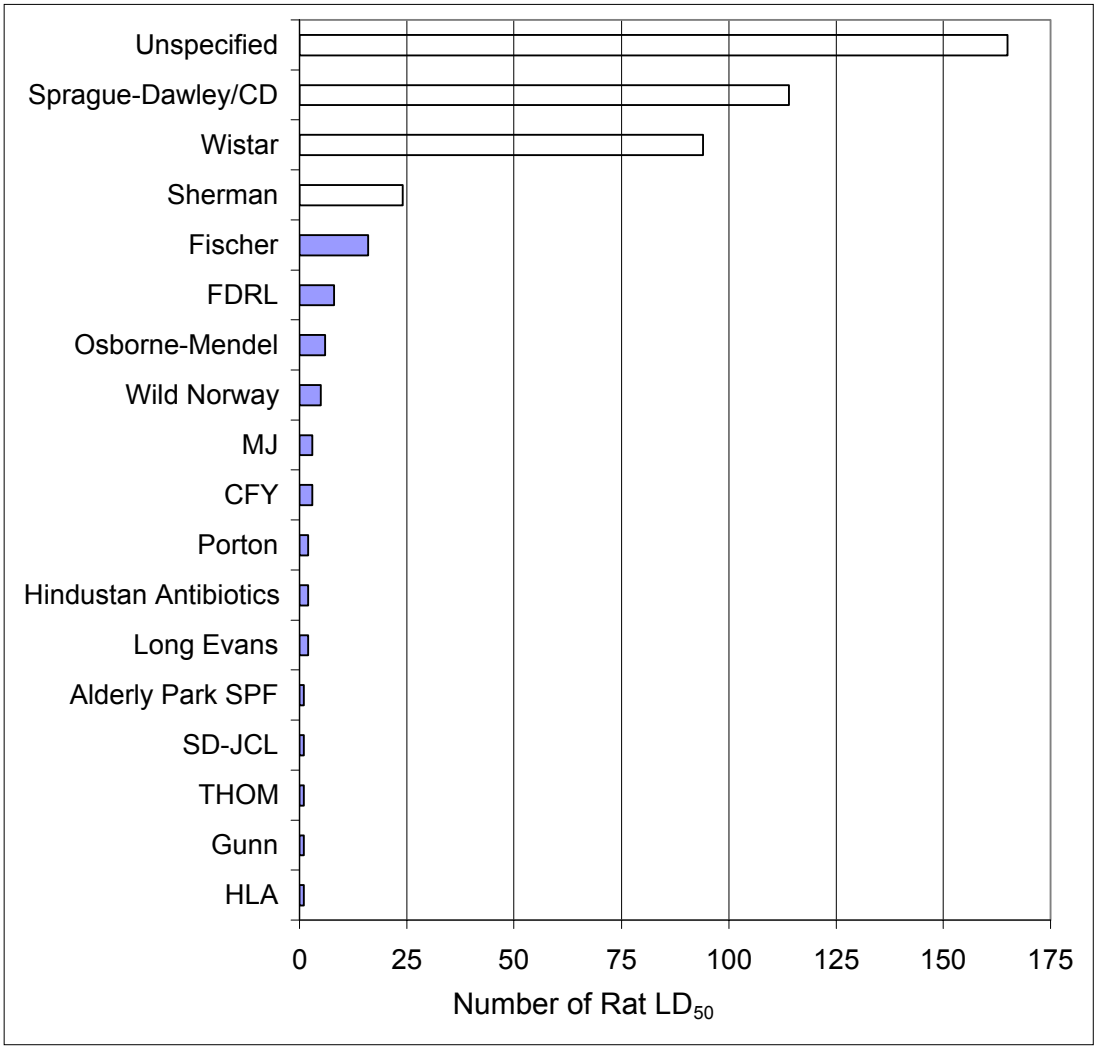
The LD_{50} data were collected using various protocols; however, information on the protocol details was often incomplete due to limited documentation in the reports. The 459 remaining data records exhibited the following characteristics:

- 64% (293/459) specified the stock or strain of rodent used. The remaining 36% (167/459) that did not specify the stock/strain described rats as rats, albino rats, white rats, rats of different strains, and mice were described as mice.
- 63% (290/459) included age or weight information for the rodents.
- 77% (354/459) specified the gender of the rodent.
- 66% (305/459) stated the method used to calculate the LD_{50}.
- 48% (221/459) reported the number of rodents used at each dose and 47% (216/459) reported the total number of rodents used.
- 26% (118/459) specified the doses used.
- 14% (66/459) quantitatively specified the purity of the reference substance used. Of the remaining records, 18% (83/459) described the purity qualitatively using such terms as “technical grade,” “pure,” “reagent grade,” and “pharmaceutical grade,” 11% (51/459) named only the source of the reference substance, and 56% (259/459) provided no information on the reference substance.
- 13% (61/459) reported the deaths at each dose.

Although many LD_{50} studies did not specify the strain or stock of rat used, the 293 studies that provided this information indicated that Sprague-Dawley/CD rats were the strain most frequently used (see **Figure H2-2**). Wistar rats were also frequently used. Strains such as Alderly Park, SD-JCL, THOM, Gunn, and HLA were the least frequently used. Of the six mouse LD_{50} values, the strain was unspecified for two studies. The other four LD_{50} values were obtained using CD-1, MS/Ae, dd, and B6D1F1(BDF1) mice.

Of the 354 studies that reported rodent gender, the most frequently used gender for both rodents was male, which was used for 193 (55%) LD_{50} values. Female rodents were used for 104 (29%) LD_{50} values, both sexes were used for 55 (16%) LD_{50} values, and rodents of unspecified gender were used for 104 (29%) LD_{50} values.

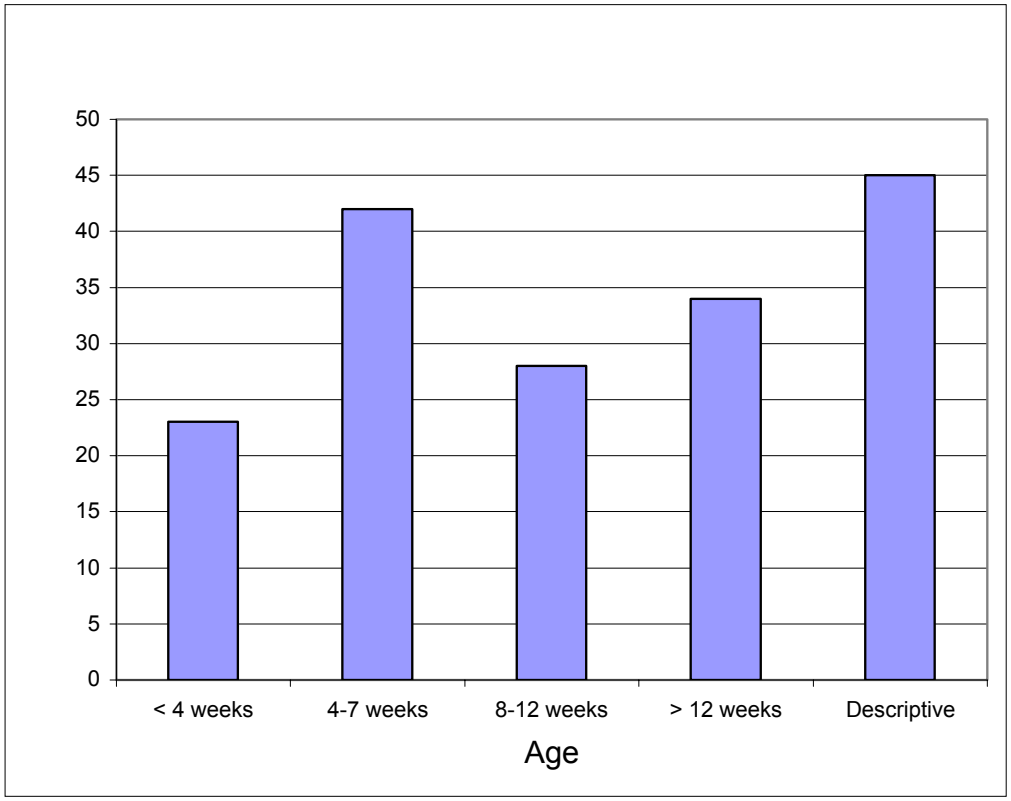
Figure H2-2 Distribution of Rat Stocks/Strains



Bars show number of rat oral LD_{50} records for each rat strain for the 453 rat values remaining after the exclusion of 30 duplicate values, two erroneous values, and six mouse values.

The age of the rodents used for the acute oral lethality studies also varied. Of the 174 LD_{50} studies that reported age, the most frequently used age was 4-7 weeks, which was reported for 42 (24%) LD_{50} values (see **Figure H2-3**). The majority of the reported ages were descriptive. Forty-five (26%) LD_{50} values used rodents that were described as young, adults, young adults, or older adults. Thirty (17%) LD_{50} studies used 8-12 week old rodents, which is the age recommended by current oral acute toxicity test guidelines (OECD 2001a, c, d; EPA 2002a). Twenty-three (13%) LD_{50} values were determined using rodents less than four weeks of age, and 34 (20%) LD_{50} values were determined using rodents greater than 12 weeks old.

Figure H2-3 Distribution of Rat and Mouse Ages



Bars show the number of rat and mouse LD_{50} records that report using animals of the specified ages. Descriptive indicates that age was described qualitatively (e.g., adult, juvenile).

The duration of animal observation was not specified for 39% (179/459) of the LD_{50} reports. Of the 280 (61%) studies that reported the duration of observation, 136 (48%) reported an observation period of 14 days, which is recommended in the current oral acute toxicity test guidelines (OECD 2001a, c, d; EPA 2002a). The second most commonly used observation period was seven days, which was reported by 59 (21%) studies. Clinical signs were reported in 30% (137/459) of the studies.

Of the 305 studies that reported the method used to calculate the LD_{50} value, the most frequently used were the graphical log-probit methods such as Litchfield and Wilcoxon (1949), with 99 (33%) LD_{50} values, and Miller and Tainter (1944), with 24 (8%) LD_{50}

values. The maximum likelihood probit method of Bliss (1938) and modifications were used for the calculation of 46 (15%) LD_{50} values. An additional 36 (12%) LD_{50} values were calculated using methods referred to in a general way as probit or log probit methods. The moving average method, such as that of Thompson (1947) or Weil (1952), was cited for 57 (19%) LD_{50} values. Thirteen (4%) LD_{50} values were described as being calculated by one method or another (e.g., by Weil or Litchfield and Wilcoxon), or by methods that were described generally, such as graphical or approximative. Some of the least frequently used methods were linear regression (six values), UDP (four values), and linear interpolation (one value). Estimates of variability such as confidence limits, standard error, or standard deviation were included in 62% (283/459) of the LD_{50} reports, but only 6% (28/459) included slopes.

H.2.2 Final Reference Values

Based on the study exclusion criteria described in **Section 4.1.2**, 73 (16%) of the 459 records identified were excluded. Thirty-one LD_{50}values were excluded because they were reported as ranges, 21 were excluded because the rats were less than four weeks old, five were excluded because the rats were feral, five were excluded because the rats were anesthetized, and four were excluded because the reference substance administered was mixed with food. Additionally, four LD_{50} values for copper sulfate pentahydrate were excluded because very low purity (i.e., ≤20%) reference substance was used. Three LD_{50} values were excluded because they were outliers at the 99% level (Dixon and Massey 1981) compared with the rest of the values for the particular reference substance. These included one ethylene glycol value of 17,800 mg/kg (range of the other 16 values=4000-9900 mg/kg), one meprobamate value of 794 mg/kg (range of other six values=1286-1522 mg/kg), and one mercury chloride value of 160 mg/kg (range of other 10 values=12-92 mg/kg). **Appendix H-1** provides the individual rationale for each LD_{50} value excluded by shading the cell that contains the reason for exclusion.

Triethylenemelamine, trichloroacetic acid, and xylene had the largest confidence limits in proportion to the geometric means. The confidence limits for triethylenemelamine and xylene were calculated from four LD_{50} values while those for trichloroacetic acid were calculated with five LD_{50} values. Nicotine and 2-propanol had the smallest confidence limits even though the number of values per reference substance were similar to that for the reference substances with large confidence limits (nicotine N=4, 2-propanol N=6).

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Appendix I

In Vitro NRU Data

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Appendix I1

3T3 NRU Reference Substance Data

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3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
ACETAMINOPHEN															
IIVS															
A1	RF	AA61HU	30.8	0.203	0.266	0.88%	2	6	0.9628	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A5
B1	DF	AA61HU	32.1	0.212	0.457	0.71%	3	5	0.9728	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES			SLS-B5
B2	DF	AA61HU	54.8	0.363	0.402	4.77%	2	5	0.9221	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES			SLS-B9
B3	DF	AA61HU	43.3	0.286	0.356	1.85%	3	5	0.9794	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES			SLS-B10
ECBC															
AA61LR-A1	RF	AA61LR	66.8	0.442	0.253	4.38%	2	0	0.9619	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-14
AA61LR-B1	DF	AA61LR	30.3	0.200	0.449	13.54%	5	3	0.9875	200, 136, 92.6, 63.0, 42.8, 29.1, 19.8, 13.5	1.47	YES			SLS-P38
AA61LR-B2	DF	AA61LR	46.1	0.305	0.298	3.30%	4	4	0.9557	200, 136, 92.6, 63.0, 42.8, 29.1, 19.8, 13.5	1.47	YES			SLS-P39
AA61LR-B3	DF	AA61LR	46.1	0.305	0.407	3.13%	4	4	0.9855	200, 136, 92.6, 63.0, 42.8, 29.1, 19.8, 13.5	1.47	YES			SLS-P42
FRAME															
FAL.3T3.PY.A1.21.10.04	RF	AA61PY	62.1	0.411	0.212	1.41%	2	6	0.9541	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.3T3.SLS.21.10.04
FAL.3T3.PY.B1.26.11.04	DF	AA61PY	92.3	0.610	0.290	3.71%	4	2	0.9374	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			FAL.3T3.SLS.26.11.04
FAL.3T3.PY.B2.02.12.04	DF	AA61PY	57.1	0.378	0.194	4.85%	6	2	0.9518	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			FAL.3T3.SLS.03.12.04
FAL.3T3.PY.B3.09.12.04	DF	AA61PY	49.1	0.325	0.416	1.16%	6	2	0.9672	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			FAL.3T3.SLS.09.12.04
ACETONITRILE															
IIVS															
A1	RF	AA61GF	NA	NA	0.393	2.29%	0.0	7	0.0319	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61GF	18100	441.25	0.305	45.66%	2	3	0.8837	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	NO	% VC difference >15	VC1 ODs lower than VC2 values; volatility issues. VC1 removed from subsequent analysis.	SLS-B6
B2	DF	AA61GF	10500	256.854	0.426	0.14%	4	1	0.9638	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	YES		OD measured 15-16 hr late; orignial reading used wrong OD wavelength; plate sealer used; outliers removed by SD; ppt in 1X C1-C4	SLS-B11
B3	DF	AA61GF	8070	196.647	0.330	3.56%	6	2	0.9540	20000, 16667,13889, 11574, 9645, 8038, 6698, 5582	1.2	YES		plate sealer used	SLS-B12
B4	DF	AA61GF	9420	229.449	0.336	0.05%	4	4	0.8516	20000, 16667,13889, 11574, 9645, 8038, 6698, 5582	1.2	YES		plate sealer used; outliers removed by SD	SLS-B13
B5	DF	AA61GF	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
ECBC															
AA61PH-A1	RF	AA61PH	NA	NA	0.309	4.26%	0	0	NA	10000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder	no toxicity detected	SLS-P1
AA61PH-A2	RF	AA61PH	NA	NA	0.308	36.98%	2	3	NA	200000, 20000, 2000, 200, 20, 2, 0.2, 0.02	10	RF	range finder		SLS-P3
AA61PH-B1(sealer)	DF	AA61PH	NA	NA	0.372	19.13%	5	2	NA	50000, 34014, 23139, 15740, 10708, 7284, 4955, 3371	1.47	NO	%VC difference > 15		SLS-P38

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61PH-B2 (sealer)	DF	AA61PH	NA	NA	0.257	29.42%	6	1	NA	50000, 34014, 23139, 15740, 10708, 7284, 4955, 3371	1.47	NO	%VC difference > 15		SLS-P39
AA61PH-B3 (sealer)	DF	AA61PH	6340	154.414	0.448	7.35%	6	2	0.9770	50000, 34014, 23139, 15740, 10708, 7284, 4955, 3371	1.47	YES			SLS-P41
AA61PH-B4 (sealer)	DF	AA61PH	6580	160.209	0.445	14.54%	4	2	0.9796	40000, 27211, 18511, 12592, 8566, 5827, 3964, 2697	1.47	YES			SLS-P43
AA61PH-B5 (sealer)	DF	AA61PH	6380	155.484	0.453	4.90%	5	3	0.9823	40000, 27211, 18511, 12592, 8566, 5827, 3964, 2697	1.47	YES			SLS-P45
FRAME															
FAL3T3.PL.A1.22-01-04	RF	AA61PL	NA	NA	0.439	1.52%	0	3	0.0000	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.22/01/04
FAL3T3.PL.B1.29-01-04	DF	AA61PL	56800	1382.569	0.404	17.45%	1	4	0.8826	100000, 46512, 21633, 10062, 4680, 2177, 1012, 471	2.15	NO	%VC difference >15	volatility problem; C1 alkaline	FAL3T3.SLS.29-01-04
FAL3T3.PL.B2.05-02-04	DF	AA61PL	6920	168.534	0.230	62.44%	2	2	0.9721	100000, 46512, 21633, 10062, 4680, 2177, 1012, 471	2.15	NO	PC failed; % VC difference > 15	problem with reservoir liners; volatility issue; VC1 <<< VC2	FAL.3T3.SLS.5/02/04
FAL3T3.PL.B4.25-02-04	DF	AA61PL	NA	NA	0.331	71.55%	3	1	NA	100000, 46512, 21633, 10062, 4680, 2177, 1012, 471	2.15	NO	%VC difference >15; possible volatility problem		FAL3T3.SLS.25.02.04
FAL3T3.PL.B5.29-04-04	DF	AA61 PL	15200	371.267	0.327	2.12%	2	4	0.8985	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	YES		heated C1-C3 to dissolve	FAL.3T3.SLS.29/04/04
FAL3T3.PL.B6.06-05-04	DF	AA61 PL	9930	241.928	0.334	5.53%	3	5	0.9631	30000, 20408, 13883, 9444, 6425, 4371, 2973, 2023	1.47	YES			FAL.3T3.SLS.06/05/04
FAL3T3.PL.B7.20/05/04	DF	AA61 PL	6490	158.011	0.344	19.62%	2	4	0.8881	30000, 13953, 6490, 3019, 1404, 653, 304, 141	2.15	NO	%VC difference >15; possible volatility problem	SD having difficulty in using plate covers for volatility problems	FAL.3T3.SLS.20/05/04
FAL3T3.PL.B8.27/05/04	DF	AA61 PL	3940	95.871	0.354	7.19%	3	3	0.9226	30000, 13953, 6490, 3019, 1404, 653, 304, 141	2.15	YES		C1-C3 heated to dissolve	FAL.3T3.SLS.27/05/04

ACETYLSALICYLIC ACID

IIVS															
A1	RF	AA61HM	480	2.662	0.371	2.14%	1	2	0.9294	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A3
B1	DF	AA61HM	344	1.911	0.413	7.89%	5	3	0.9635	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	YES			SLS-B5
B2	DF	AA61HM	467	2.590	0.394	1.04%	4	4	0.9853	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	YES			SLS-B9
B3	DF	AA61HM	392	2.174	0.383	1.33%	4	4	0.9724	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	YES			SLS-B10
ECBC															
AA61ME-A1	RF	AA61ME	175	0.969	0.256	7.03%	1	6	0.7065	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P9
AA61ME-B1	DF	AA61ME	589	3.268	0.344	6.13%	2	2	0.9566	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P30
AA61ME-B2	DF	AA61ME	711	3.947	0.304	4.93%	2	6	0.9182	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P32
AA61ME-B3	DF	AA61ME	637	3.534	0.345	0.84%	2	4	0.9244	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P34
FRAME															
FAL.3T3.JA.A1.21.05.04	RF	AA61JA	1110	6.169	0.190	4.35%	0	1	0.5653	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points 0 - 50%		FAL.3T3.SLS.21.05.04
FAL.3T3.JA.B1.04.06.04	DF	AA61JA	1290	7.149	0.358	12.22%	2	6	0.9869	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.3T3.SLS.04.06.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.JA.B2.18.06.04	RF	AA61JA	1500	8.342	0.471	8.60%	1	5	0.9217	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES		outlier removed by SD from VC1	FAL.3T3.SLS.18.06.04
FAL.3T3.JA.B3.08.07.04	DF	AA61JA	912	5.061	0.262	0.73%	3	5	0.9499	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.3T3.SLS.08.07.04
AMINOPTERIN															
IIVS															
A1	RF	AA61JD	0.006	0.00001	0.449	1.25%	6	1	0.8361	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A2
B1	DF	AA61JD	0.006	0.00001	0.310	1.69%	4	4	0.8810	0.02, 0.013, 0.0089, 0.0059, 0.0040, 0.0026, 0.0018, 0.0012	1.5	YES			SLS-B1
B2	DF	AA61JD	0.004	0.00001	0.402	1.66%	5	3	0.8854	0.02, 0.013, 0.0089, 0.0059, 0.0040, 0.0026, 0.0018, 0.0012	1.5	YES			SLS-B2
B3	DF	AA61JD	0.003	0.00001	0.461	0.11%	6	2	0.8529	0.02, 0.013, 0.0089, 0.0059, 0.0040, 0.0026, 0.0018, 0.0012	1.5	NO	PC failed		SLS-B3
B4	DF	AA61JD	0.005	0.00001	0.300	0.33%	5	1	0.8025	0.02, 0.013, 0.0089, 0.0059, 0.0040, 0.0026, 0.0018, 0.0012	1.5	YES			SLS-B4
ECBC															
AA61MB-A1	RF	AA61MB	0.012	0.00003	0.373	14.44%	6	2	0.6985	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	low r2; range finder		SLS-P4
AA61MB-A2	RF	AA61MB	0.014	0.00003	0.470	22.09%	6	1	0.7532	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	NO	low r2;% VC difference > 15; range finder		SLS-P5
AA61MB-B1	DF	AA61MB	0.007	0.00002	0.435	3.5%	4	1	0.8625	0.1000, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			SLS-P7
AA61MB-B2	DF	AA61MB	0.004	0.00001	0.400	5.46%	5	1	0.8409	0.0200, 0.0136, 0.0093, 0.0063, 0.0043, 0.0029, 0.0020, 0.0013	1.47	NO	PC failed		SLS-P9
AA61MB-B3	DF	AA61MB	0.005	0.00001	0.383	11.29%	5	1	0.8251	0.0200, 0.0136, 0.0093, 0.0063, 0.0043, 0.0029, 0.0020, 0.0013	1.47	YES			SLS-P11
AA61MB-B4	DF	AA61MB	0.005	0.00001	0.544	7.46%	5	2	0.8840	0.0200, 0.0136, 0.0093, 0.0063, 0.0043, 0.0029, 0.0020, 0.0013	1.47	YES			SLS-P14
FRAME															
A1PU190603	RF	AA61PU	0.146	0.00033	0.550	2.01%	6	0	0.6490	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	PC failed; no points between 50 - 90%; low r2		A1SLS190603
FAL.3T3.PU.A2.26.06.03	RF	AA61PU	NA	NA	0.446	4.4%	8	0	0.0669	3.50, 2.38, 1.62, 1.10, 0.75, 0.51, 0.35, 0.24	1.47	NO	no points between 10 - 50%; low r2; range finder		FAL.3T3.SLS.A2.26.06.03
FAL.3T3.PU.B1.03.07.03	DF	AA61PU	NA	NA	0.453	0.09%	8	0	NA	0.100, 0.047, 0.022, 0.010, 0.005, 0.002, 0.001, 0.0005	2.13	NO	PC failed; no points between 10 - 50; r2 not available		FAL.3T3.SLS.B1.03.07.03
FAL.3T3.B2.PU.10.07.03	DF	AA61PU	NA	NA	0.451	3.11%	8	0	0.0018	0.100, 0.047, 0.022, 0.010, 0.005, 0.002, 0.001, 0.0005	2.13	NO	no points between 50 - 90%; low r2		FAL.3T3.SLS.10.07.03
FAL.3T3.B7.PU.17.10.03	DF	AA61PU	0.00583	0.00001	0.302	10.79%	1	4	0.8196	0.010, 0.005, 0.002, 0.0010, 0.0005, 0.0002, 0.0001, 0.00005	2.5	YES			FAL.3T3.SLS.171003
FAL.3T3.B8.PU.30.10.03	DF	AA61PU	0.0129	0.00003	0.361	0.21%	1	4	0.9443	0.022, 0.010, 0.005, 0.0022, 0.0010, 0.0005, 0.0002, 0.0001	2.2	YES			FAL.3T3.SLS.301003
FAL.3T3.B9.PU.31.10.03	DF	AA61PU	0.0166	0.00004	0.289	8.80%	2	2	0.8698	0.046, 0.021, 0.010, 0.0046, 0.0022, 0.001, 0.0005, 0.0002	2.2	YES			FAL.3T3.SLS.301003 (should be 311003)

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
5-AMINOSALICYLIC ACID															
IIVS															
A1	RF	AA61GZ	NA	NA	0.448	0.95%	0	5	0.7520	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A3
B1	DF	AA61GZ	1360	8.872	0.447	3.00%	1	7	0.9462	1500, 1154, 888, 683, 525, 404, 311, 239	1.3	YES			SLS-B6
B2	DF	AA61GZ	1610	10.520	0.451	0.98%	0	8	0.9642	1500, 1154, 888, 683, 525, 404, 311, 239	1.3	NO	no points between 0 - 50%	plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B3	DF	AA61GZ	1710	11.144	0.349	4.42%	2	6	0.9177	2500, 1786, 1276, 911, 651, 465, 332, 237	1.4	YES		ppt in 2X C1-C3	SLS-B12
B4	DF	AA61GZ	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B5	DF	AA61GZ	1600	10.472	0.409	0.65%	2	6	0.9854	2500, 1786, 1276, 911, 651, 465, 332, 237	1.4	YES		ppt in 2X C1-C2	SLS-B15
ECBC															
AA61KD-A1	RF	AA61KD	NA	NA	0.318	12.19%	0	4	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-11
AA61KD-B1	DF	AA61KD	1530	10.024	0.709	2.06%	1	7	0.9218	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			SLS-P46
AA61KD-B2	DF	AA61KD	1240	8.110	0.413	0.16%	2	6	0.9375	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			SLS-P47
AA61KD-B3	DF	AA61KD	1630	10.642	0.386	0.26%	2	6	0.9711	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			SLS-P49
FRAME															
FAL.3T3.PA.A1.21.05.04	RF	AA61PA	NA	NA	0.394	4.59%	0	1	0.5658	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.21.05.04
FAL.3T3.PA.B1.04.06.04	DF	AA61PA	1770	11.535	0.501	0.57%	1	7	0.9637	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.3T3.SLS.04.06.04
FAL.3T3.PA.B2.18.06.04	DF	AA61PA	2010	13.123	0.491	14.06%	1	4	0.8978	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.3T3.SLS.18.06.04
FAL.3T3.PA.B3.08.07.04	DF	AA61PA	2430	15.850	0.343	1.34%	1	4	0.8650	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.3T3.SLS.08.07.04
AMITRIPTYLINE HCL															
IIVS															
A1	RF	AA61RF	5.45	0.017	0.327	1.25%	1	2	0.9939	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1 and 1X C1	SLS-A1
B1	DF	AA61RF	8.83	0.03	0.349	0.19%	2	5	0.9858	25.0, 16.7, 11.1, 7.41, 4.94, 3.29, 2.19, 1.46	1.5	YES			SLS-B1
B2	DF	AA61RF	8.35	0.03	0.344	1.92%	2	2	0.9464	25.0, 16.7, 11.1, 7.41, 4.94, 3.29, 2.19, 1.46	1.5	YES			SLS-B2
B3	DF	AA61RF	6.24	0.02	0.357	0.02%	2	2	0.9701	25.0, 16.7, 11.1, 7.41, 4.94, 3.29, 2.19, 1.46	1.5	YES			SLS-B3
ECBC															
AA61PR-A1	RF	AA61PR	10.6	0.034	0.352	8.18%	0	5	0.8920	1000, 100,10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1 and 1X C1	SLS-P4
AA61PR-B1	DF	AA61PR	6.26	0.020	0.384	2.37%	2	4	0.9661	80.0, 37.2, 17.3, 8.05, 3.74, 1.74, 0.81, 0.38	2.15	YES			SLS-P20
AA61PR-B2	DF	AA61PR	4.55	0.014	0.451	1.05%	2	5	0.9214	15.0, 10.2, 6.94, 4.72, 3.21, 2.19, 1.49, 1.01	1.47	YES			SLS-P22
AA61PR-B3	DF	AA61PR	7.28	0.023	0.577	2.79%	2	4	0.9701	15.0, 10.2, 6.94, 4.72, 3.21, 2.19, 1.49, 1.01	1.47	YES			SLS-P24
FRAME															
FAL.3T3.LE.A1.090104	RF	AA61LE	12.9	0.041	0.463	1.62%	1	3	0.9739	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	FAL.3T3.SLS.09/01/04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL3T3.LE.B1.16.01.04	DF	AA61 LE	10.4	0.033	0.500	7.09%	3	4	0.9391	100, 46.5, 21.6, 10.1, 4.7, 2.2, 1.0, 0.5	2.15	YES			FAL.3T3.SLS.16/01/04
FAL3T3.LE.B2.23.01.04	DF	AA61LE	6.48	0.021	0.347	13.33%	5	2	0.9709	50.0, 34.0, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			FAL3T3.23-01-04
FAL3T3.LE.B3.30.01.04	DF	AA61LE	NA	NA	0.262	13.73%	5	3	NA	50.0, 34.0, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	NO	SD rejects this experiment	serious NR crystal problem; SD rejects this experiment	FAL.3T3.SLS.29/01/04
FAL3T3.LE.B4.06-02-04	DF	AA61LE	6.70	0.021	0.325	5.48%	5	3	0.9586	50.0, 34.0, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES		possible NR crystals present; blanks slightly higher than usual	FAL.3T3.SLS.06/02/04
ARSENIC III TRIOXIDE															
IIVS															
A1 Preliminary	RF	AA61FX	1.50	0.008	0.409	2.18%	0	1	0.9861	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A1
B1	DF	AA61FX	3.17	0.016	0.529	5.69%	1	2	0.9787	100, 46.4, 21.6, 10.0, 4.64, 2.16, 1.00, 0.46	2.16	YES		not fully soluble at 200 ug/ml; part. observed at 100 ug/ml	SLS-B1
B2	DF	AA61FX	2.47	0.012	0.485	1.29%	1	2	0.9875	100, 46.4, 21.6, 10.0, 4.64, 2.16, 1.00, 0.46	2.16	YES		not fully soluble at 200 ug/ml; part. observed at 100 ug/ml	SLS-B2
B3	DF	AA61FX	6.63	0.034	0.599	6.19%	1	3	0.9597	100, 46.4, 21.6, 10.0, 4.64, 2.16, 1.00, 0.46	2.16	YES		not fully soluble at 200 ug/ml; part. observed at 100 ug/ml	SLS-B3
ECBC															
ECBC-3T3-Ib-01 AA61KU-A1	RF	AA61KU	18.3	0.093	0.414	3.18%	1	2	0.6456	25, 2.5, 0.25, 0.025, 0.0025,0.00025, 0.000025, 0.0000025	10	RF	range finder		SLS-P1
ECBC-3T3-Ib-02 AA61KU-B1	DF	AA61KU	2.39	0.012	0.340	0.32%	3	0	0.8812	50, 34, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	NO	No points between 50 and 90%; PC failed		SLS-P3
ECBC-3T3-Ib-03 AA61KU-B2	DF	AA61KU	2.57	0.013	0.405	4.55%	3	1	0.9221	34.2, 23.2, 15.8, 10.8, 7.3, 5.0, 3.4, 2.3	1.47	NO	PC failed		SLS-P4
ECBC-3T3-Ib-04 AA61KU-B3	DF	AA61KU	3.07	0.016	0.777	7.74%	3	2	0.9511	25.0, 17.0, 11.6, 7.9, 5.4, 3.6, 2.5, 1.7	1.47	YES			SLS-P5
ECBC-3T3-Ib-05 AA61KU-B4	DF	AA61KU	2.53	0.013	0.419	0.20%	4	1	0.9580	25.0, 17.0, 11.6, 7.9, 5.4, 3.6, 2.5, 1.7	1.47	YES			SLS-P7
ECBC-3T3-Ib-06 AA61KU-B5	DF	AA61KU	2.74	0.014	0.606	3.92%	2	2	0.9663	25.0, 17.0, 11.6, 7.9, 5.4, 3.6, 2.5, 1.7	1.47	YES			SLS-P9
ECBC-3T3-Ib-07 AA61KU-B6	DF	AA61KU	1.28	0.006	0.393	6.66%	3	1	0.9680	15.0, 10.2, 6.9, 4.7, 3.2, 2.2, 1.5, 1.0	1.47	YES			SLS-P12
FRAME															
1b3T3RF01FALNC	RF	AA61NC	6.85	0.035	0.426	3.32%	1	4	0.9380	100, 20, 4, 0.8, 0.16, 0.032, 0.0064, 0.00128	5	RF	range finder		1b3T3CTRFALSLS 12/17/02
1b3T3RF02FALNC	RF	AA61NC	2.77	0.014	0.543	8.42%	0	0	0.6786	50, 34, 23.1, 15.7, 10.7, 7.3, 5, 3.4	1.47	RF	range finder	NR crystals in plate	1b3T3CTRFALSLS 1/7/03
1b3T3RF02FALNC	RF	AA61NC	1.48	0.007	0.247	12.73%	2	3	0.8760	10, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	RF	range finder	NR crystals in plate; stopped after 1 h	1b3T3CTRFALSLS 1/8/03
1b3T3DF01FALNC	DF	AA61NC	0.328	0.002	0.669	4.88%	1	0	0.5431	24, 16.33, 11.11, 7.56, 5.14, 3.5, 2.38, 1.61	1.47	NO	No points between 50 & 90% viability; r2 < 0.8	Didn't reach 50% viability	1b3T3CTRFALSLS 1/14/03
1b3T3DF02FALNC	DF	AA61NC	1.74	0.009	0.363	3.42%	3	0	0.9517	28.5, 19.39, 13.19, 8.97, 6.1, 4.15, 2.82, 1.92	1.47	NO	No points between 50 & 90% viability; PC failed	Didn't reach 50% viability	1b3T3CTRFALSLS 1/15/03
1b3T3DF03FALNC	DF	AA61NC	1.05	0.005	0.742	0.84%	3	3	0.9163	7.000, 4.762, 3.239, 2.204, 1.499, 1.020, 0.694, 0.472	1.47	YES			1b3T3CTRFALSLS 1/21/03
1b3T3DF04FALNC	DF	AA61NC	1.39	0.007	0.303	15.26%	2	4	0.9591	7, 4.76, 3.24, 2.20, 1.50, 1.02, 0.69, 0.47	1.47	NO	NR crystals in plate; stopped after 1 h; PC failed		1b3T3CTRFALSLS 1/28/03
1b3T3DF09FALNC	DF	AA61NC	1.25	0.006	0.624	1.40%	1	3	0.9671	7, 4.76, 3.24, 2.20, 1.50, 1.02, 0.69, 0.47	1.47	NO	PC failed		1b3T3CTRFALSLS 1/29/03

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
1b3T3DF06FALNC	DF	AA61NC	0.984	0.005	0.569	0.76%	1	2	0.9099	2.500, 1.701, 1.157, 0.787, 0.535, 0.364, 0.248, 0.169	1.47	YES			1b3T3CTRFALSLS 2/403
1b3T3DF07FALNC	DF	AA61NC	1.00	0.005	0.639	1.80%	2	3	0.9303	5.000, 3.401, 2.314, 1.574, 1.071, 0.728, 0.496, 0.337	1.47	YES			1b3T3CTRFALSLS 2/5/03
1b3T3DF07(2)FALNC	DF	AA61NC	1.14	0.006	0.651	2.48%	2	2	0.9256	7, 4.76, 3.24, 2.20, 1.50, 1.02, 0.69, 0.47	1.47	YES			1b3T3CTRFALSLS 2/5/03
ATROPINE SULFATE															
IIVS															
A1	RF	AA61NE	50.4	0.072	0.391	0.62%	1	2	0.9941	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A4
B1	DF	AA61NE	63.8	0.092	0.485	4.11%	3	5	0.8808	300, 167, 92.6, 51.4, 28.6, 15.9, 8.82, 4.90	1.8	YES		outlier removed by study dire	SLS-B4
B2	DF	AA61NE	71.1	0.102	0.374	1.70%	3	5	0.9230	300, 167, 92.6, 51.4, 28.6, 15.9, 8.82, 4.90	1.8	YES		G11 in VC2 not used; rec'd extra 100ul medium during seeding process;SD removed	SLS-B7
B3	DF	AA61NE	75.0	0.108	0.436	3.00%	2	6	0.9070	300, 167, 92.6, 51.4, 28.6, 15.9, 8.82, 4.90	1.8	YES		SD note: response curves in 3 valid DF similar & don't follow classic Hill response curve	SLS-B8
ECBC															
AA61KX-A1	RF	AA61KX	87.9	0.127	0.390	11.37%	1	5	0.9664	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P13
AA61KX-B1	DF	AA61KX	31.3	0.045	0.510	7.40%	3	3	0.9452	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			SLS-P31
AA61KX-B2	DF	AA61KX	43.4	0.062	0.465	9.34%	3	4	0.9483	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			SLS-P33
AA61KX-B3	DF	AA61KX	87.5	0.126	0.686	5.74%	3	4	0.9275	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			SLS-P35
FRAME															
FAL.3T3.FU.A1.10.09.04	RF	AA61FU	461	0.664	0.384	4.08%	1	0	0.9358	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50 - 100%		FAL.3T3.SLS.10.09.04
FAL.3T3.FU.B1.16.09.04	DF	AA61FU	160	0.231	0.350	1.76%	5	3	0.9137	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		outlier removed by SD	FAL.3T3.SLS.16.09.04
FAL.3T3.FU.B2.15.10.04	DF	AA61FU	153	0.221	0.342	2.06%	4	4	0.9807	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			FAL.3T3.SLS.15.10.04
FAL.3T3.FU.B3.28.10.04	DF	AA61FU	85.5	0.123	0.184	5.35%	5	3	0.9528	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			FAL.3T3.SLS.28.10.04
BORIC ACID															
IIVS															
A1	RF	AA61LD	979	15.842	0.433	3.67%	1	6	0.9184	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A4
B1	DF	AA61LD	1090	17.571	0.403	0.04%	4	4	0.9456	5000, 3125, 1953, 1221, 763, 477, 298, 186	1.6	YES			SLS-B6
B2	DF	AA61LD	685	11.087	0.486	2.50%	5	3	0.9462	5000, 3125, 1953, 1221, 763, 477, 298, 186	1.6	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B3	DF	AA61LD	1830	29.635	0.349	0.52%	2	4	0.9129	5000, 3125, 1953, 1221, 763, 477, 298, 186	1.6	YES			SLS-B12
ECBC															
AA61JH-A1	RF	AA61JH	897	14.514	0.329	0.46%	2	6	0.8984	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P12

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61JH-B1	DF	AA61JH	1150	18.570	0.477	1.66%	3	5	0.9684	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P31
AA61JH-B2	DF	AA61JH	1290	20.932	0.423	0.14%	4	4	0.9524	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P33
AA61JH-B3	DF	AA61JH	2050	33.098	0.691	5.22%	3	3	0.9571	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P35
FRAME															
FAL.3T3.GR.A1.10.09.04	RF	AA61GR	2000	32.270	0.394	4.32%	1	1	0.8608	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.10.09.04
FAL.3T3.GR.B1.16.09.04	DF	AA61GR	4320	69.791	0.351	10.82%	2	3	0.8630	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			FAL.3T3.SLS.16.09.04
FAL.3T3.GR.B2.23.09.04	DF	AA61GR	4450	71.912	0.336	3.84%	2	4	0.8582	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES		outlier removed bySD	FAL.3T3.SLS.23.09.04
FAL.3T3.GR.B3.14.10.04	DF	AA61GR	3190	51.618	0.319	3.58%	3	5	0.7925	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			FAL.3T3.SLS.14.10.04
BUSULFAN															
IIVS															
A1	RF	AA61RL	29.2	0.118	0.387	12.48%	1	6	0.8879	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A1
B1	DF	AA61RL	41.7	0.169	0.425	3.61%	3	5	0.8760	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.4	YES			SLS-B5
B2	DF	AA61RL	44.9	0.18	0.332	5.19%	5	3	0.8920	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES			SLS-B9
B3	DF	AA61RL	44.6	0.18	0.332	3.79%	4	4	0.8775	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES		plate sealer used	SLS-B10
ECBC															
AA61LH-A1	RF	AA61LH	97.3	0.395	0.360	3.64%	1	5	0.8554	1000, 100,10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-P4
AA61LH-B1	DF	AA61LH	57.3	0.233	0.293	3.70%	3	5	0.8885	500, 233, 108, 50.3, 23.4, 10.88, 5.06, 2.35	2.15	YES			SLS-P18
AA61LH-B2	DF	AA61LH	44.6	0.181	0.385	6.29%	3	5	0.8764	500, 233, 108, 50.3, 23.4, 10.88, 5.06, 2.35	2.15	YES			SLS-P20
AA61LH-B3	DF	AA61LH	19.4	0.079	0.463	0.58%	5	3	0.8778	500, 233, 108, 50.3, 23.4, 10.88, 5.06, 2.35	2.15	YES			SLS-P21
FRAME															
FAL.3T3.JE.A1.09/01/04	RF	AA61JE	38.7	0.156	0.677	5.72%	1	4	0.9065	250, 25, 2.5, 0.25, 0.025, 0.0025, 0.00025, 0.000025	10	RF	range finder		FAL.3T3.SLS.09/01/04
FAL.3T3.JE.A2.16/01/04	DF	AA61JE	528	2.145	0.597	9.65%	1	7	0.7176	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.3T3.SLS.16/01/04
FAL.3T3.JE.B1.23/01/04	DF	AA61JE	234	0.952	0.361	10.07%	1	6	0.9558	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		morphological changes seen at C5 but not noted in NRU	FAL3T3.23-01-04
FAL.3T3.JE.B2.30/01/04	DF	AA61JE	NA	NA	0.266	3.00%	0	6	NA	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	no points between 0-50; SD rejects this experiment	serious NR crystal problem; SD rejects this experiment	FAL.3T3.SLS.29/01/04
FAL.3T3.JE.B3.06-02-04	DF	AA61JE	202	0.819	0.308	7.09%	1	7	0.8537	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		possible NR crystals present; blanks slightly higher than usual	FAL.3T3.SLS.06/02/04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
CADMIUM II CHLORIDE															
IIVS															
A1	RF	AA61NK	0.462	0.003	0.442	2.10%	1	3	0.9959	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A1
B1	DF	AA61NK	1.31	0.007	0.325	0.39%	2	5	0.9811	3.0, 2.0, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES			SLS-B1
B2	DF	AA61NK	0.575	0.003	0.382	8.48%	3	4	0.9735	3.0, 2.0, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES			SLS-B2
B3	DF	AA61NK	0.529	0.003	0.407	1.25%	4	3	0.9907	3.0, 2.0, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	NO	PC failed		SLS-B3
B4	DF	AA61NK	0.565	0.003	0.336	4.71%	3	4	0.9832	3.0, 2.0, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES			SLS-B4
ECBC															
AA61KR-A1	RF	AA61KR	0.620	0.003	0.346	0.53%	0	0	0.9671	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	no points between 10 -90%; range finder		SLS-P4
AA61KR-B1	DF	AA61KR	0.514	0.003	0.542	7.85%	2	4	0.8434	2.0, 1.361, 0.926, 0.630, 0.428, 0.291, 0.198, 0.135	1.47	YES			SLS-P6
AA61KR-B2	DF	AA61KR	0.530	0.003	0.496	3.06%	3	4	0.9625	2.0, 1.361, 0.926, 0.630, 0.428, 0.291, 0.198, 0.135	1.47	YES			SLS-P7
AA61KR-B3	DF	AA61KR	0.406	0.002	0.389	3.87%	2	3	0.9474	2.0, 1.361, 0.926, 0.630, 0.428, 0.291, 0.198, 0.135	1.47	YES			SLS-P10
FRAME															
A1JP190603	RF	AA61JP	0.973	0.005	0.523	1.02%	1	0	0.9777	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		A1SLS190603
FAL.3T3.JP.B1 .26.06.03	RF	AA61JP	0.547	0.003	0.463	3.71%	1	2	0.9748	5.0, 3.4, 2.3, 1.5, 1.1, 0.7, 0.5, 0.3	1.47	YES			FAL.3T3.SLS.A2.26.06.03
FAL.3T3.JP.B2.03.07.03	DF	AA61JP	0.817	0.004	0.364	4.50%	1	1	0.9422	3.0, 2.04, 1.39, 0.94, 0.64, 0.44, 0.30, 0.20	1.47	NO	PC failed		FAL.3T3.SLS.B1.03.07.03
FAL.3T3.B3.JP.10.07.03	DF	AA61JP	0.343	0.002	0.484	1.25%	2	2	0.9894	3.0, 2.04, 1.39, 0.94, 0.64, 0.44, 0.30, 0.20	1.47	YES			FAL.3T3.SLS.10.07.03
FAL.3T3.B4.JP.17.07.03	DF	AA61JP	0.309	0.002	0.549	0.47%	2	2	0.9837	3, 2.04, 1.39, 0.94, 0.64, 0.44, 0.30, 0.20	1.47	YES			FAL.3T3.SLS.17.07.03
CAFFEINE															
IIVS															
A1	RF	AA61JM	176	0.905	0.439	6.89%	1	1	0.9381	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61JM	183	0.941	0.510	0.72%	4	4	0.9939	1000, 625, 391, 244, 153, 95.4, 59.6, 37.3	1.6	YES			SLS-B4
B2	DF	AA61JM	208	1.073	0.379	8.66%	4	4	0.9793	1000, 625, 391, 244, 153, 95.4, 59.6, 37.3	1.6	YES		outlier removed bySD	SLS-B7
B3	DF	AA61JM	183	0.944	0.452	1.60%	4	4	0.9857	1000, 625, 391, 244, 153, 95.4, 59.6, 37.3	1.6	YES			SLS-B8
ECBC															
AA61NU-A1	RF	AA61NU	119	0.613	0.457	8.10%	1	5	0.9548	10000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P3
AA61NU-B1	DF	AA61NU	130	0.668	0.469	0.04%	3	4	0.9366	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P17
AA61NU-B2	DF	AA61NU	148	0.760	0.539	2.01%	3	5	0.9798	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P19
AA61NU-B3	DF	AA61NU	122	0.631	0.543	0.37%	3	5	0.9791	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P22

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.GW.A1.09/01/04	RF	AA61GW	198	1.018	0.632	5.54%	1	6	0.8800	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.09/01/04
FAL.3T3.GW.A2.16.01.04 revised by NICEATM; bottom set to 0 as constant	DF	AA61GW	67.9	0.350	1.046	2.64%	6	2	0.9544	5000, 2325.6, 1081.7, 503.1, 234.0, 108.8, 50.6, 23.5	2.15	YES			FAL.3T3.SLS.16/01/04
FAL.3T3.GW.B1.23.01.04 revised by NICEATM; bottom set to 0 as constant	DF	AA61GW	228	1.174	0.562	0.19%	3	4	0.9827	5000, 1582, 501, 158, 50.1, 15.9, 5.02, 1.59	3.16	YES			FAL3T3.23-01-04
FAL.3T3.GW.B2.30.01.04	DF	AA61GW	NA	NA	0.315	1.72%	2	4	NA	5000, 1587, 504, 160, 51, 16, 5.1, 1.6	3.15	NO	SD rejects this experiment	serious NR crystal problem; SD rejects this experiment	FAL.3T3.SLS.29/01/04
FAL.3T3.GW.B3.06-02-04	DF	AA61GW	176	0.907	0.460	3.57%	3	5	0.9731	5000, 1587, 504, 160, 51, 16, 5.1, 1.6	3.15	YES			FAL.3T3.SLS.06/02/04
CARBAMAZEPINE															
IIVS															
A1	RF	AA61NB	NA	NA	0.281	6.74%	0	4	NA	10.0, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-A5
B1	DF	AA61NB	164	0.694	0.397	2.68%	0	8	0.5447	50.0, 38.5, 29.6, 22.8, 17.5, 13.5, 10.4, 7.97	1.3	NO	no points between 0 - 50%		SLS-B6
B2	DF	AA61NB	88.7	0.375	0.381	8.51%	6	1	0.9179	300, 250, 208, 174, 145, 121, 100, 83.7	1.2	YES		C1 data removed from Hill analyses; plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B3	DF	AA61NB	104	0.441	0.318	1.57%	3	5	0.9379	200, 154, 118, 91.0, 70.0, 53.9, 41.4, 31.9	1.3	YES			SLS-B13
B4	DF	AA61NB	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4 (should be B5)	DF	AA61NB	82.6	0.350	0.403	5.68%	4	4	0.9465	200, 154, 118, 91.0, 70.0, 53.9, 41.4, 31.9	1.3	YES			SLS-B15
ECBC															
AA61LX-A1	RF	AA61LX	88.9	0.376	0.438	9.61%	1	0	0.8266	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-16
AA61LX-B1	DF	AA61LX	93.8	0.397	0.601	3.55%	3	5	0.9413	250, 170, 116, 78.7, 53.5, 36.4, 24.8, 16.9	1.47	YES			SLS-P45
AA61LX-B2	DF	AA61LX	85.1	0.360	0.614	1.82%	2	6	0.9155	250, 170, 116, 78.7, 53.5, 36.4, 24.8, 16.9	1.47	YES			SLS-P46
AA61LX-B3	DF	AA61LX	70.1	0.297	0.314	9.73%	3	5	0.9105	170, 116, 78.7, 53.5, 36.4, 24.8, 16.8, 11.5	1.47	YES			SLS-P48
FRAME															
FAL.3T3.HD.A1.21.10.04	RF	AA61HD	107	0.451	0.190	1.01%	1	1	0.9436	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	outliers removed by SD; ppt in 1X C1 and 2X C1	FAL.3T3.SLS.21.10.04
FAL.3T3.HD.B1.11.11.04	DF	AA61HD	217	0.917	0.217	2.35%	2	1	0.7684	1000, 318, 101, 32.0, 10.2, 3.2, 1.0, 0.3	3.15	YES		ppt in 2X C1-C2; &1X in C1	FAL.3T3.SLS.10.11.04
FAL.3T3.HD.B3.18.11.04 (should be B2 and 19.11.04)	DF	AA61HD	130	0.550	0.237	4.35%	3	2	0.9861	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		ppt in 2X C1-C2 & 1X in C1-C2	FAL.3T3.SLS.19.11.04
FAL.3T3.HD.B3.18.11.04	DF	AA61HD	110	0.466	0.241	0.24%	3	1	0.9107	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		ppt in 2X C1-C2 & 1X in C1-C3;	FAL.3T3.SLS.25.11.04
CARBON TETRACHLORIDE															
IIVS															
A1	RF	AA61JK	NA	NA	0.349	15.06%	0	2	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C3	SLS-A2
B1	DF	AA61JK	NA	NA	0.391	6.82%	0	5	NA	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	NO	no points between 0-50%	ppt in 2X C1-C3	SLS-B6

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B2	DF	AA61JK	NA	NA	0.394	7.97%	0	1	NA	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	NO	no points between 0-50%	ppt in 2X C1-C8; no toxicity detected	SLS-B9
B3	DF	AA61JK	NA	NA	0.368	3.76%	0	5	NA	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	NO	no points between 0-50%	ppt in 2X C1-C8; some toxicity detected; C1 has lower toxicity than C2	SLS-B10
ECBC															
AA61NZ-A1	RF	AA61NZ	NA	NA	0.328	16.23%	0	6	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P9
AA61NZ-A2	RF	AA61NZ	NA	NA	0.419	7.89%	0	1	NA	3000, 300, 30, 3, 0.3, 0.03, 0.003, 0.0003	10	RF	range finder		SLS-P19
AA61NZ-B1	DF	AA61NZ	NA	NA	0.416	2.67%	0	8	NA	4500, 3719, 3074, 2540, 2099, 1735, 1434, 1185	1.21	NO	no points between 0 - 50%		SLS-P65
AA61NZ-B2	DF	AA61NZ	NA	NA	0.567	3.83%	0	7	NA	7000, 5785, 4781, 3951, 3266, 2699, 2230, 1843	1.21	NO	no points between 0 - 50%	dilution factor is 1.21; no points between 0-50%; test would pass due to dilution factor	SLS-P67
AA61NZ-B3	DF	AA61NZ	NA	NA	0.536	8.16%	0	7	NA	7000, 5785, 4781, 3951, 3266, 2699, 2230, 1843	1.21	NO	no points between 0 - 50%	ppt in 2X C1 - C5; oily	SLS-P73
FRAME															
FAL.3T3.HC.A1.30/04/04	RF	AA61HC	NA	NA	0.179	1.46%	0	1	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.3T3.SLS.30/04/04
FAL.3T3.HC.B1.06/05/0404	DF	AA61HC	NA	NA	0.218	2.75%	0	0	NA	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 0 - 100%	SD ends testing and returns as non-toxic at 2500ug/ml	FAL.3T3.SLS.06/05/04
FAL.3T3.HC.B2.26.11.04	DF	AA61HC	NA	NA	0.253	12.16%	0	5	NA	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 0 - 50%	the toxicity curve appears reversed; higher conc. less toxic than lower conc.	FAL.3T3.SLS.26.11.04
FAL.3T3.HC.B3.03.12.04	DF	AA61HC	2430	15.776	0.179	3.22%	1	0	0.5412	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 50 - 100%		FAL.3T3.SLS.03.12.04
FAL.3T3.HC.B4.09.12.04	DF	AA61HC	NA	NA	0.286	7.61%	0	6	NA	2500, 2066, 1708, 1411, 1166, 964, 797, 658	1.21	NO	no points between 0 - 50%		FAL.3T3.SLS.09.12.04

CHLORAL HYDRATE

IIVS															
A1	RF	AA61FJ	56.2	0.340	0.469	87.75%	2	4	0.9868	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	VC1 ODs < VC2 ODs; VC1 removed from subsequent analysis; volatility issues.	SLS-A2
B1	DF	AA61FJ	156	0.943	0.509	2.80%	2	6	0.9655	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES			SLS-B4
B2	DF	AA61FJ	193	1.165	0.336	2.36%	2	5	0.9653	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES		outliers removed by SD; plate sealer used	SLS-B7
B3	DF	AA61FJ	162	0.981	0.447	5.20%	2	6	0.9613	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES		plate sealer used	SLS-B8
ECBC															
AA61KB-A1	RF	AA61KB	NA	NA	0.189	94.19%	3	0	NA	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder	probable volatility problem; VC1 <<< VC2	SLS-P6
AA61KB-A2	RF	AA61KB	107	0.648	0.295	0.25%	0	1	NA	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-P7
AA61KB-B1	DF	AA61KB	160	0.965	0.474	0.63%	3	5	0.9590	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			SLS-P22
AA61KB-B2	DF	AA61KB	160	0.969	0.703	2.49%	3	5	0.9682	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			SLS-P24
AA61KB-B3	DF	AA61KB	133	0.806	0.588	0.17%	3	5	0.9604	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			SLS-P26
FRAME															

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.LK.A1.01/04/04	RF	AA61LK	711	4.300	0.271	69.44%	2	0	0.2684	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	possible volatility problem	FAL.3T3.SLS.01/04/04
FAL.3T3.LK.B1.29/04/04	DF	AA61LK	243	1.470	0.287	5.23%	2	2	0.9262	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		NR crystals; high background	FAL.3T3.SLS.29/04/04
FAL.3T3.LK.B2.06/05/04	DF	AA61LK	265	1.605	0.313	8.07%	4	4	0.9706	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			FAL.3T3.SLS.06/05/04
FAL.3T3.LK.B3.20/05/04	DF	AA61LK	1450	8.739	0.347	12.28%	0	1	0.9010	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	NO	no points between 0 - 50%	curve very different compared to other curves	FAL.3T3.SLS.20/05/04
FAL.3T3.LK.B4.27/05/04	DF	AA61LK	215	1.302	0.412	4.39%	4	4	0.9575	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			FAL.3T3.SLS.27/05/04
CHLORAMPHENICOL															
IIVS															
A1	RF	AA61GJ	98.9	0.306	0.323	27.15%	1	1	0.1298	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A1
B1	DF	AA61GJ	187	0.579	0.307	4.85%	2	6	0.9661	558, 310, 172, 95.7, 53.2, 29.5, 16.4, 9.11	1.8	YES			SLS-B1
B2	DF	AA61GJ	148	0.458	0.421	0.91%	3	5	0.9649	558, 310, 172, 95.7, 53.2, 29.5, 16.4, 9.11	1.8	YES			SLS-B2
B3	DF	AA61GJ	142	0.439	0.428	0.12%	3	5	0.9668	558, 310, 172, 95.7, 53.2, 29.5, 16.4, 9.11	1.8	NO	PC failed		SLS-B3
B3 with plate cover	DF	AA61GJ	171	0.529	0.345	4.49%	2	5	0.9683	558, 310, 172, 95.7, 53.2, 29.5, 16.4, 9.11	1.8	NO	PC failed		SLS-B3
B4	DF	AA61GJ	133	0.412	0.350	3.69%	3	5	0.9171	593, 329, 183, 102, 56.5, 31.4, 17.4, 9.69	1.8	YES			SLS-B4
ECBC															
AA61JS-A1	RF	AA61JS	54.5	0.169	0.401	14.20%	1	4	0.7119	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	low r2; range finder		SLS-P4
AA61JS-B1	DF	AA61JS	88.5	0.274	0.440	20.33%	2	3	0.8484	1000, 300, 100, 30, 10, 3, 1, 0.3	3.33	NO	% VC difference > 15; range finder		SLS-P6
AA61JS-B2	DF	AA61JS	39.1	0.121	0.461	1.90%	2	4	0.9618	1000, 465.1, 216.3, 100.6, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P7
AA61JS-B3	DF	AA61JS	61.1	0.189	0.395	1.46%	3	4	0.8537	1000, 465.1, 216.3, 100.6, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P10
AA61JS-B4	DF	AA61JS	55.1	0.171	0.504	2.80%	3	4	0.9541	1000, 465.1, 216.3, 100.6, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P14
AA61JS-B5	DF	AA61JS	68.5	0.212	0.448	5.20%	3	4	0.9401	1000, 465.1, 216.3, 100.6, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P15
FRAME															
A1MU190603	RF	AA61MU	568	1.758	0.550	1.44%	1	0	0.9021	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	PC failed; no points between 50 - 90%; range finder		A1SLS190603
FAL.3T3.MU.B1.26.06.03	DF	AA61MU	276	0.854	0.491	13.66%	5	3	0.8425	1500, 1020, 690, 470, 320, 220, 150, 100	1.47	YES			FAL.3T3.SLS.A2.26.06.03
FAL.3T3.MU.B2.03.07.03	DF	AA61MU	520	1.609	0.306	3.63%	2	2	0.8810	1250, 580, 270, 125, 58.5, 27.2, 12.6, 5.9	2.15	NO	PC failed		FAL.3T3.SLS.B1.03.07.03
FAL.3T3.B3.MU.10.07.03	DF	AA61MU	NA	NA	0.486	1.00%	0	2	NA	50.0, 34.0, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	NO	no points between 10 - 50%		FAL.3T3.SLS.10.07.03
FAL.3T3.B3.MU.17.07.03 (should be B4?)	DF	AA61MU	237	0.733	0.455	1.91%	3	2	0.9782	2500, 1160, 540, 251, 117, 54.4, 25.3, 11.7	2.15	YES			FAL.3T3.SLS.17.07.03
FAL.3T3.B4.MU.25.07.03 (should be B5?)	DF	AA61MU	385	1.191	0.379	0.65%	2	2	0.9291	2500, 1160, 540, 251, 117, 54.4, 25.3, 11.7	2.15	YES			FAL.3T3.SLS.25.07.03
FAL.3T3.B5.MU.070803 (should be B6?)	DF	AA61MU	64.4	0.199	0.721	1.63%	4	4	0.8501	2500, 1160, 540, 251, 117, 54.4, 25.3, 11.7	2.15	NO	PC failed		FAL.3T3.SLS.070803
FAL.3T3.MU.B7.120903	DF	AA61MU	193	0.597	0.363	0.80%	4	4	0.9490	2500, 1162, 540, 251, 117, 54.4, 25.3, 11.7	2.15	YES			FAL.3T3.SLS.120903

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
CITRIC ACID															
IIVS															
A1	RF	AA61MH	1030	5.376	0.363	7.76%	1	2	0.8924	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61MH	681	3.54	0.389	3.15%	2	3	0.9722	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES			SLS-B1
B2	DF	AA61MH	942	4.90	0.379	5.58%	1	4	0.9742	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES			SLS-B2
B3	DF	AA61MH	971	5.05	0.381	1.66%	1	4	0.9858	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES			SLS-B3
ECBC															
AA61HH-A1	RF	AA61HH	409	2.130	0.341	0.32%	2	5	0.9275	10000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1	SLS-P1
AA61HH-B1	DF	AA61HH	598	3.115	0.299	2.62%	4	4	0.9879	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES		ppt in 1X C1	SLS-P5
AA61HH-B2	DF	AA61HH	325	1.692	0.418	9.20%	4	2	0.9800	4651, 2163, 1006, 468, 218, 101, 47.1, 21.9	2.15	YES		ppt in 1X C1	SLS-P8
AA61HH-B3	DF	AA61HH	497	2.585	0.423	1.95%	3	5	0.9732	4651, 2163, 1006, 468, 218, 101, 47.1, 21.9	2.15	YES			SLS-P17
FRAME															
FAL.3T3.RB.A1.08/01/04	RF	AA61RB	1050	5.489	0.557	1.11%	1	1	0.8824	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.080104
FAL3T3.RB.A2.15-01-04	DF	AA61RB	668	3.479	0.730	5.04%	4	4	0.9467	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	NO	PC failed;		FAL.3T3.SLS.15/01/04
FAL3T3.RB.B1.22-01-04	DF	AA61RB	1080	5.617	0.411	1.38%	3	2	0.9403	5000, 2325.6, 1081.7, 503.1, 234.0, 108.8, 50.6, 23.5	2.15	YES			FAL.3T3.SLS.22/01/04
FAL3T3.RB.B2.29-01-04	DF	AA61RB	1050	5.476	0.423	12.11%	4	4	0.9575	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	YES		pH 3 fpr C1; SD suggests high pH may be cause of toxicity for this concentration;	FAL3T3.SLS.29-01-04
FAL3T3.RB.B3.05.02.04	DF	AA61RB	345	1.797	0.344	4.03%	7	0	0.8104	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	NO	PC failed; no points between 50-100	problem with reservoir liners; SD incorrectly determined 4 points between 50-100 instead of 0 points	FAL.3T3.SLS.5/02/04
FAL3T3.RB.25-02-04	DF	AA61RB	1100	5.721	0.481	11.65%	4	4	0.9805	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	YES		definitive test B4	FAL3T3.SLS.25.02.04
FAL3T3.RB.B5.17.03.04	DF	AA61RB	1360	7.087	0.304	6.25%	2	2	0.9139	5000, 2325.6, 1081.7, 503.1, 234.0, 108.8, 50.6, 23.5	2.15	YES			FAL.3T3.SLS.17/03/04
COLCHICINE															
IIVS															
A1	RF	AA61FL	0.027	0.0001	0.514	1.69%	5	1	0.9699	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61FL	0.028	0.0001	0.416	3.16%	4	4	0.9768	0.1, 0.067, 0.044, 0.030, 0.020, 0.013, 0.0088, 0.0059	1.49	YES			SLS-B1
B2	DF	AA61FL	0.028	0.0001	0.527	2.34%	4	4	0.9809	0.1, 0.067, 0.044, 0.030, 0.020, 0.013, 0.0088, 0.0059	1.49	YES			SLS-B2
B3	DF	AA61FL	0.037	0.0001	0.578	6.33%	3	2	0.9522	0.1, 0.067, 0.044, 0.030, 0.020, 0.013, 0.0088, 0.0059	1.49	NO	PC failed		SLS-B3

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B4	DF	AA61FL	0.028	0.0001	0.406	0.86%	4	2	0.9508	0.1, 0.067, 0.044, 0.030, 0.020, 0.013, 0.0088, 0.0059	1.49	YES			SLS-B4
ECBC															
AA61JZ-A1	RF	AA61JZ	0.008	0.0000	0.369	3.91%	2	0	0.9383	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	PC failed; no points between 50 - 90%; range finder		SLS-P2
AA61JZ-B2	DF	AA61JZ	0.023	0.0001	0.595	8.49%	6	2	0.8811	0.200, 0.136, 0.093, 0.063,0.043, 0.029, 0.020, 0.013	1.47	YES			SLS-P8
AA61JZ-B3	DF	AA61JZ	0.018	0.0001	0.494	0.43%	6	2	0.9020	0.136, 0.093, 0.063, 0.043, 0.029, 0.020, 0.013, 0.009	1.47	YES			SLS-P9
AA61JZ-B4	DF	AA61JZ	0.019	0.0001	0.549	0.68%	4	2	0.9658	0.136, 0.093, 0.063, 0.043, 0.029, 0.020, 0.013, 0.009	1.47	YES			SLS-P12
AA61JZ-B5	DF	AA61JZ	0.022	0.0001	0.664	1.90%	6	1	0.9584	0.136, 0.093, 0.063, 0.043, 0.029, 0.020, 0.013, 0.009	1.47	YES			SLS-P13
FRAME															
FAL.3T3.A1.NW.200603	RF	AA61NW	0.088	0.0003	0.699	5.16%	6	0	0.4881	10000,1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	no points between 50 - 90%; low r2; range finder		FAL.3T3.SLS2.A1.200603
FAL.3T3.A2.NW.27.06.03	RF	AA61NW	NA	NA	0.519	0.16%	8	0	0.2194	2.50, 1.16, 0.54, 0.25, 0.12, 0.05, 0.025, 0.012	2.15	RF	no points between 50 - 90%; low r2		FAL.3T3.SLS.A2.27.06.03
FAL.3T3.B1.NW.04.07.03	DF	AA61NW	0.184	0.0006	0.503	2.71%	5	1	0.7952	2.50, 1.16, 0.54, 0.25, 0.12, 0.05, 0.025, 0.012	2.15	NO	PC failed; low r2		FAL.3T3.SLS.04.07.03
FAL.3T3.B2.NW.11.07.03	DF	AA61NW	0.046	0.0001	0.532	4.41%	6	2	0.8093	2.50, 1.16, 0.54, 0.25, 0.12, 0.05, 0.025, 0.012	2.15	YES			FAL.3T3.SLS.11.07.03
FAL.3T3.B3.NW.18.07.03 (recalculated to fit bottom)	DF	AA61NW	0.127	0.0004	0.481	5.60%	5	2	0.8882	5.00, 2.33, 1.08, 0.50, 0.234, 0.109, 0.051, 0.024	2.15	YES			FAL.3T3.SLS.18.07.03
FAL.3T3.B5.NW.25.07.03	DF	AA61NW	0.106	0.0003	0.397	3.23%	5	3	0.8590	2.50, 1.16, 0.54, 0.25, 0.12, 0.05, 0.025, 0.012	2.15	YES			FAL.3T3.SLS.25.07.03
CUPRIC SULFATE PENTAHYDRATE															
IIVS															
A1	RF	AA61LA	4.02	0.016	0.496	4.40%	2	5	0.9647	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A2
B1	DF	AA61LA	4.26	0.017	0.395	23.78%	3	1	0.6017	10.0, 7.14, 5.10, 3.64, 2.60, 1.86, 1.33, 0.949	1.4	NO	% VC difference > 15	excessive variability within treatment and cotrol groups	SLS-B4
B2	DF	AA61LA	4.58	0.018	0.463	1.04%	3	3	0.9765	10.0, 7.14, 5.10, 3.64, 2.60, 1.86, 1.33, 0.949	1.4	YES			SLS-B6
B3	DF	AA61LA	4.84	0.019	0.418	0.86%	3	3	0.9887	10.0, 7.14, 5.10, 3.64, 2.60, 1.86, 1.33, 0.949	1.4	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B4	DF	AA61LA	7.73	0.031	0.375	2.20%	1	2	0.8726	10.0, 7.14, 5.10, 3.64, 2.60, 1.86, 1.33, 0.949	1.4	YES			SLS-B12
ECBC															
AA61HX-A1	RF	AA61HX	50.7	0.203	0.461	2.51%	2	1	0.9661	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-P8
AA61HX-B1	DF	AA61HX	86.3	0.346	0.604	0.57%	3	3	0.9913	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		ppt in 2X C1	SLS-P26
AA61HX-B2	DF	AA61HX	81.7	0.327	0.668	3.02%	3	3	0.9623	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	YES			SLS-P28
AA61HX-B3	DF	AA61HX	80.2	0.321	0.447	5.54%	5	3	0.9336	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	YES			SLS-P29
FRAME															

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.LP.A1.21.05.04	RF	AA61LP	85.9	0.344	0.266	1.47%	2	0	0.5809	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points 50 - 100%		FAL.3T3.SLS.21.05.04
FAL.3T3.LP.B1.04.06.04	DF	AA61LP	99.1	0.397	0.415	9.90%	6	1	0.9314	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			FAL.3T3.SLS.04.06.04
FAL.3T3.LP.B2.17/06/04	DF	AA61LP	204	0.816	0.492	0.27%	3	1	0.9641	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			FAL.3T3.SLS.17.06.04
FAL.3T3.LP.B3.09.07.04	DF	AA61LP	106	0.425	0.408	0.31%	5	0	0.9552	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	NO	no points between 50 - 100%		FAL.3T3.SLS.09.07.04
FAL.3T3.LP.B4.14.10.04	DF	AA61LP	101	0.404	0.304	1.01%	3	0	0.9749	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	NO	no points between 50 - 100%		FAL.3T3.SLS.14.10.04
FAL.3T3.LP.B5.15.10.04	DF	AA61LP	138	0.552	0.303	7.50%	4	0	0.9352	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	NO	no points between 50 - 100%	outlier removed bySD	FAL.3T3.SLS.15.10.04
FAL.3T3.LP.B6.21.10.04	DF	AA61LP	NA	NA	0.284	10.97%	7	0	0.0000	500, 413, 342, 282, 233, 193, 159, 132	1.21	NO	no points between 50 - 100%		FAL.3T3.SLS.21.10.04
FAL.3T3.LP.B7.28.10.04	DF	AA61LP	91.8	0.368	0.211	3.94%	4	1	0.9658	250, 207, 171, 141, 117, 96.4, 79.7, 65.8	1.21	YES			FAL.3T3.SLS.28.10.04
FAL.3T3.LP.B8.04.11.04	DF	AA61LP	97.9	0.392	0.329	2.47%	5	2	0.9464	250, 207, 171, 141, 117, 96.4, 79.7, 65.8	1.21	YES		outlier removed bySD	FAL.3T3.SLS.04.11.04
CYCLOHEXIMIDE															
IIVS															
A1	RF	AA61GL	0.0873	0.0003	0.403	1.70%	5	1	0.9733	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A4
B1	DF	AA61GL	0.101	0.0004	0.500	4.13%	6	2	0.9567	2.00, 1.11, 0.617, 0.343, 0.191, 0.106, 0.059, 0.033	1.8	YES			SLS-B4
B2	DF	AA61GL	0.136	0.0005	0.363	2.02%	5	3	0.9053	2.00, 1.11, 0.617, 0.343, 0.191, 0.106, 0.059, 0.033	1.8	YES		outlier removed bySD	SLS-B7
B3	DF	AA61GL	0.0887	0.0003	0.444	0.43%	6	2	0.9577	2.00, 1.11, 0.617, 0.343, 0.191, 0.106, 0.059, 0.033	1.8	YES			SLS-B8
ECBC															
AA61KK-A1	RF	AA61KK	0.102	0.0004	0.377	10.52%	5	0	0.9586	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P13
AA61KK-B1	DF	AA61KK	0.11	0.0004	0.659	9.97%	5	3	0.9666	3.00, 1.40, 0.649, 0.302,0.140, 0.065, 0.030, 0.014	2.15	YES			SLS-P37
AA61KK-B2	DF	AA61KK	0.0767	0.0003	0.412	3.79%	5	3	0.9698	3.00, 1.40, 0.649, 0.302,0.140, 0.065, 0.030, 0.014	2.15	YES			SLS-P40
AA61KK-B3	DF	AA61KK	0.187	0.0007	0.553	9.02%	4	4	0.9535	3.00, 1.40, 0.649, 0.302,0.140, 0.065, 0.030, 0.014	2.15	YES			SLS-P41
FRAME															
FAL.3T3.PF.A1.10.09.04	RF	AA61PF	1.89	0.0067	0.435	1.28%	4	2	0.9465	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.10.09.04
FAL.3T3.PF.B1.16.09.04	DF	AA61PF	0.0796	0.0003	0.334	6.16%	8	0	0.9819	465, 148, 46.9, 14.9, 4.7, 1.5, 0.476, 0.151	3.15	NO	no points between 50 - 100%		FAL.3T3.SLS.16.09.04
FAL.3T3.PF.B2.15.10.04	DF	AA61PF	1.12	0.0040	0.333	0.40%	4	2	0.8800	50.0, 15.9, 5.04, 1.60, 0.508, 0.161, 0.0512, 0.0162	3.15	YES			FAL.3T3.SLS.15.10.04
FAL.3T3.PF.28.10.04	DF	AA61PF	0.00946	0.0000	0.272	2.42%	8	0	0.9126	50.0, 15.9, 5.04, 1.60, 0.508, 0.161, 0.0512, 0.0162	3.15	NO	no points between 0 - 50%	outlier removed bySD	FAL.3T3.SLS.28.10.04
FAL.3T3.PF.B4.04.11.04	DF	AA61PF	0.221	0.0008	0.282	7.83%	5	2	0.9566	50.0, 15.9, 5.04, 1.60, 0.508, 0.161, 0.0512, 0.0162	3.15	YES			FAL.3T3.SLS.04.11.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.PF.B5.11.11.04	DF	AA61PF	0.601	0.0021	0.266	5.33%	4	2	0.9235	50.0, 15.9, 5.04, 1.60, 0.508, 0.161, 0.0512, 0.0162	3.15	YES		outlier removed bySD	FAL.3T3.SLS.10.11.04
DIBUTYL PHTHALATE															
IIVS															
A1	RF	AA61FD	13.5	0.048	0.371	2.38%	2	1	0.9701	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2 and 1X C1-C2	SLS-A3
B1	DF	AA61FD	19.5	0.070	0.474	7.57%	4	2	0.9692	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 2X C1 and 1X C1	SLS-B5
B2	DF	AA61FD	20.4	0.073	0.393	4.01%	4	4	0.9786	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 2X C1-C4; ppt in 1X C1	SLS-B9
B3	DF	AA61FD	22.2	0.080	0.338	1.43%	4	4	0.9749	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 1X C1-C2	SLS-B10
ECBC															
AA61JX-A1	RF	AA61JX	127	0.458	0.245	7.41%	0	2	0.9266	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1 and 1X C1; higher than usual blank OD	SLS-P10
AA61JX-B1	DF	AA61JX	NA	NA	0.643	4.39%	NA	NA	NA	300, 204, 139, 94.4, 64.2, 43.7, 29.7, 20.2	1.47	NO	odd toxicity curve; couldn't accurately calculate ICx values	toxicity curve goes up at the higher concentrations	SLS-P44
AA61JX-B2	DF	AA61JX	NA	NA	0.551	4.45%	N/A	N/A	NA	300, 204, 139, 94.4, 64.2, 43.7, 29.7, 20.2	1.47	NO	odd toxicity curve; couldn't accurately calculate ICx values	toxicity curve goes up at the higher concentrations	SLS-P46
AA61JX-B3	DF	AA61JX	NA	NA	0.627	6.38%	0	8	NA	60.0, 40.8, 27.8, 18.9, 12.8, 8.74, 5.95, 4.05	1.47	NO	PC failed; no points between 0 - 50%		SLS-P60
AA61JX-B4	DF	AA61JX	19.8	0.071	0.491	8.85%	5	3	0.8450	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES			SLS-P63
AA61JX-B5	DF	AA61JX	27.7	0.099	0.442	3.39%	3	5	0.7470	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES		1X C1-C4 have small globules; highest conc. (C7, C8) less toxicity than C3-C6	SLS-P67
AA61JX-B6	DF	AA61JX	22.9	0.082	0.342	4.56%	4	3	0.9178	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES		1X C1-C4 have small globules; highest conc. (C7, C8) less toxicity than C3-C4	SLS-P69
FRAME															
FAL.3T3.MK.A1.21.05.04	RF	AA61MK	104	0.372	0.225	1.44%	1	1	0.7617	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1-C2	FAL.3T3.SLS.21.05.04
FAL.3T3.MK.B1.04.06.04	DF	AA61MK	306	1.100	0.429	4.08%	3	5	0.8027	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		ppt in 1X C1-C8 and 2X C1-C2	FAL.3T3.SLS.04.06.04
FAL.3T3.MK.B2.17.06.04	DF	AA61MK	74.6	0.268	0.410	0.20%	5	3	0.9555	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 1X C1-C4 and 2X C1-C2	FAL.3T3.SLS.17.06.04
FAL.3T3.MK.B3.09.07.04	DF	AA61MK	190	0.683	0.304	0.47%	4	4	0.9592	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES		ppt in 1X C1-C8 and 2X C1-C2	FAL.3T3.SLS.09.07.04
FAL.3T3.MK.B4.25.11.04	DF	AA61MK	192	0.689	0.319	2.64%	3	5	0.9167	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES		ppt in 2X C1; ppt in 1X C1-C3;	FAL.3T3.SLS.25.11.04
DICHLORVOS															
IIVS															
A1	RF	AA61NP	8.66	0.039	0.341	83.42%	1	1	0.9677	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	VC1 Ods < VC2 ODs; VC1 removed from subsequent analysis; volatility issues.	SLS-A3
B1	DF	AA61NP	16.9	0.076	0.347	8.46%	3	5	0.9602	70.0, 38.9, 21.6, 12.0, 6.67, 3.70, 2.06, 1.14	1.8	YES			SLS-B1
B2	DF	AA61NP	17.3	0.078	0.321	0.23%	3	3	0.9593	70.0, 38.9, 21.6, 12.0, 6.67, 3.70, 2.06, 1.14	1.8	YES			SLS-B2

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B3	DF	AA61NP	20.7	0.093	0.366	4.92%	3	2	0.9733	70.0, 38.9, 21.6, 12.0, 6.67, 3.70, 2.06, 1.14	1.8	YES			SLS-B3
ECBC															
AA61PZ-A1	RF	AA61PZ	NA	NA	0.121	98.38%	3	0	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	probable volatility problem; VC1 <<< VC2; higher than usual blank OD	SLS-P10
AA61PZ-A2 (sealer)	RF	AA61PZ	13.7	0.062	0.473	4.53%	0	5	0.9461	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P43
AA61PZ-B1 (sealer)	DF	AA61PZ	NA	NA	0.242	11.12%	3	4	NA	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	PC failed		SLS-P50
AA61PZ-B2 (sealer)	DF	AA61PZ	NA	NA	0.256	6.09%	4	4	NA	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	PC failed		SLS-P52
AA61PZ-B3 (sealer)	DF	AA61PZ	12.1	0.055	0.503	12.85%	2	5	0.9711	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P54
AA61PZ-B4 (sealer)	DF	AA61PZ	NA	NA	0.322	25.27%	2	5	NA	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	PC failed; % VC difference > 15		SLS-P56
AA61PZ-B5 (sealer)	DF	AA61PZ	5.90	0.027	0.298	7.56%	3	4	0.9166	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P58
AA61PZ-B6 (sealer)	DF	AA61PZ	11.1	0.050	0.421	3.01%	3	4	0.9466	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	PC failed		SLS-P60
AA61PZ-B7 (sealer)	DF	AA61PZ	11.5	0.052	0.347	2.26%	2	5	0.9275	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		C1 conc. seems to interact with NR; toxicity curve going in opposite direction at this point	SLS-P62
FRAME															
FAL.3T3.HS.A1.21.05.04	RF	AA61HS	57.8	0.262	0.119	90.83%	2	0	0.1864	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; % VC difference > 15; no points between 50 - 100%	volatility problem	FAL.3T3.SLS.21.05.04
FAL.3T3.HS.B1.04.06.04	DF	AA61HS	35.0	0.158	0.371	5.60%	3	3	0.9832	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			FAL.3T3.SLS.04.06.04
FAL.3T3.HS.B2.18.06.04	DF	AA61HS	30.9	0.140	0.685	1.85%	3	4	0.9772	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			FAL.3T3.SLS.18.06.04
FAL.3T3.HS.B3.08.07.04	DF	AA61HS	32.5	0.147	0.209	11.98%	2	2	0.9328	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			FAL.3T3.SLS.08.07.04

DIETHYL PHTHALATE

IIVS															
A1	RF	AA61NX	276	1.242	0.232	5.28%	1	1	0.1408	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	the solvent controls treated with 1% DMSO instead of 0.5%	SLS-A4
B1	DF	AA61NX	135	0.607	0.369	9.08%	3	2	0.9536	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 2X C1-C2	SLS-B5
B2	DF	AA61NX	97.1	0.437	0.338	6.11%	4	3	0.9853	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 2X C1-C2	SLS-B9
B3	DF	AA61NX	87.1	0.392	0.342	4.96%	5	3	0.9870	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 2X C1-C3	SLS-B10
ECBC															
AA61GA-A1	RF	AA61GA	115	1.086	0.230	6.44%	1	1	0.9260	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P12
AA61GA-B1	DF	AA61GA	119	0.536	0.323	6.29%	4	4	0.9776	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P32
AA61GA-B2	DF	AA61GA	68.1	0.306	0.324	4.70%	5	3	0.9414	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P34
AA61GA-B3	DF	AA61GA	69.5	0.313	0.552	0.35%	5	3	0.9527	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P36
FRAME															
FAL.3T3.KZ.A1.10.09.04	RF	AA61KZ	148	0.666	0.259	12.82%	1	2	0.7507	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	FAL.3T3.SLS.10.09.04
FAL.3T3.KZ.B1.16.09.04	DF	AA61KZ	176	0.791	0.239	15.05%	3	3	0.9712	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		outlier removed bySD; ppt in 2X C1-C2	FAL.3T3.SLS.16.09.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.KZ.B2.15.10.04	DF	AA61KZ	160	0.720	0.244	1.62%	3	5	0.9759	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		outlier removed by SD	FAL.3T3.SLS.15.10.04
FAL.3T3.KZ.B3.28.10.04	DF	AA61KZ	104	0.469	0.185	4.87%	3	3	0.9759	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		ppt in 2X C1-C2	FAL.3T3.SLS.28.10.04
DIGOXIN															
IIVS															
A1	RF	AA61MF	310	0.398	0.350	0.21%	1	1	0.9022	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	*ppt in 2X C1 and in 1X C1*	SLS-A3
B1	DF	AA61MF	269	0.344	0.427	6.41%	1	3	0.8853	1000, 588, 346, 204, 120, 70.4, 41.4, 24.4	1.7	YES		ppt in 1X C1-C3 & 2X C1; SD removed C1 & C2 from PRISM to get Hill analysis; ppt in 1X C1 and C2 caused upswing in the toxicity curve	SLS-B5
B2	DF	AA61MF	NA	NA	0.308	9.13%	0	3	NA	400, 267, 178, 119, 79.0, 52.7, 35.1, 23.4	1.5	NO	no points between 0 - 50%	ppt in 1X C1	SLS-B13
B3	DF	AA61MF	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61MF	365	0.467	0.296	2.70%	0	4	0.5436	1000, 556, 309, 171, 95, 53, 29.4, 16.3	1.8	YES		ppt in 2X C1 and 1X C1-C2; SD removed C1 & C2 from PRISM analyses; no points left between 0-50% viability; SD accepts test	SLS-B15
B5	DF	AA61MF	1500	1.925	0.335	4.20%	0	4	0.3342	1000, 556, 309, 171, 95, 53, 29.4, 16.3	1.8	NO	no points between 0 - 50%	ppt in 2X C1; ppt in 1X C1-C2; SD ends testing of chemical; solubility limits have been reached	SLS-B16
ECBC															
AA61PP-A1	RF	AA61PP	123	0.157	0.238	3.43%	1	4	0.8888	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1 and 2X C1; higher than usual blank OD	SLS-P10
AA61PP-B1	DF	AA61PP	NA	NA	0.344	6.81%	0	6	NA	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	NO	no points between 0 - 50%	crystals in !x C1-C2; not like NR crystals	SLS-P40
AA61PP-B2	DF	AA61PP	475	0.609	0.463	6.42%	2	3	0.8877	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	YES		crystals in 1X C1-C2; not like NR crystals; C1 toxicity less than C2	SLS-P42
AA61PP-B3	DF	AA61PP	204	0.261	0.452	4.45%	5	3	0.6366	1000, 680, 463, 315, 214, 146, 99, 67	1.47	YES		ppt in 2X C1; ppt in 1X C1-C5 (large chemical crystals in wells);	SLS-P72
AA61PP-B4	DF	AA61PP	373	0.478	0.452	10.52%	2	6	0.6692	1000, 680, 463, 315, 214, 146, 99, 67	1.47	YES		ppt in 2X C1-C2; ppt in 1X C1-C4	SLS-P74
FRAME															
FAL.3T3.HN.A1.27/05/04	RF	AA61HN	918	1.176	0.381	7.78%	1	0	0.6117	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points 50 - 100%	ppt in 1X C1-C3	FAL.3T3.SLS.27/05/04
FAL.3T3.HN.B1.04/06/04 FAULT	DF	AA61HN	873	1.118	0.419	6.70%	1	2	0.8308	750, 347, 162, 75.0, 35.0, 16.3, 7.6, 3.5	2.15	YES		ppt in 1X C1-C3; dilution factor not provided	FAL.3T3.SLS.04.06.04
FAL.3T3.HN.B2.18/06/04	RF	AA61HN	387	0.496	0.451	4.84%	NA	NA	0.5399	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	can't determine which points are true toxicity points	ppt in 2X C1 and ppt in 1X C1-C3	FAL.3T3.SLS.18.06.04
FAL.3T3.HN.B3.09/07/04	DF	AA61HN	75900	97.141	0.317	1.92%	0	6	0.8417	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.1	2.15	NO	no points between 0 - 50%	ppt in 2X C1 and ppt in 1X C1-C5	FAL.3T3.SLS.09.07.04
FAL.3T3.HN.B4.16/07/04	DF	AA61HN	NA	NA	0.262	0.86%	0	4	0.3528	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	NO	no points between 0 - 50%	ppt in 2X C1-C2 and ppt in 1X C1-C4	FAL.3T3.SLS.16.07.04
FAL.3T3.HN.B5.17.09.04	DF	AA61HN	NA	NA	0.304	0.27%	0	4	NA	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	NO	no points between 0 - 50%	problem with stimulation of NRU; toxicity increases then as conc. rises NRU also rises & IC50 not reached	FAL.3T3.SLS.17.09.04
FAL.3T3.HN.B6.23.09.04	DF	AA61HN	582	0.745	0.310	3.38%	2	2	0.6844	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		outlier removed by SD; ppt in 2X C1-C2; ppt in 1X C1-C4	FAL.3T3.SLS.23.09.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.HN.B7.14.10.04	DF	AA61HN	1220	1.568	0.322	4.77%	1	7	0.4589	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		outlier removed bySD; ppt in 2X C1-C3; ppt in 1X C1-C6	FAL.3T3.SLS.14.10.04

DIMETHYLFORMAMIDE

IIVS

A1	RF	AA61FN	6870	93.990	0.392	1.04%	1	5	0.7331	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A5
B1	DF	AA61FN	5060	69.196	0.485	3.39%	4	4	0.9915	20000, 13333, 8889, 5926, 3951, 2634, 1756, 1171	1.5	YES			SLS-B4
B2	DF	AA61FN	4940	67.621	0.375	8.71%	4	4	0.9900	20000, 13333, 8889, 5926, 3951, 2634, 1756, 1171	1.5	YES			SLS-B7
B3	DF	AA61FN	4700	64.281	0.413	5.07%	4	4	0.9892	20000, 13333, 8889, 5926, 3951, 2634, 1756, 1171	1.5	YES			SLS-B8

ECBC

AA61MW-A1	RF	AA61MW	6410	87.717	0.522	4.15%	1	2	0.9137	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-15
AA61MW-B1	DF	AA61MW	4750	65.025	0.522	3.30%	4	4	0.9866	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			SLS-P42
AA61MW-B2	DF	AA61MW	5680	77.639	0.697	1.26%	3	5	0.9610	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			SLS-P43
AA61MW-B3	DF	AA61MW	5600	76.574	0.616	0.92%	4	4	0.9830	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			SLS-P45

FRAME

FAL.3T3.KF.A1.21.10.04	RF	AA61KF	8990	123.050	0.315	7.39%	1	0	0.3085	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50-100%		FAL.3T3.SLS.21.10.04
FAL.3T3.KF.B1.11.11.04	DF	AA61KF	5180	70.808	0.276	14.58%	4	2	0.9649	50000, 23256, 10817, 5031, 2340, 1088, 506, 236	2.15	YES			FAL.3T3.SLS.10.11.04
FAL.3T3.KF.B2.18.11.04	DF	AA61KF	673	9.206	0.305	26.13%	3	2	0.9507	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	NO	% VC difference >15	ppt in 2X C1; concentraton range may be off by factor of 10; C1 probably 50000	FAL.3T3.SLS.19.11.04
FAL.3T3.KF.B3.25.11.04	DF	AA61KF	6080	83.192	0.382	0.74%	2	3	0.9630	50000, 23256, 10817, 5031, 2340, 1088, 506, 236	2.15	YES		ppt in 2X C1	FAL.3T3.SLS.25.11.04
FAL.3T3.KF.B4.26.11.04	DF	AA61KF	5190	70.971	0.381	9.84%	2	3	0.8958	50000, 23256, 10817, 5031, 2340, 1088, 506, 236	2.15	YES		ppt in 2X C1	FAL.3T3.SLS.26.11.04

DIQUAT DIBROMIDE MONOHYDRATE

IIVS

A1	RF	AA61GN	4.65	0.013	0.448	3.20%	2	4	0.9862	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A3
B1	DF	AA61GN	3.83	0.011	0.485	2.39%	3	4	0.9675	10.0, 7.14, 5.10, 3.64, 2.60, 1.86, 1.33, 0.949	1.4	YES			SLS-B4
B2	DF	AA61GN	6.04	0.017	0.353	0.24%	2	3	0.9379	10.0, 7.14, 5.10, 3.64, 2.60, 1.86, 1.33, 0.949	1.4	YES			SLS-B7
B3	DF	AA61GN	6.31	0.017	0.442	3.25%	2	4	0.9544	10.0, 7.14, 5.10, 3.64, 2.60, 1.86, 1.33, 0.949	1.4	YES			SLS-B8

ECBC

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61KS-A1	RF	AA61KS	5.48	0.015	0.301	6.79%	2	1	0.9864	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P11
AA61KS-B1	DF	AA61KS	3.47	0.010	0.518	5.60%	4	3	0.9823	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P31
AA61KS-B2	DF	AA61KS	3.26	0.009	0.423	8.46%	4	3	0.9818	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P33
AA61KS-B3	DF	AA61KS	4.89	0.013	0.721	3.07%	5	3	0.9904	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P35
FRAME															
FAL.3T3.NV.A1.21.05.04	RF	AA61NV	9.05	0.025	0.484	4.80%	2	0	0.9320	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.21.05.04
FAL.3T3.NV.B1.04.06.04	DF	AA61NV	76.7	0.212	0.468	11.19%	1	1	0.7598	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			FAL.3T3.SLS.04.06.04
FAL.3T3.NV.B2.18.06.04	DF	AA61NV	20.4	0.056	0.720	0.86%	8	0	0.9479	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES		C8 % viability < 20; used lowest dilution factor; pass even though not enough points between 0-100%	FAL.3T3.SLS.18.06.04
FAL.3T3.NV.B3.08.07.04	DF	AA61NV	NA	NA	0.370	4.61%	6	0	NA	100, 46.5, 21.6, 10.1, 4.7, 2.2, 1.01, 0.47	2.15	NO	no points between 50 - 100%		FAL.3T3.SLS.08.07.04
FAL.3T3.NV.B4.16.07.04	DF	AA61NV	11.1	0.031	0.384	6.76%	2	1	0.8922	100, 31.6, 10.0, 3.2, 1.0, 0.3, 0.100, 0.032	3.16	YES			FAL.3T3.SLS.16.07.04

DISULFOTON

IIVS															
A1	RF	AA61FC	95.1	0.346	0.255	12.80%	2	1	0.4754	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	*the solvent controls were treated with 1% DMSO, rather than 0.5%;*	SLS-A4
B1	DF	AA61FC	25.4	0.093	0.437	5.32%	5	3	0.9601	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES		ppt in 2X C1-C4; outlier removed by SD	SLS-B5
B2	DF	AA61FC	46.3	0.169	0.269	7.62%	3	4	0.9111	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES		ppt in 2X C1-C4	SLS-B13
B3	DF	AA61FC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61FC	138	0.504	0.294	0.57%	1	7	0.9243	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES		ppt in 2X C1	SLS-B15
B5	DF	AA61FC	31.8	0.116	0.259	11.99%	5	3	0.9540	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES		ppt in 2x C1-C4	SLS-B16
ECBC															
AA61NY-A1	RF	AA61NY	NA	NA	0.247	1.18%	0	6	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; PC failed; no points between 0-50%	ppt in 2X C1-C2	SLS-P51
AA61NY-B1	DF	AA61NY	155	0.564	0.379	1.45%	3	4	0.9199	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 2X C1-C4; ppt in 1X C1-C3	SLS-P67
AA61NY-B2	DF	AA61NY	NA	NA	0.356	4.07%	0	8	NA	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	no points between 0 - 50%	small pieces of chemical in 1X C1-C4	SLS-P69
AA61NY-B3	DF	AA61NY	54.6	0.199	0.398	0.71%	4	4	0.9654	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		small globules in 1X C1-C5 & 2X C1-C3; SD removed C1 from PRISM analysis; C1 toxicity < C2	SLS-P72
AA61NY-B4	DF	AA61NY	201	0.734	0.406	2.30%	1	7	0.8792	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		small globules in 1X C1-C6; ppt in 2X C1-C5; SD removed C1 & C2 from PRISM analysis; C1 & C2 toxicity < C3	SLS-P74
FRAME															
FAL.3T3.LC.A1.10.09.04	RF	AA61LC	1070	3.914	0.258	10.84%	0	3	0.3989	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 -- 50%	ppt in 1X C1	FAL.3T3.SLS.10.09.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.LC.B1.16.09.04	DF	AA61LC	11200	40.793	0.254	2.23%	1	6	0.8311	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		outlier removed bySD; ppt in 1X C1 and 2X C1-C5; IC50 out of synch with other IC50s	FAL.3T3.SLS.16.09.04
FAL.3T3.LC.B2.15.10.04	DF	AA61LC	NA	NA	0.257	1.49%	0	8	0.4810	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 0 - 50%	ppt in 1X C1-C6;	FAL.3T3.SLS.15.10.04
FAL.3T3.LC.B3.19.11.04	DF	AA61LC	NA	NA	0.260	13.68%	0	6	0.6459	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 0 - 50%	ppt in 1X C1-C6; ppt in 2X C1-C3	FAL.3T3.SLS.19.11.04
ENDOSULFAN															
IIVS															
A1	RF	AA61HZ	1.3	0.003	0.366	49.45%	1	6	0.9673	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	VC1 Ods < VC2 ODs; VC1 removed from subsequent analysis; ppt in 2X C1 and 1X C1;volatility issues	SLS-A2
B1	DF	AA61HZ	5.35	0.013	0.397	1.30%	3	5	0.9207	30.0, 16.7, 9.26, 5.14, 2.86, 1.59, 0.882, 0.490	1.8	YES		ppt in 2X C1; outlier removed by SD; plate sealer	SLS-B5
B2	DF	AA61HZ	13.6	0.033	0.261	20.27%	3	3	0.9195	50.0, 27.8, 15.4,8.57, 4.76, 2.65, 1.47, 0.817	1.8	NO	% VC difference > 15	ppt in 2X C1; very high OD value in VC1	SLS-B13
B3	DF	AA61HZ	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61HZ	1.64	0.004	0.302	42.29%	6	2	0.7300	50.0, 27.8, 15.4,8.57, 4.76, 2.65, 1.47, 0.817	1.8	NO	% VC difference > 15	ppt in 2X C1-C2; low ODs in VC1; used VC2 value for viability calculations	SLS-B15
B5	DF	AA61HZ	2.52	0.006	0.256	3.03%	6	2	0.6745	50.0, 27.8, 15.4,8.57, 4.76, 2.65, 1.47, 0.817	1.8	YES		ppt in 2X C1-C2	SLS-B16
B6	DF	AA61HZ	2.95	0.007	0.256	14.77%	5	2	0.7624	50.0, 27.8, 15.4,8.57, 4.76, 2.65, 1.47, 0.817	1.8	YES		ppt in 2X C1-C2	SLS-B17
ECBC															
AA61LG-A1	RF	AA61LG	NA	NA	0.237	18.26%	2	2	NA	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; PC failed; % VC difference > 15	ppt in 2X C1	SLS-P51
AA61LG-B1	DF	AA61LG	NA	NA	0.383	25.75%	5	2	NA	80.0, 37.2, 17.3, 8.05, 3.74, 1.74, 0.81, 0.38	2.15	NO	% VC difference > 15	ppt in 2X C1	SLS-P55
AA61LG-B2 (sealer)	DF	AA61LG	NA	NA	0.445	5.26%	7	1	NA	60.0, 27.9, 13.0, 6.04, 2.81, 1.31, 0.61, 0.28	2.15	NO	PC failed	ppt in 2X C1	SLS-P56
AA61LG-B3 (sealer)	DF	AA61LG	4.15	0.010	0.217	8.65%	3	5	0.9066	30.0, 14.0, 6.49, 3.02, 1.40, 0.65, 0.30, 0.14	2.15	YES			SLS-P58
AA61LG-B4 (sealer)	DF	AA61LG	2.98	0.007	0.319	13.07%	3	5	0.8831	30.0, 14.0, 6.49, 3.02, 1.40, 0.65, 0.30, 0.14	2.15	YES			SLS-P63
AA61LG-B5 (sealer)	DF	AA61LG	8.68	0.021	0.338	4.57%	2	6	0.9264	30.0, 14.0, 6.49, 3.02, 1.40, 0.65, 0.30, 0.14	2.15	YES		ppt in 2X C1-C3	SLS-P64
FRAME															
FAL.3T3.PW.A1.01/04/04	RF	AA61PW	52500	128.974	0.209	16.91%	0	2	0.3175	10.0, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001,	10	RF	range finder	possible volatility problem	FAL.3T3.SLS.01/04/04
FAL.3T3.PW.B1.29/04/04 (should be A2)	DF	AA61PW	0.249	0.001	0.261	24.71%	2	5	0.4825	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	NO	%VC difference > 15	NR crystals; high background	FAL.3T3.SLS.29/04/04
FAL.3T3.PW.B2.29/04/04 (should be B1)	DF	AA61PW	22.9	0.056	0.241	29.31%	2	6	0.3954	50.0, 34.0, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	NO	%VC difference > 15	possible volatility problem	FAL.3T3.SLS.07/05/04
FAL.3T3.PW.B2.20/05/04	DF	AA61PW	32.7	0.080	0.324	10.51%	1	7	0.3827	50.0, 34.0, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			FAL.3T3.SLS.20/05/04
FAL.3T3.PW.B3.27/05/04	DF	AA61PW	6.47	0.016	0.444	1.54%	6	2	0.7075	50.0, 34.0, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			FAL.3T3.SLS.27/05/04
FAL.3T3.PW.B4.17/06/04	DF	AA61PW	11.2	0.028	0.396	5.49%	7	1	0.7541	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			FAL.3T3.SLS.17.06.04
FAL.3T3.PW.B5.24/06/04	DF	AA61PW	10.4	0.026	0.408	5.45%	1	6	0.8455	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.3T3.SLS.24.06.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
EPINEPHRINE BITARTRATE															
IIVS															
A1	RF	AA61LT	34.4	0.103	0.460	5.31%	0	2	0.9689	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A2
B1	DF	AA61LT	61.8	0.185	0.429	3.46%	1	6	0.8482	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.4	YES			SLS-B1
B2	DF	AA61LT	65.5	0.196	0.413	3.71%	0	6	0.8365	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.4	YES		SMT accepts this test in spite of no points between 0-50%; agreed to on 8/12/04	SLS-B2
B3	DF	AA61LT	62.8	0.188	0.388	2.42%	2	6	0.8693	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.4	YES			SLS-B3
ECBC															
AA61HW-A1	RF	AA61HW	25.4	0.076	0.280	0.36%	2	1	0.9466	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P5
AA61HW-B1	DF	AA61HW	58.5	0.175	0.682	5.06%	1	6	0.8963	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			SLS-P23
AA61HW-B2	DF	AA61HW	46.8	0.140	0.582	3.32%	2	6	0.9135	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			SLS-P27
AA61HW-B3	DF	AA61HW	49.3	0.148	0.440	2.56%	1	6	0.9306	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			SLS-P29
FRAME															
FAL.3T3.RK.A1.01/04/04	RF	AA61RK	37.2	0.112	0.361	17.45%	3	0	0.8041	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	possible volatility problem	FAL.3T3.SLS.01/04/04
FAL.3T3.RK.B1.29/04/04	DF	AA61RK	79.4	0.238	0.349	2.51%	1	0	0.9283	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	no points between 50 - 100%		FAL.3T3.SLS.29/04/04
FAL.3T3.RK.B2.06/05/04	DF	AA61RK	70.5	0.211	0.341	4.84%	2	1	0.9573	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES			FAL.3T3.SLS.06/05/04
FAL.3T3.RK.B3.20/05/04	DF	AA61RK	62.2	0.187	0.407	6.36%	1	0	0.9364	200, 165, 137, 113, 93.3, 77.1, 63.7, 52.7	1.21	YES		lowest dilution factor used; SMT will accept this test	FAL.3T3.SLS.20/05/04
FAL.3T3.RK.B4.27/05/04	DF	AA61RK	57.4	0.172	0.490	12.09%	2	1	0.8531	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES			FAL.3T3.SLS.27/05/04
ETHANOL															
IIVS															
A2	RF	AA61FH	NA	NA	0.416	0.36%	0	8	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A3
B1	DF	AA61FH	12500	270.758	0.154	83.09%	4	1	0.9319	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	NO	% VC difference > 15	VC1 ODs < VC2 ODs; VC1 removed from subsequent analysis; volatility issues.	SLS-B6
B2	DF	AA61FH	7140	155.089	0.400	8.34%	4	0	0.9518	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	NO	no points between 50--100%	ppt in 1X C1-C3; plates read 15-16 hr late; orignial reading used wrong OD wavelength; outliers removed by SD; plate sealer used	SLS-B11
B3	DF	AA61FH	5200	112.871	0.388	2.54%	8	0	0.8605	20000, 16667, 13889, 11574, 9645, 8038, 6698, 5582	1.2	NO	no points between 50--100%;	plate sealer used	SLS-B12
B4	DF	AA61FH	6760	146.751	0.384	4.75%	6	2	0.8518	20000, 16667, 13889, 11574, 9645, 8038, 6698, 5582	1.2	YES		plate sealer used; outliers removed bySD	SLS-B13
B5	DF	AA61FH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B6	DF	AA61FH	6070	131.699	0.458	1.89%	4	4	0.9316	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES			SLS-B15
B7	DF	AA61FH	6410	139.182	0.322	8.21%	4	3	0.9515	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES		plate sealer used; outliers removed by SD	SLS-B16

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
ECBC															
AA61JU-A1	RF	AA61JU	NA	NA	0.322	0.67%	0	2	NA	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder	no toxicity detected	SLS-P6
AA61JU-A2	RF	AA61JU	NA	NA	0.193	57.54%	2	2	NA	100000, 10000, 1000, 100, 10, 10, 0.1, 0.01	10	RF	range finder	probable volatility problem; VC1 <<< VC2	SLS-P8
AA61JU-B1(sealer)	DF	AA61JU	NA	NA	0.134	49.27%	6	1	NA	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	NO	% VC difference > 15	volatility problem	SLS-P37
AA61JU-B2 (sealer)	DF	AA61JU	NA	NA	0.255	10.33%	4	0	NA	68027, 46277, 31481, 21416, 14568, 9910, 6742, 4586	1.47	NO	no points between 50 - 100%		SLS-P39
AA61JU-B3(sealer)	DF	AA61JU	NA	NA	0.218	19.55%	8	0	NA	40000, 33058, 27321, 22579, 18660, 15422, 12745, 10533	1.21	NO	no points between 50 - 100%		SLS-P47
AA61JU-B4 (sealer)	DF	AA61JU	NA	NA	0.234	15.04%	7	0	NA	30000, 24793, 20490, 16934, 13995, 11566, 9559, 7900	1.21	NO	PC failed	dilution factor is 1.21; no points between 50-100%; test would pass due to dilution factor	SLS-P50
AA61JU-B5 (sealer)	DF	AA61JU	NA	NA	0.250	9.95%	7	1	NA	20000, 16529, 13660, 11289, 9330, 7711, 6373, 5267	1.21	NO	PC failed		SLS-P52
AA61JU-B6 (sealer)	DF	AA61JU	5400	117.107	0.556	6.34%	3	5	0.8953	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	NO	PC failed		SLS-P60
AA61JU-B7 (sealer)	DF	AA61JU	6300	136.641	0.478	17.05%	3	5	0.9477	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	NO	% VC difference > 15		SLS-P62
AA61JU-B8 (sealer)	DF	AA61JU	4860	105.580	0.389	3.72%	3	5	0.9188	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	YES			SLS-P64
AA61JU-B9 (sealer)	DF	AA61JU	7310	158.702	0.416	6.29%	2	6	0.8826	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	YES			SLS-P66
AA61JU-B10 (sealer)	DF	AA61JU	3910	84.836	0.393	5.15%	4	4	0.9316	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	YES			SLS-P68
FRAME															
FAL.3T3.PC.A1.30/04/04	RF	AA61PC	NA	NA	0.224	10.66%	0	1	0.0000	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.30/04/04
FAL.3T3.PC.B1.06/05/04	DF	AA61PC	NA	NA	0.190	26.31%	0	5	0.7166	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	no points between 0 - 50%; %VC difference > 15		FAL.3T3.SLS.06/05/04
FAL.3T3.PC.B2.20/05/04	DF	AA61PC	14200	308.732	0.223	34.53%	3	3	0.8898	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	%VC difference > 15; volatility problem		FAL.3T3.SLS.20/05/04
FAL.3T3.PC.B2.27/05/04 should be B3	DF	AA61PC	8300	180.128	0.412	19.58%	4	3	0.9538	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	%VC difference > 15; volatility problem		FAL.3T3.SLS.27/05/04
FAL.3T3.PC.B4.17/06/04	DF	AA61PC	44000	954.073	0.462	10.31%	0	0	0.9212	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	no points between 0 - 100%		FAL.3T3.SLS.17.06.04
FAL.3T3.PC.B5.24/06/04	DF	AA61PC	7110	154.377	0.311	6.43%	6	2	0.9785	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	YES			FAL.3T3.SLS.24.06.04
FAL.3T3.PC.B6.08.07.04	DF	AA61PC	9480	205.865	0.234	14.05%	4	4	0.8796	40000, 27211, 18511, 12592, 8566, 5827, 3964, 2697	1.47	YES			FAL.3T3.SLS.08.07.04
FAL.3T3.PC.B7.16.07.04	DF	AA61PC	8670	188.184	0.308	13.82%	4	4	0.9668	40000, 27211, 18511, 12592, 8566, 5827, 3964, 2697	1.47	YES			FAL.3T3.SLS.16.07.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
ETHYLENE GLYCOL															
IIVS															
A1 Preliminary	RF	AA61HR	15700	252.899	0.430	9.87%	0	1	0.5803	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61HR	27100	436.534	0.489	7.90%	2	2	0.9878	100000, 56250, 31600, 17800, 10000, 5600, 3160, 1770	1.78	YES			SLS-B1
B2	DF	AA61HR	22400	360.825	0.505	4.97%	2	3	0.9713	100000, 56250, 31600, 17800, 10000, 5600, 3160, 1770	1.78	YES			SLS-B2
B3	DF	AA61HR	28200	454.253	0.573	5.77%	2	5	0.9449	100000, 56250, 31600, 17800, 10000, 5600, 3160, 1770	1.78	YES			SLS-B3
ECBC															
ECBC-3T3-lb-01 AA61LM-A1	RF	AA61LM	13000	209.407	0.288	17.62%	0	3	0.05128	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P1
ECBC-3T3-lb-02 AA61LM-A2	RF	AA61LM	18000	289.948	0.238	13.45%	0	3	0.7979	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	No points between 10 and 50%; r2 < 0.8; PC failed; range finder		SLS-P3
ECBC-3T3-lb-03 AA61LM-B1	DF	AA61LM	21200	341.495	0.408	19.53%	3	2	0.9087	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	NO	VC difference > 15%: PC failed		SLS-P4
ECBC-3T3-lb-04 AA61LM-B2	DF	AA61LM	19200	309.278	0.839	4.60%	3	3	0.9718	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	YES			SLS-P5
ECBC-3T3-lb-05 AA61LM-B3	DF	AA61LM	16100	259.343	0.445	8.06%	3	3	0.9290	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	YES			SLS-P7
ECBC-3T3-lb-06 AA61LM-B4	DF	AA61LM	19900	320.554	0.554	2.47%	3	3	0.9186	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	YES			SLS-P9
ECBC-3T3-lb-07 AA61LM-B5	DF	AA61LM	16500	265.786	0.480	16.31%	3	3	0.9611	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	NO	VC difference > 15%		SLS-P12
ECBC-3T3-lb-08 AA61LM-B6	DF	AA61LM	18100	291.559	0.529	1.25%	3	3	0.9695	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	YES			SLS-P13
FRAME															
A1 1b3T3RF01FALPD	RF	AA61PD	NA	NA	0.527	11.89%	0	0	NA	985, 98.5, 9.9, 1.0, 0.1, 0.0099, 0.0010, 0.0001		RF	range finder		1b3T3CRTFALSLS 12/4/02
A2 1b3T3RF02FALPD	RF	AA61PD	34800	560.567	0.449	6.05%	1	0	0.9623	263510, 52702, 10540.4, 2108.1, 421.6, 84.3, 16.9, 3.4		NO	no points between 50 anad 100%		1b3T3CRTFALSLS 12/10/02
1b3T3DF01FALPD	DF	AA61PD	34200	550.902	0.443	1.22%	2	3	0.9645	182500, 124150, 85460, 57450, 39080, 26590, 18090, 12304		NO	PC failed		1b3T3CRTFALSLS 12/17/02
1b3T3DF02FALPD	DF	AA61PD	36500	587.951	0.612	12.90%	2	5	0.9340	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742		YES		NR crystals in plate	1b3T3CRTFALSLS 1/7/03
1b3T3DF03FALPD	DF	AA61PD	40500	652.384	0.306	12.08%	1	4	0.8911	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742		NO	NR crystals in plate; stopped after 1 h; PC failed		1b3T3CRTFALSLS 1/8/03
1b3T3DF04FALPD	DF	AA61PD	27200	438.144	0.489	11.17%	2	5	0.9232	85300, 58027, 39474, 26853, 18268, 12427, 8454, 5751		YES			1b3T3CRTFALSLS 1/14/03
1b3T3DF05FALPD	DF	AA61PD	41700	671.714	0.463	6.48%	1	5	0.9483	100000, 68100, 46100, 31500, 21400, 14600, 9900, 6700		NO	PC failed		1b3T3CRTFALSLS 1/15/03
1b3T3DF06FALPD	DF	AA61PD	23600	380.155	0.557	13.34%	4	3	0.8834	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742		YES			1b3T3CRTFALSLS 1/21/03

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
1b3T3DF07FALPD	DF	AA61PD	39300	633.054	0.281	12.56%	2	3	0.8509	100000, 68100, 46100, 31500, 21400, 14600, 9900, 6700		YES			1b3T3CRTFALSLS 2/26/03
FENPROPATHRIN															
IIVS															
A1	RF	AA61HY	15.3	0.044	0.359	2.00%	2	6	0.9682	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2 and 1X C1-C2	SLS-A1
B1	DF	AA61HY	17.7	0.051	0.454	5.40%	4	4	0.9881	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 2X C1-C3; ppt in 1X C1-C3	SLS-B5
B2	DF	AA61HY	18.1	0.05	0.362	1.43%	4	3	0.9827	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 2X C1-C4; ppt in 1X C1-C3	SLS-B9
B3	DF	AA61HY	14.4	0.04	0.371	0.16%	5	3	0.9848	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 2X C1-C4; ppt in 1X C1-C2	SLS-B10
ECBC															
AA61LJ-A1	RF	AA61LJ	29.5	0.084	0.290	2.90%	2	2	0.8956	1000, 100,10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2 and 1X C1-C2	SLS-P2
AA61LJ-B1	DF	AA61LJ	20.3	0.058	0.316	1.48%	6	2	0.9404	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	YES		slope & IC50 similar for B1, B2, and B3; ppt does not appear to be a factor;ppt in 2X C1-C3; ppt in 1X C1-C3	SLS-P6
AA61LJ-B2	DF	AA61LJ	22.3	0.064	0.254	6.77%	3	4	0.9379	60.0, 40.8, 27.8, 18.9, 12.9, 8.7, 6.0, 4.1	1.47	YES		slope & IC50 similar for B1, B2, and B3; ppt does not appear to be a factor;ppt in 2X C1-C3	SLS-P7
AA61LJ-B3	DF	AA61LJ	25.1	0.072	0.471	3.22%	2	5	0.9274	60.0, 40.8, 27.8, 18.9, 12.9, 8.74, 5.95, 4.05	1.47	YES		slope & IC50 similar for B1, B2, and B3; ppt does not appear to be a factor	SLS-16
FRAME															
FAL.3T3.PT.A1.080104	RF	AA61PT	142	0.405	0.407	9.41%	1	4	0.6639	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	well B7 outlier; no cells; not removed by SD	FAL.3T3.SLS.080104
FAL3T3.PT.A2.15-01-04	DF	AA61PT	54.7	0.157	0.386	2.12%	5	3	0.9203	1000, 680, 465, 216, 100, 46.5, 21.6, 10.1	2.15	NO	PC failed;	ppt in 1X C1-C5; ppt in 2X C1-C5	FAL.3T3.SLS.15/01/04
FAL3T3.PT.B1.22-01-04	DF	AA61PT	59.7	0.171	0.310	1.66%	5	3	0.8978	1000, 680, 465, 216, 100, 46.5, 21.6, 10.1	2.15	YES		ppt in 1X C1-C6 and 2X C1-C5	FAL.3T3.SLS.22/01/04
FAL3T3.PT.B2.29-01-04	DF	AA61PT	69.0	0.198	0.362	7.84%	3	2	0.9594	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 1X C1-C2; ppt in 2X C1-C4	FAL3T3.SLS.29-01-04
FAL3T3.PT.B3.05.02.04	DF	AA61PT	21.6	0.062	0.259	4.89%	4	1	0.9415	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	PC failed;	problem with reservoir liners; ppt in 1X C1-C5 & 2X C1-C4	FAL.3T3.SLS.5/02/04
FAL3T3.PT.B4.25-02-04	DF	AA61 PT	29.8	0.085	0.523	3.33%	4	2	0.9173	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 1X C1-C3 & 2X C1-C2	FAL3T3.SLS.25.02.04
FAL3T3.PT.B5.17.03.04	DF	AA61PT	10.9	0.031	0.238	10.23%	3	3	0.8792	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 1X C1-C4 & 2X C1-C4	FAL.3T3.SLS.17/03/04
GIBBERELLIC ACID															
IIVS															
A1	RF	AA61RE	NA	NA	0.403	3.68%	0	4	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A4
B1	DF	AA61RE	13300	38.322	0.557	0.22%	0	8	0.4182	5000, 3846, 2959, 2276, 2276, 1751, 1347, 1036, 797	1.3	NO	no points between 0-50%		SLS-B6
B2	DF	AA61RE	7830	22.618	0.457	1.39%	1	7	0.9631	10000, 7692, 5917, 4552, 3501, 2693, 2072, 1594	1.3	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength; ppt in 2X C1-C3	SLS-B11

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B3	DF	AA61RE	6840	19.745	0.340	7.57%	2	6	0.9288	10000, 7692, 5917, 4552, 3501, 2693, 2072, 1594	1.3	YES		ppt in 2X C1-C4; outlier removed bySD because well didn't receive 50 ul of growth medium during refeeding	SLS-B12
B4	DF	AA61RE	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B5	DF	AA61RE	8300	23.958	0.413	2.36%	1	7	0.8974	10000, 7692, 5917, 4552, 3501, 2693, 2072, 1594	1.3	YES		ppt in 2X C1-C3	SLS-B15
ECBC															
AA61FR-A1	RF	AA61FR	NA	NA	0.472	0.90%	0	7	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P14
AA61FR-B1	DF	AA61FR	NA	NA	0.385	3.16%	0	8	NA	5000, 4132, 3415, 2822, 2333, 1928, 1593, 1317	1.21	NO	no points between 0 - 50%		SLS-P47
AA61FR-B2	DF	AA61FR	9020	26.028	0.430	3.16%	1	7	0.8611	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES		ppt in 2X C1	SLS-P65
AA61FR-B3	DF	AA61FR	7820	22.566	0.436	1.89%	2	5	0.9515	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES		ppt in 2X C1-C4; ppt in 1X C1	SLS-P67
AA61FR-B4	DF	AA61FR	7240	20.914	0.356	2.99%	3	4	0.9605	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES		ppt in 2X C1-C4	SLS-P69
FRAME															
FAL.3T3.GY.A1.10.09.04	RF	AA61GY	78.3	0.226	0.293	5.52%	2	0	0.9008	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50 - 100%		FAL.3T3.SLS.10.09.04
FAL.3T3.GY.B1.16.09.04	DF	AA61GY	NA	NA	0.317	23.98%	0	0	0.0000	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	no points between 0 - 100%; %VC difference >15		FAL.3T3.SLS.16.09.04 addendum lists incorrect PC
FAL.3T3.GY.B2.15.10.04	DF	AA61GY	NA	NA	0.286	4.02%	0	4	0.0000	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	NO	no points between 0 - 50%	outlier removed by SD	FAL.3T3.SLS.15.10.04
FAL.3T3.GY.B3.25.11.04	DF	AA61GY	NA	NA	0.342	6.74%	0	2	0.0000	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	NO	no points between 0 - 50%		FAL.3T3.SLS.25.11.04

GLUTETHIMIDE

IIVS															
A1	RF	AA61NN	80.5	0.371	0.294	7.00%	2	6	0.9499	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-A5
B1	DF	AA61NN	139	0.640	0.374	6.19%	3	5	0.9421	500, 313, 195, 122, 76.3, 47.7, 29.8, 18.6	1.6	YES			SLS-B6
B2	DF	AA61NN	119	0.548	0.263	2.36%	4	4	0.9536	500, 313, 195, 122, 76.3, 47.7, 29.8, 18.6	1.6	YES		outliers removed bySD	SLS-B13
B3	DF	AA61NN	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61NN	122	0.561	0.350	9.49%	4	4	0.9580	500, 313, 195, 122, 76.3, 47.7, 29.8, 18.6	1.6	YES			SLS-B15
B5	DF	AA61NN	121	0.558	0.339	0.00%	4	4	0.9484	500, 313, 195, 122, 76.3, 47.7, 29.8, 18.6	1.6	YES			SLS-B17
ECBC															
AA61FE-A1	RF	AA61FE	256	1.177	0.486	2.55%	1	7	0.9256	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-16
AA61FE-B1	DF	AA61FE	160	0.736	0.605	10.75%	5	3	0.9842	1000, 680, 463, 315, 214, 146, 99, 67	1.47	YES		ppt in 2X C1-C2; appear oily	SLS-P44
AA61FE-B2	DF	AA61FE	174	0.800	0.575	4.13%	5	3	0.9784	1000, 680, 463, 315, 214, 146, 99, 67	1.47	YES		ppt in 2X C1-C2; appear oily	SLS-P46
AA61FE-B3	DF	AA61FE	167	0.767	0.256	3.42%	5	3	0.9456	1000, 680, 463, 315, 214, 146, 99, 67	1.47	YES			SLS-P48
FRAME															
FAL.3T3.KY.A1.21.10.04	RF	AA61KY	508	2.339	0.227	5.39%	1	1	0.8073	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	FAL.3T3.SLS.21.10.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.KY.B1.11.11.04	DF	AA61KY	303	1.396	0.268	4.20%	3	5	0.9424	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		ppt in 2X C1	FAL.3T3.SLS.10.11.04
FAL.3T3.KY.B2.19.11.04	DF	AA61KY	262	1.208	0.207	3.18%	2	5	0.9086	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		ppt in 2X C1	FAL.3T3.SLS.19.11.04
FAL.3T3.KY.B3.25.11.04	DF	AA61KY	288	1.327	0.350	10.56%	2	5	0.7829	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			FAL.3T3.SLS.25.11.04
GLYCEROL															
IIVS															
A1	RF	AA61JF	NA	NA	0.402	3.00%	0	4	0.5520	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61JF	38200	414.75	0.453	1.93%	3	5	0.9665	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	YES			SLS-B6
B2	DF	AA61JF	28800	313.175	0.460	1.18%	4	4	0.9609	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B3	DF	AA61JF	16500	178.973	0.392	5.33%	4	2	0.9540	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	YES		outlier removed bySD	SLS-B12
ECBC															
AA61HG-A1	RF	AA61HG	31800	344.975	0.345	4.28%	0	4	0.3823	10000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P1
AA61HG-A2	RF	AA61HG	1870	20.314	0.446	5.59%	1	2	0.9208	10000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P3
AA61HG-B1	DF	AA61HG	23400	254.558	0.471	0.04%	4	4	0.9245	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			SLS-P17
AA61HG-B2	DF	AA61HG	18800	204.544	0.434	1.94%	4	3	0.9732	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			SLS-P23
AA61HG-B3	DF	AA61HG	11600	125.831	0.341	18.42%	6	2	0.9815	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	NO	%VC difference > 15		SLS-P30
AA61HG-B4	DF	AA61HG	17800	193.102	0.642	0.19%	4	4	0.9798	68027, 46277, 31481, 21416, 14568, 9910, 6742, 4586	1.47	YES			SLS-P36
FRAME															
FAL.3T3.RA.A1.08/01/04	RF	AA61RA	NA	NA	0.777	13.65%	0	8	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	no points between 0-50; range finder	straight line; no toxicity	FAL.3T3.SLS.080104
FAL3T3.RA.A2.15-01-04	DF	AA61RA	11400	123.819	0.717	1.03%	2	6	0.6816	100000, 31646, 10014, 3169, 1003, 317, 100, 31.8	3.16	NO	PC failed;		FAL.3T3.SLS.15/01/04
FAL3T3.RA.B1.22-01-04	DF	AA61RA	5710	62.057	0.447	3.38%	3	5	0.9498	100000, 31646, 10014, 3169, 1003, 317, 100, 31.8	3.16	YES			FAL.3T3.SLS.22/01/04
FAL3T3.RA.B2.29-01-04	DF	AA61RA	71800	779.449	0.481	1.42%	1	7	0.9674	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES		little variation in curve; no acidity at C1; morpholog. score didn't match NRU which was lower than expected; affect lysosomes?	FAL3T3.SLS.29-01-04
FAL3T3.RA.B3.05.02-04	DF	AA61RA	18900	205.016	0.370	3.33%	4	4	0.8908	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	NO	PC failed;	problem with reservoir liners	FAL.3T3.SLS.5/02/04
FAL3T3.RA.B4.25-02-04	DF	AA61RA	49200	534.303	0.513	2.62%	2	3	0.9772	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			FAL3T3.SLS.25.02.04
FAL3T3.RA.B5.17-03-04	DF	AA61RA	28800	313.175	0.438	7.92%	4	4	0.9627	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			FAL.3T3.SLS.17/03/04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
HALOPERIDOL															
IIVS															
A1	RF	AA61LW	7.60	0.020	0.290	0.23%	0	1	0.4600	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	the solvent controls were treated with 1% DMSO, rather than 0.5%.; ppt in 1X C1-C2	SLS-A4
B1	DF	AA61LW	5.98	0.016	0.399	1.50%	3	5	0.9242	10.0, 7.69, 5.92, 4.55, 3.50, 2.69, 2.07, 1.59	1.3	YES			SLS-B5
B2	DF	AA61LW	5.69	0.015	0.318	4.32%	4	4	0.9350	20.0, 14.3, 10.2, 7.29, 5.21, 3.72, 2.66, 1.90	1.4	YES			SLS-B13
B3	DF	AA61LW	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61LW	4.73	0.013	0.358	6.35%	3	4	0.9252	20.0, 14.3, 10.2, 7.29, 5.21, 3.72, 2.66, 1.90	1.4	YES			SLS-B15
ECBC															
AA61JC-A1	RF	AA61JC	3.45	0.009	0.346	9.78%	2	5	0.9328	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	ppt in 2X C1 and 1X C1	SLS-P14
AA61JC-B1	DF	AA61JC	5.01	0.013	0.454	8.40%	3	4	0.9612	20.0, 13.6, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES			SLS-P38
AA61JC-B2	DF	AA61JC	4.89	0.013	0.320	12.12%	4	4	0.8878	20.0, 13.6, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES			SLS-P39
AA61JC-B3	DF	AA61JC	6.07	0.016	0.433	1.12%	2	5	0.9620	20.0, 13.6, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES			SLS-P42
FRAME															
FAL.3T3.PM.A1.10.09.04	RF	AA61PM	NA	NA	0.373	3.11%	0	1	0.0000	100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; no points between 0 -- 50%	ppt in 1X C1	FAL.3T3.SLS.10.09.04
FAL.3T3.PM.B1.16.09.04	DF	AA61PM	NA	NA	0.269	3.91%	0	0	0.0000	250, 170, 116, 78.7, 53.5, 36.4, 24.8, 16.9	1.47	NO	no points between 50 - 100%	ppt in 2X C1	FAL.3T3.SLS.16.09.04
FAL.3T3.PM.B2.23.09.04	DF	AA61PM	10.1	0.027	0.199	6.98%	1	0	0.8164	25.0, 11.6, 5.4, 2.5, 1.2, 0.544, 0.253, 0.118	2.15	NO	no points between 50 - 100%	ppt in 2X C1	FAL.3T3.SLS.23.09.04
FAL.3T3.PM.B3.14.10.04	DF	AA61PM	8.75	0.023	0.232	1.04%	2	2	0.9504	25.0, 17.0, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	YES		ppt in 2X C1	FAL.3T3.SLS.14.10.04
FAL.3T3.PM.B4.21.10.04	DF	AA61PM	7.60	0.020	0.251	12.27%	3	1	0.9286	25.0, 17.0, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	YES		ppt in 2X C1	FAL.3T3.SLS.21.10.04
FAL.3T3.PM.B5.04.11.04	DF	AA61PM	7.63	0.020	0.190	12.15%	3	3	0.9797	25.0, 17.0, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	YES		outlier removed bySD	FAL.3T3.SLS.04.11.04
HEXACHLOROPHENE															
IIVS															
A1	RF	AA61JN	3.21	0.008	0.353	1.04%	1	2	0.9799	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-A1
B1	DF	AA61JN	2.90	0.01	0.440	0.93%	5	3	0.9582	20.0, 12.5, 7.81, 4.88, 3.05, 1.91, 1.19, 0.745	1.6	YES			SLS-B5
B2	DF	AA61JN	3.39	0.01	0.367	0.61%	4	4	0.9595	20.0, 12.5, 7.81, 4.88, 3.05, 1.91, 1.19, 0.745	1.6	YES			SLS-B9
B3	DF	AA61JN	2.88	0.01	0.341	3.03%	5	3	0.9868	20.0, 12.5, 7.81, 4.88, 3.05, 1.91, 1.19, 0.745	1.6	YES			SLS-B10
ECBC															
AA61ND-A1	RF	AA61ND	9.47	0.023	0.329	9.12%	2	2	0.9700	1000, 100,10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2 and ppt in 1X C1	SLS-P4
AA61ND-B1	DF	AA61ND	7.81	0.019	0.293	3.27%	3	3	0.9653	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P18
AA61ND-B2	DF	AA61ND	3.70	0.009	0.426	9.89%	3	3	0.9878	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P20
AA61ND-B3	DF	AA61ND	3.56	0.009	0.371	3.77%	5	3	0.9882	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P21
FRAME															

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.HB.A1.09/01/04	RF	AA61HB	9.80	0.024	0.387	8.80%	1	4	0.9858	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	did'nt dissolve properly; top 2 conc. prepared from stock & C2 from C1. C3 prepared by diluting stock and C4-8 from the respective C3-7 (from SD); ppt at 100 ug/mL.	FAL.3T3.SLS.09/01/04
FAL.3T3.HB.B1.16.01.04	DF	AA61 HB	7.35	0.018	0.558	6.11%	4	3	0.9833	100, 47.0, 22.0, 10.0, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.3T3.SLS.16/01/04
FAL.3T3.HB.B2.23.01.04	DF	AA61HB	4.59	0.011	0.393	5.57%	3	3	0.8846	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL3T3.23-01-04
FAL.3T3.HB.B3.30.01.04	DF	AA61HB	NA	NA	0.264	12.04%	2	6	NA	100, 47.0, 22.0, 10.0, 4.68, 2.18, 1.01, 0.47	2.15	NO	SD rejects this experiment	serious NR crystal problem; SD rejects this experiment	FAL.3T3.SLS.29/01/04
FAL.3T3.HB.B4.06-02-04	DF	AA61HB	4.10	0.010	0.455	3.82%	5	3	0.9631	100, 47.0, 22.0, 10.0, 4.68, 2.18, 1.01, 0.47	2.15	YES		possible NR crystals present; blanks slightly higher than usual	FAL.3T3.SLS.06/02/04
LACTIC ACID															
IIVS															
A1	RF	AA61FW	1710	18.940	0.443	13.41%	1	2	0.8766	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A2
B1	DF	AA61FW	3020	33.525	0.447	0.32%	1	2	0.9050	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	YES		plate sealer used	SLS-B4
B2	DF	AA61FW	3210	35.594	0.371	3.03%	0	5	0.9595	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	NO	no points between 0-50%		SLS-B7
B3	DF	AA61FW	2770	30.787	0.422	6.41%	0	5	0.9166	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	NO	no points between 0-50%		SLS-B8
B4	DF	AA61FW	2840	31.577	0.494	1.43%	2	5	0.8914	5000, 4167, 3472,2894,2411, 2009, 1674, 1395	1.2	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B5	DF	AA61FW	2510	27.821	0.349	3.18%	2	5	0.8772	5000, 4167, 3472,2894,2411, 2009, 1674, 1395	1.2	YES		outliers removed bySD	SLS-B12
ECBC															
AA61NL-A1	RF	AA61NL	1890	20.959	0.260	14.18%	1	1	0.8301	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1	SLS-P5
AA61NL-B1	DF	AA61NL	2630	29.199	0.587	4.77%	3	5	0.9427	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P26
AA61NL-B2	DF	AA61NL	2940	32.687	0.526	1.28%	3	5	0.9463	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P27
AA61NL-B3	DF	AA61NL	3260	36.172	0.441	1.38%	3	4	0.9660	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P29
FRAME															
FAL.3T3.JT.A1.01/04/04	RF	AA61JT	5750	63.881	0.314	3.27%	1	0	0.7232	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.01/04/04
FAL.3T3.JT.B1.29/04/04	DF	AA61JT	3000	33.294	0.315	0.03%	2	2	0.9638	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES		NR crystals; high background	FAL.3T3.SLS.29/04/04
FAL.3T3.JT.B2.07/05/04	DF	AA61JT	3590	39.845	0.361	17.30%	4	2	0.9759	10000, 7519, 5653, 4251, 3196, 2403, 1807, 1358	1.33	NO	%VC difference > 15	possible volatility problem	FAL.3T3.SLS.07/05/04
FAL.3T3.JT.B3.20/05/04	DF	AA61JT	4100	45.538	0.377	2.39%	4	1	0.9730	10000, 7519, 5653, 4251, 3196, 2403, 1807, 1358	1.33	YES			FAL.3T3.SLS.20/05/04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.JT.B4.27/05/04	DF	AA61JT	3360	37.271	0.363	1.72%	4	4	0.8950	10000, 7519, 5653, 4251, 3196, 2403, 1807, 1358	1.33	YES			FAL.3T3.SLS.27/05/04
LINDANE															
IIVS															
A1	RF	AA61PJ	15.9	0.055	0.403	35.64%	1	7	0.9488	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	VC1 ODs < VC2 ODs; VC1 removed from subsequent analysis; ppt in 2X C1; volatility issues.	SLS-A3
B1	DF	AA61PJ	39.0	0.134	0.403	5.91%	2	4	0.9245	100, 55.6, 30.9, 17.1, 9.53, 5.29, 2.94, 1.63	1.8	YES		ppt in 2X C1-C2; plate sealer used	SLS-B5
B2	DF	AA61PJ	51.2	0.176	0.244	10.19%	3	4	0.9211	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES		SD removed C1 from Hill function due to upswing in response curve; C1 toxicity< C2-C4; plate sealer used; ppt in 2X C1-C4	SLS-B13
B3	DF	AA61PJ	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61PJ	35.2	0.121	0.239	1.50%	4	3	0.9526	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES		ppt in 2X C1-C5; ppt in 1X C1-C3	SLS-B15
B5	DF	AA61PJ	288	0.989	0.251	0.40%	1	5	0.8492	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES		ppt in 2X C1-C3	SLS-B16
B6	DF	AA61PJ	38.8	0.133	0.324	4.98%	4	3	0.8974	500, 278, 154, 85.7, 47.6, 26.5, 14.7, 8.17	1.8	YES		ppt in 2X C1-C6	SLS-18
ECBC															
AA61FK-A1	RF	AA61FK	38.9	0.134	0.191	14.03%	2	6	0.9093	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2; ppt in 1X C1; higher than usual blank OD	SLS-P10
AA61FK-B1	DF	AA61FK	42.9	0.147	0.242	3.72%	3	5	0.9082	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES		ppt in 2X C1-C3; ppt in 1X C1-C2	SLS-P65
AA61FK-B2	DF	AA61FK	262	0.902	0.340	0.96%	2	6	0.8636	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES		ppt in 2X C1-C3; ppt in 1X C1	SLS-P66
AA61FK-B3	DF	AA61FK	71.0	0.244	0.240	5.46%	3	4	0.8190	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES		ppt in 2X C1-C3; ppt in 1X C1-C3; SD removed data for C1 from PRISM analysis	SLS-P69
FRAME															
FAL.3T3.KN.A1.27/05/04	RF	AA61KN	37.1	0.127	0.252	24.49%	2	2	0.7351	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; % VC difference > 15	ppt in 1X C1; volatility proble	FAL.3T3.SLS.27/05/04
FAL.3T3.KN.B1.04/06/04	DF	AA61KN	125	0.431	0.363	11.01%	3	5	0.7052	2500, 791, 250, 79.2, 25.1, 7.9, 2.5, 0.8	3.16	YES		odd graph; ppt in 2X C1-C4 and ppt in 1X C1	FAL.3T3.SLS.04.06.04
FAL.3T3.KN.B2.18/06/04	DF	AA61KN	45.5	0.156	0.404	11.01%	4	0	0.8725	1500, 475, 150, 47.5, 15.0, 4.76, 1.51, 0.48	3.16	NO	no points between 50 - 100%	ppt in 1X C1-C3 and 2X C1-C2	FAL.3T3.SLS.18.06.04
FAL.3T3.KN.B3.24.06.04	DF	AA61KN	153	0.528	0.355	17.86%	3	1	0.9198	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	% VC difference > 15	volatility problem; ppt in 2X C1-C3	FAL.3T3.SLS.24.06.04
FAL.3T3.KN.B4.08.07.04	DF	AA61KN	308	1.060	0.250	11.89%	1	7	0.7219	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES		ppt in 2X C1-C3 and ppt in 1X C1-C3	FAL.3T3.SLS.08.07.04
FAL.3T3.KN.B5.09.07.04	DF	AA61KN	303	1.041	0.333	4.48%	2	6	0.7443	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES		ppt in 2X C1-C5 and ppt in 1X C1-C3	FAL.3T3.SLS.09.07.04
FAL.3T3.KN.B6.16.07.04	DF	AA61KN	329	1.131	0.238	6.21%	2	3	0.9111	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES		ppt in 2X C1-C4 and ppt in 1X C1-C2	FAL.3T3.SLS.16.07.04
LITHIUM I CARBONATE															
IIVS															
A1	RF	AA61RN	625	8.459	0.557	5.35%	0	2	-0.1197	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A1
B1	DF	AA61RN	877	11.869	0.378	2.23%		6		300, 214, 153, 109, 78.1,55.8, 39.8, 28.5	1.4	NO	no points between 0.1 - 50%; low r2		SLS-B1

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B2	DF	AA61RN	NA	NA	0.499	7.37%	0	4	0.2402	300, 214, 153, 109, 78.1,55.8, 39.8, 28.5	1.4	NO	No points between 0.1 - 50%; low r2		SLS-B2
B3	DF	AA61RN	2.74	0.037	0.573	2.02%	0	3	-0.0036	300, 214, 153, 109, 78.1,55.8, 39.8, 28.5	1.4	NO	no points 0.1- 50%; PC failed		SLS-B3
B4	DF	AA61RN	NA	NA	0.500	8.09%	0	5	NA	300, 214, 153, 109, 78.1,55.8, 39.8, 28.5	1.4	NO	no points between 0.1 - 50%; low r2		SLS-B4
ECBC															
AA61RR-A1	RF	AA61RR	NA	NA	0.363	7.10%	0	0	0.2245	100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	no points between 10 - 90%; low r2; range finder		SLS-P1
AA61RR-A2	RF	AA61RR	561	7.592	0.387	11.51%	0	3	0.2234	500, 50, 5.0, 0.5, 0.05, 0.005, 0.0005	10	NO	no points between 10 - 50%; low r2; range finder		SLS-P3
AA61RR-B1	DF	AA61RR	656	8.878	0.574	2.50%	1	5	0.7540	750, 510.2, 347.1, 236.1, 160.6, 109.3, 74.3, 50.6	1.47	NO	low r2	cloudy stock solution	SLS-P6
AA61RR-B2	DF	AA61RR	762	10.313	0.568	2.56%	1	5	0.7590	750, 510.2, 347.1, 236.1, 160.6, 109.3, 74.3, 50.6	1.47	NO	low r2		SLS-P8
AA61RR-B3	DF	AA61RR	574	7.768	0.545	0.11%	2	6	0.8864	1102.5, 750, 510.2, 347.1, 236.1, 160.6, 109.3, 74.3	1.47	YES			SLS-P10
AA61RR-B4	DF	AA61RR	630	8.526	0.608	3.32%	2	4	0.9561	1102.5, 750, 510.2, 347.1, 236.1, 160.6, 109.3, 74.3	1.47	YES		ppts. In C1-C3	SLS-P15
AA61RR-B5	DF	AA61RR	498	6.740	0.195	1.42%	2	5	0.9176	1102.5, 750, 510.2, 347.1, 236.1, 160.6, 109.3, 74.3	1.47	YES		ppts. In C1-C3	SLS-P16
FRAME															
FAL.3T3.A1.RM.200603	RF	AA61RM	28200	381.648	0.729	6.72%	0	0	0.2031	100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	no points between 10 - 90%; low r2; range finder		FAL.3T3.SLS2.A1.200603
FAL.3T3.RM.B1.04.07.03	DF	AA61RM	0.002	0.000	0.509	0.53%	0	0	-0.3160	250, 170, 115.7, 78.7, 53.5, 36.4, 24.8, 16.9	1.47	NO	PC failed; no points between 10 - 90%	straight cytotoxicity line; can't perform proper calculations	FAL.3T3.SLS.04.07.03
FAL.3T3.B2.RM.11.07.03	DF	AA61RM	NA	NA	0.490	2.31%	0	0	NA	250, 170, 115.7, 78.7, 53.5, 36.4, 24.8, 16.9	1.47	NO	PC failed; no points between 10 - 90%		FAL.3T3.SLS.11.07.03
FAL.3T3.B3.RM.18.07.03	DF	AA61RM	NA	NA	0.517	2.17%	0	0	NA	250, 170, 115.7, 78.7, 53.5, 36.4, 24.8, 16.9	1.47	NO	no points between 10 - 90%; No toxicity		FAL.3T3.SLS.18.07.03
FAL.3T3.RM.B4.070803	DF	AA61RM	24.7	0.334	0.738	5.09%	0	8	0.6965	1000, 680, 462, 314, 214, 145, 99.1, 67.4	1.47	NO	PC failed; no points between 0 & 50% viability; cytotoxicity curve goes in opposite direction		FAL.3T3.SLS.070803
FAL.3T3.RM.B5.080803	DF	AA61RM	1190	16.105	0.474	18.96%	1	7	0.2883	1000, 680, 462, 314, 214, 145, 99.1, 67.4	1.47	NO	PC failed; low r2; % VC difference > 15		FAL.3T3.SLS.080803

MEPROBAMATE

IIVS															
A1	RF	AA61LS	390	1.786	0.329	5.97%	1	7	0.9290	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A1
B1	DF	AA61LS	395	1.811	0.544	1.09%	3	5	0.9490	2000, 1111, 617, 343, 191, 106, 58.8, 32.7	1.8	YES		ppt in 2X C1	SLS-B5
B2	DF	AA61LS	385	1.762	0.367	1.27%	3	5	0.9715	2000, 1111, 617, 343, 191, 106, 58.8, 32.7	1.8	YES			SLS-B9
B3	DF	AA61LS	377	1.726	0.381	5.07%	3	5	0.9719	2000, 1111, 617, 343, 191, 106, 58.8, 32.7	1.8	YES			SLS-B10
ECBC															

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61RJ-A1	RF	AA61RJ	283	1.297	0.266	3.58%	1	5	0.8633	1000, 100,10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P2
AA61RJ-B1	DF	AA61RJ	309	1.416	0.336	9.11%	2	6	0.8967	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P6
AA61RJ-B2	DF	AA61RJ	344	1.577	0.285	3.34%	3	4	0.9449	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P8
AA61RJ-B3	DF	AA61RJ	407	1.866	0.345	0.70%	3	5	0.8884	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES		outlier not removed from from C6	SLS-P18
FRAME															
FAL.3T3.HV.A1.080104	RF	AA61HV	798	3.655	0.505	5.60%	1	1	0.8944	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.3T3.SLS.080104
FAL3T3.HV.A2.15-01-04	DF	AA61HV	1030	4.720	0.526	6.07%	2	6	0.9564	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	PC failed;		FAL.3T3.SLS.15/01/04
FAL3T3.HV.B1.22-01-04	DF	AA61HV	984	4.508	0.311	13.54%	2	5	0.7904	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		solubility a problem above 2500 ug/ml	FAL.3T3.SLS.22/01/04
FAL3T3.HV.B2.29-01-04	DF	AA61HV	904	4.139	0.377	3.07%	3	5	0.9632	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL3T3.SLS.29-01-04
FAL3T3.HV.B3.05.02.04	DF	AA61HV	80	0.366	0.341	11.28%	8	0	0.5764	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	PC failed; no points between 50-100	problem with reservoir liners	FAL.3T3.SLS.5/02/04
FAL.3T3.HV.B4.25.02.04	DF	AA61HV	927	4.246	0.437	3.66%	3	5	0.9673	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL3T3.SLS.25.02.04
FAL3T3.HV.B5.17.03.04	DF	AA61HV	692	3.169	0.378	0.13%	4	4	0.9275	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.3T3.SLS.17/03/04

MERCURY II CHLORIDE

IIVS															
A1	RF	AA61MX	1.21	0.004	0.316	58.59%	1	4	0.9661	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	VC1 ODs < VC2 ODs; VC1 removed from subsequent analysis; volatility issues.	SLS-A1
B1	DF	AA61MX	3.39	0.012	0.320	2.63%	1	6	0.9147	10.0, 5.56, 3.09, 1.71, 0.953, 0.529, 0.294, 0.163	1.8	YES			SLS-B1
B2	DF	AA61MX	3.50	0.013	0.311	5.10%	1	1	0.9564	10.0, 5.56, 3.09, 1.71, 0.953, 0.529, 0.294, 0.163	1.8	YES			SLS-B2
B3	DF	AA61MX	3.63	0.013	0.346	7.05%	2	5	0.9477	10.0, 5.56, 3.09, 1.71, 0.953, 0.529, 0.294, 0.163	1.8	YES			SLS-B3
ECBC															
AA61KP-A1	RF	AA61KP	NA	NA	0.152	58.91%	2	2	0.9275	1000, 100,10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	low ODs for VC1; ppt in C1	SLS-P2
AA61KP-A2	RF	AA61KP	1.43	0.005	0.373	3.96%	0	1	0.9241	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-P4
AA61KP-B1	DF	AA61KP	3.26	0.012	0.278	2.28%	2	1	0.8937	10.0, 4.65, 2.16, 1.01, 0.47, 0.22, 0.10, 0.05	2.15	YES			SLS-P18
AA61KP-B2	DF	AA61KP	3.61	0.013	0.353	5.88%	2	5	0.9465	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			SLS-P20
AA61KP-B3	DF	AA61KP	3.48	0.013	0.384	6.51%	2	5	0.9682	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			SLS-P21
FRAME															
FAL.3T3.HA.A1.080104	RF	AA61HA	4.11	0.015	0.399	9.97%	1	0	0.9558	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	no points between 50-100 range finder	ppt in 1000ug/ml	FAL.3T3.SLS.080104
FAL3T3.HA.A2.15-01-04	DF	AA61HA	6.77	0.025	0.363	6.52%	2	6	0.8549	10.0, 7.7, 5.9, 4.6, 3.5, 2.7, 2.1, 1.6	1.3	NO	PC failed;		FAL.3T3.SLS.15/01/04
FAL3T3.HA.B1.22-01-04	DF	AA61HA	5.71	0.021	0.371	3.49%	1	6	0.8036	10.0, 7.7, 5.9, 4.6, 3.5, 2.7, 2.1, 1.6	1.3	YES			FAL.3T3.SLS.22/01/04
FAL3T3.HA.B2.29-01-04	DF	AA61HA	7.98	0.029	0.481	1.42%	1	5	0.9674	10.0, 7.7, 5.9, 4.6, 3.5, 2.7, 2.1, 1.6	1.3	YES			FAL3T3.SLS.29-01-04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL3T3.HA.B3.05.02.04	DF	AA61HA	0.967	0.004	0.380	7.96%	8	0	0.8305	10.0, 7.7, 5.9, 4.6, 3.5, 2.7, 2.1, 1.6	1.3	NO	PC failed; no points between 50-100	problem with reservoir liners	FAL.3T3.SLS.5/02/04
FAL3T3.HA.B4.17.03.04	DF	AA61HA	4.28	0.016	0.223	2.28%	3	5	0.9519	10, 7.63, 5.83, 4.45, 3.40, 2.59, 1.98, 1.51	1.31	YES			FAL.3T3.SLS.17/03/04
METHANOL															
IIVS															
A1	RF	AA61FZ	NA	NA	0.256	7.10%	0	6	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A5
B1	DF	AA61FZ	NA	NA	0.380	5.76%	0	2	0.4933	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	NO	no points between 0 - 50%		SLS-B9
B2	DF	AA61FZ	NA	NA	0.284	2.14%	0	1	NA	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	NO	no points between 0 - 50%		SLS-B13
B3	DF	AA61FZ	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61FZ	NA	NA	0.400	2.42%	0	2	NA	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	NO	no points between 0 - 50%		SLS-B15
ECBC															
AA61MJ-A1	RF	AA61MJ	NA	NA	0.443	6.04%	0	3	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-16
AA61MJ-B1	DF	AA61MJ	NA	NA	0.709	5.19%	0	8	NA	3000, 2479, 2049, 1693, 1400, 1157, 956, 790	1.21	NO	no points between 0 - 50%	no toxicity detected; need larger conc; dilut. factor 1.21	SLS-P44
AA61MJ-B2	DF	AA61MJ	NA	NA	0.512	2.61%	0	7	NA	3500, 2893, 2391, 1976, 1633, 1349, 1115, 922	1.21	NO	no points between 0 - 50%	no toxicity was detected	SLS-P72
AA61MJ-B3	DF	AA61MJ	NA	NA	0.375	14.56%	0	0	NA	3500, 2893, 2391, 1976, 1633, 1349, 1115, 922	1.21	NO	no points between 0 - 100%	no toxicity was detected	SLS-P74
FRAME															
FAL.3T3.RG.A1.21.10.04	RF	AA61RG	NA	NA	0.203	7.09%	0	0	NA	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0-100%		FAL.3T3.SLS.21.10.04
FAL.3T3.RG.B1.04.11.04	DF	AA61RG	NA	NA	0.175	6.75%	0	0	NA	2500, 1701, 1157, 787, 535, 264, 248, 169	1.47	NO	no points between 0-100%		FAL.3T3.SLS.04.11.04
FAL.3T3.RG.B2.25.11.04	DF	AA61RG	NA	329085	0.258	0.36%	0	0	-1.2340	2500, 2066, 1708, 1411, 1166, 964, 797, 658	1.21	NO	no points between 0-100%		FAL.3T3.SLS.25.11.04
FAL.3T3.RG.B3.26.11.04	DF	AA61RG	NA	NA	0.263	5.11%	0	0	NA	2500, 2066, 1708, 1411, 1166, 964, 797, 658	1.21	NO	no points between 0-100%	no toxicity detected; 1.21 dilut. factor doesn't affect acceptability; outlier removed bySD	FAL.3T3.SLS.26.11.04
NICOTINE															
IIVS															
A1	RF	AA61HL	339	2.089	0.457	7.49%	0	5	0.9490	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A4
B1	DF	AA61HL	422	2.600	0.539	5.98%	1	2	0.9929	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES			SLS-B4
B2	DF	AA61HL	508	3.133	0.392	3.15%	2	0	0.8900	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	NO	no points between 50 - 100%		SLS-B7
B3	DF	AA61HL	513	3.162	0.469	2.05%	2	1	0.9111	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES			SLS-B8
B4	DF	AA61HL	415	2.558	0.440	1.63%	3	2	0.9953	1000, 833, 694, 579, 482, 402, 335, 279	1.2	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
ECBC															
AA61NA-A1	RF	AA61NA	NA	NA	0.410	19.57%	0	5	NA	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P49
AA61NA-B1	DF	AA61NA	NA	NA	0.532	3.40%	3	5	NA	500, 413, 342, 282, 233, 193, 159, 132	1.21	NO	PC failed		SLS-P53
AA61NA-B2 (sealer)	DF	AA61NA	292	1.803	0.603	4.02%	3	5	0.8541	500, 413, 342, 282, 233, 193, 159, 132	1.21	YES			SLS-P54

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61NA-B3 (sealer)	DF	AA61NA	NA	NA	0.399	9.58%	4	4	NA	500, 413, 342, 282, 233, 193, 159, 132	1.21	NO	PC failed		SLS-P56
AA61NA-B4 (sealer)	DF	AA61NA	325	2.004	0.451	5.32%	3	5	0.7971	500, 413, 342, 282, 233, 193, 159, 132	1.21	YES			SLS-P58
AA61NA-B5 (sealer)	DF	AA61NA	199	1.227	0.536	5.08%	5	3	0.8836	500, 413, 342, 282, 233, 193, 159, 132	1.21	YES			SLS-P62
FRAME															
FAL.3T3.KL.A1.10.09.04	RF	AA61KL	582	3.589	0.402	9.59%	1	0	0.9633	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50 - 100%		FAL.3T3.SLS.10.09.04
FAL.3T3.KL.B1.16.09.04	DF	AA61KL	460	2.838	0.375	1.07%	3	0	0.9720	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	NO	no points between 50 - 100%		FAL.3T3.SLS.16.09.04
FAL.3T3.KL.B2.23.09.04	DF	AA61KL	481	2.964	0.356	3.30%	4	2	0.9817	1000, 826, 683, 565, 467, 386, 319, 263	1.21	YES		outlier removed by SD	FAL.3T3.SLS.23.09.04
FAL.3T3.KL.B3.14.10.04	DF	AA61KL	499	3.076	0.359	4.34%	2	1	0.9323	1000, 826, 683, 565, 467, 386, 319, 263	1.21	YES			FAL.3T3.SLS.14.10.04
FAL.3T3.KL.B4.04.11.04	DF	AA61KL	255	1.574	0.227	6.96%	6	2	0.9486	750, 620, 512, 423, 350, 289, 239, 197	1.21	YES		outlier removed by SD	FAL.3T3.SLS.04.11.04

PARAQUAT

IIVS															
A1	RF	AA61GD	14.1	0.055	0.454	0.61%	1	1	0.9683	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A2
B1	DF	AA61GD	7.91	0.031	0.491	0.08%	6	2	0.9744	86.8 ,54.3, 33.9, 21.2, 13.3, 8.28, 5.18, 3.24	1.6	YES			SLS-B4
B2	DF	AA61GD	22.7	0.088	0.386	6.81%	3	5	0.9777	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES			SLS-B7
B3	DF	AA61GD	39.4	0.153	0.478	2.70%	2	6	0.9759	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES			SLS-B8
ECBC															
AA61MP-A1	RF	AA61MP	11.9	0.046	0.345	1.23%	1	1	0.9870	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P5
AA61MP-B1	DF	AA61MP	23.6	0.092	0.654	5.58%	2	5	0.9673	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P23
AA61MP-B2	DF	AA61MP	13.1	0.051	0.632	7.30%	3	5	0.9128	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P26
AA61MP-B3	DF	AA61MP	27.1	0.105	0.622	7.05%	2	5	0.9779	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P27
FRAME															
FAL.3T3.HP.A1.01/04/04	RF	AA61HP	62.4	0.243	0.396	1.11%	3	0	0.8909	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.01/04/04
FAL.3T3.HP.B1.29/04/04	DF	AA61HP	39.8	0.155	0.275	10.84%	1	0	0.8164	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	no points between 50 - 100%	NR crystals; high background	FAL.3T3.SLS.29/04/04
FAL.3T3.HP.B2.07/05/04	DF	AA61HP	7.35	0.029	0.347	7.92%	4	2	0.9791	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.3T3.SLS.07/05/04
FAL.3T3.HP.B3.20/05/04	DF	AA61HP	40.2	0.156	0.360	0.93%	4	1	0.9192	100, 75.2, 56.5, 42.5, 32.0, 24.0, 18.1, 13.6	1.33	YES			FAL.3T3.SLS.20/05/04
FAL.3T3.HP.B4.27/05/04	DF	AA61HP	27.0	0.105	0.425	2.86%	4	4	0.9183	100, 75.2, 56.5, 42.5, 32.0, 24.0, 18.1, 13.6	1.33	YES		outlier removed by SD	FAL.3T3.SLS.27/05/04

PARATHION

IIVS															
A1	RF	AA61PS	50.6	0.174	0.402	5.64%	1	3	0.9458	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C3	SLS-A3
B1	DF	AA61PS	20.7	0.071	0.435	5.34%	3	3	0.8956	300, 167, 92.6, 51.4, 28.6, 15.9, 8.82, 4.90	1.8	YES		SD removed C1 & C2 from Hill function in PRISM due to upswing in response curve	SLS-B5

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B2	DF	AA61PS	27.5	0.095	0.348	13.21%	5	3	0.9431	300, 167, 92.6, 51.4, 28.6, 15.9, 8.82, 4.90	1.8	YES		ppt in 2X C1-C4	SLS-B9
B3	DF	AA61PS	17.9	0.062	0.353	5.03%	3	3	0.9864	300, 167, 92.6, 51.4, 28.6, 15.9, 8.82, 4.90	1.8	YES		ppt in 2X C1-C4 & 1X C1-C2; SD removed C1 & C2 from Hill function in PRISM due to ppts & flat response curve	SLS-B10
ECBC															
AA61MD-A1	RF	AA61MD	NA	NA	0.329	7.11%	2	6	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; PC failed	ppt in 1X C1 & 2X C1-C2 (cloudy)	SLS-P50
AA61MD-B1	DF	AA61MD	18	0.062	0.648	8.54%	3	5	0.9126	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P55
AA61MD-B2	DF	AA61MD	13.6	0.047	0.418	10.70%	3	5	0.8929	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		ppt in 2X C1	SLS-P57
AA61MD-B3	DF	AA61MD	36.4	0.125	0.321	10.09%	2	5	0.9559	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P59
FRAME															
FAL.3T3.KE.A1.28.05.04	RF	AA61KE	407	1.399	0.396	3.60%	1	4	0.9626	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1-C2	FAL.3T3.SLS.28.05.04
FAL.3T3.KE.B1.04.06.04	DF	AA61KE	51.9	0.178	0.330	7.50%	5	3	0.7298	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 2X C1-C4; ppt in 1X C1-C2	FAL.3T3.SLS.04.06.04
FAL.3T3.KE.B2.18.06.04	DF	AA61KE	NA	0.121	0.714	6.99%	3	0	0.8485	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	no points between 50 - 100%	ppt in 2X C1-C2; tox.curve going in wrong direction; SD suggests ppt is cause of bad curve	FAL.3T3.SLS.18.06.04
FAL.3T3.KE.B3.09.07.04	DF	AA61KE	123	0.423	0.283	6.74%	2	6	0.9136	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 2X C1	FAL.3T3.SLS.09.07.04
FAL.3T3.KE.B4.16.07.04	DF	AA61KE	247	0.847	0.263	3.84%	2	3	0.8273	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 2X C1-C2; ppt in 1X C1-C2	FAL.3T3.SLS.16.07.04
PHENOBARBITAL															
IIVS															
A1	RF	AA61FG	77.4	0.333	0.283	14.30%	1	5	0.9228	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A5
B1	DF	AA61FG	813	3.500	0.359	8.28%	0	8	0.4992	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	NO	no points between 0 - 50%		SLS-B6
B2	DF	AA61FG	379	1.633	0.342	1.76%	3	5	0.9762	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B2 (should be B3)	DF	AA61FG	624	2.686	0.302	13.14%	2	6	0.8659	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES		outliers removed bySD	SLS-B13
B4	DF	AA61FG	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B5	DF	AA61FG	497	2.138	0.335	2.24%	3	5	0.9744	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES			SLS-B15
B6	DF	AA61FG	405	1.742	0.302	1.43%	3	5	0.9775	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES			SLS-B16
ECBC															
AA61KV-A1	RF	AA61KV	351	1.510	0.324	3.98%	1	2	0.9078	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P68
AA61KV-B1	DF	AA61KV	624	2.686	0.405	1.63%	3	5	0.9793	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	YES			SLS-P70
AA61KV-B2	DF	AA61KV	505	2.173	0.412	8.99%	3	5	0.7926	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	YES			SLS-P71
AA61KV-B3	DF	AA61KV	773	3.327	0.410	6.44%	2	6	0.9504	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	YES			SLS-P72
FRAME															
FAL.3T3.NJ.A1.21.10.04	RF	AA61NJ	796	3.428	0.169	1.18%	1	0	0.5114	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50-100%		FAL.3T3.SLS.21.10.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.NJ.B1.11.11.04	DF	AA61NJ	435	1.871	0.311	4.08%	4	4	0.8929	2000, 1361, 926, 630, 428, 291, 198,135	1.47	YES		ppt in 2X C1	FAL.3T3.SLS.10.11.04
FAL.3T3.NJ.B2.25.11.04	DF	AA61NJ	832	3.582	0.295	9.31%	2	2	0.8514	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 2X C1	FAL.3T3.SLS.25.11.04
FAL.3T3.NJ.B3.26.11.04	DF	AA61NJ	912	3.927	0.204	1.06%	2	2	0.9435	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 2X C1	FAL.3T3.SLS.26.11.04
PHENOL															
IIVS															
A1	RF	AA61PG	3.12	0.033	0.418	100.04%	2	4	0.9933	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	VC1 ODs < VC2 ODs; VC1 removed from subsequent analysis; volatility issues.	SLS-A3
B1	DF	AA61PG	NA	NA	0.465	1.52%	0	3	0.5739	20.0, 12.5, 7.81, 4.88, 3.05, 1.91, 1.19, 0.745	1.6	NO	no points between 0 - 50%	plate sealer used	SLS-B4
B2	DF	AA61PG	54.8	0.583	0.422	2.43%	3	5	0.9767	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES		plate sealer used	SLS-B6
B3	DF	AA61PG	33.6	0.357	0.392	34.71%	4	4	0.9925	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	NO	% VC difference > 15	plates read 15-16 hr late; orignial reading used wrong OD wavelength; VC2 used to calculate viability; volatility problem	SLS-B11
B4	DF	AA61PG	65.9	0.700	0.337	1.73%	3	5	0.9669	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES			SLS-B12
B5	DF	AA61PG	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B6	DF	AA61PG	53.6	0.569	0.411	3.44%	3	5	0.9775	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES			SLS-B15
ECBC															
AA61FV-A1	RF	AA61FV	NA	NA	0.140	99.80%	4	0	NA	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder	probable volatility problem; VC1 <<< VC2	SLS-11
AA61FV-A2 (sealer)	RF	AA61FV	56.0	0.595	0.430	3.64%	2	1	0.8997	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P45
AA61FV-B1(sealer)	DF	AA61FV	50.6	0.537	0.305	4.48%	4	4	0.9861	1000, 465, 216, 101, 46.8, 21.8, 10.1	2.15	YES			SLS-P47
AA61FV-B2 (sealer)	DF	AA61FV	NA	NA	0.280	0.51%	5	3	NA	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	PC failed		SLS-P50
AA61FV-B3 (sealer)	DF	AA61FV	NA	NA	0.341	7.61%	4	4	NA	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	NO	PC failed		SLS-P52
AA61FV-B4 (sealer)	DF	AA61FV	60.8	0.646	0.552	2.48%	3	3	0.9615	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			SLS-P54
AA61FV-B5 (sealer)	DF	AA61FV	NA	NA	0.354	3.58%	4	4	NA	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	NO	PC failed		SLS-P56
AA61FV-B6 (sealer)	DF	AA61FV	39.1	0.415	0.416	4.85%	4	4	0.9808	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			SLS-P58
FRAME															
FAL.3T3.MS.A1.21.05.04	RF	AA61MS	10.4	0.110	0.176	99.85%	2	1	0.4657	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	volatility problem	FAL.3T3.SLS.21.05.04
FAL.3T3.MS.B1.17/06/04	DF	AA61MS	NA	NA	0.387	40.08%	2	1	NA	1000, 317, 100, 31.7, 10.0, 3.2, 1.0, 0.3	3.16	NO	% VC difference > 15		FAL.3T3.SLS.17.06.04
FAL.3T3.MS.B2.24/06/04	DF	AA61MS	375	3.984	0.154	18.61%	1	4	0.9472	1000, 317, 100, 31.7, 10.0, 3.2, 1.0, 0.3	3.16	NO	% VC difference > 15	used plate sealer; volatility problem	FAL.3T3.SLS.24.06.04
FAL.3T3.MS.B3.08.07.04	DF	AA61MS	142	1.504	0.308	26.58%	4	1	0.9369	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.1	2.15	NO	% VC difference > 15		FAL.3T3.SLS.08.07.04
FAL.3T3.MS.B4.09.07.04	DF	AA61MS	37.8	0.401	0.301	25.71%	3	2	0.5823	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.1	2.15	NO	% VC difference > 15		FAL.3T3.SLS.09.07.04
FAL.3T3.MS.B5.16.07.04	DF	AA61MS	110	1.168	0.360	7.07%	3	2	0.9794	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.1	2.15	YES			FAL.3T3.SLS.16.07.04
FAL.3T3.MS.B6.17.09.04	DF	AA61MS	124	1.322	0.530	17.30%	3	2	0.9579	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.1	2.15	NO	% VC difference > 15	did not use plate sealer	FAL.3T3.SLS.17.09.04
FAL.3T3.MS.B7.23.09.04	DF	AA61MS	126	1.335	0.313	7.13%	3	2	0.9717	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.1	2.15	YES		outlier removed bySD	FAL.3T3.SLS.23.09.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.MS.B8.14.10.04	DF	AA61MS	116	1.231	0.234	27.97%	4	2	0.9535	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.1	2.15	NO	% VC difference > 15	volatility problem	FAL.3T3.SLS.14.10.04
FAL.3T3.MS.B9.21.10.04	DF	AA61MS	77.3	0.821	0.339	13.82%	4	3	0.9581	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			FAL.3T3.SLS.21.10.04
PHENYLTHIOUREA															
IIVS															
A1	RF	AA61PV	49.4	0.325	0.369	1.67%	2	3	0.8971	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A3
B1	DF	AA61PV	113	0.741	0.446	4.65%	3	4	0.9548	300, 188, 117, 73.2, 45.8, 28.6, 17.9, 11.2	1.6	YES			SLS-B5
B2	DF	AA61PV	83.9	0.552	0.262	5.15%	6	2	0.9737	1000, 625, 391,244, 153, 95.4, 59.6, 37.3	1.6	YES			SLS-B13
B3	DF	AA61PV	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B4	DF	AA61PV	70.0	0.460	0.335	8.85%	6	2	0.9580	1000, 625, 391,244, 153, 95.4, 59.6, 37.3	1.6	YES			SLS-B15
ECBC															
AA61LN-A1	RF	AA61LN	NA	#VALUE!	0.284	5.42%	2	6	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; PC failed		SLS-P51
AA61LN-B1	DF	AA61LN	NA	#VALUE!	0.350	14.50%	4	4	NA	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	NO	PC failed		SLS-P53
AA61LN-B2	DF	AA61LN	48.6	0.320	0.613	11.02%	4	4	0.9747	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			SLS-P55
AA61LN-B3	DF	AA61LN	9.11	0.060	0.601	9.45%	5	3	0.9428	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			SLS-P57
AA61LN-B4	DF	AA61LN	32.7	0.215	0.374	8.69%	4	4	0.9730	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			SLS-P59
FRAME															
FAL.3T3.JB.A1.27/05/04	RF	AA61JB	34.0	0.223	0.302	7.47%	2	1	0.9382	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.3T3.SLS.27/05/04
FAL.3T3.JB.B1.04/06/04	DF	AA61JB	164	1.075	0.320	7.72%	3	5	0.8392	2500, 791, 250, 79.2, 25.1, 7.9, 2.5, 0.8	3.16	YES		ppt in 2X C1	FAL.3T3.SLS.04.06.04
FAL.3T3.JB.B2.18/06/04	DF	AA61JB	288	1.891	0.388	2.92%	2	3	0.9514	2500, 791, 250, 79.2, 25.1, 7.9, 2.5, 0.8	3.16	YES		ppt in 2X C1	FAL.3T3.SLS.18.06.04
FAL.3T3.JB.B3.08.07.04	DF	AA61JB	264	1.736	0.250	6.43%	2	6	0.8568	2500, 791, 250, 79.2, 25.1, 7.9, 2.5, 0.8	3.16	YES		ppt in 2X C1	FAL.3T3.SLS.08.07.04
PHYSOSTIGMINE															
IIVS															
A1	RF	AA61NF	673	2.444	0.262	11.85%	1	4	0.9016	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A5
B1	DF	AA61NF	75.9	0.275	0.517	10.55%	2	0	0.8901	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	NO	no points between 50 - 100%		SLS-B5
B2	DF	AA61NF	30.1	0.109	0.411	6.64%	2	4	0.9115	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES			SLS-B6
B3	DF	AA61NF	19.8	0.072	0.338	7.64%	2	3	0.9406	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES		outliers removed bySD	SLS-B13
B4	DF	AA61NF	NA	NA	NA	NA	NA	NA	NA	NA	NA	NO	PC failed		SLS-B14
B5	DF	AA61NF	16.0	0.058	0.350	0.98%	3	2	0.9600	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES			SLS-B15
B6	DF	AA61NF	15.8	0.057	0.365	1.30%	4	2	0.9575	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES			SLS-B16
ECBC															
AA61FT-A1	RF	AA61FT	NA	NA	0.281	7.43%	2	4	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; PC failed		SLS-P51
AA61FT-B1	DF	AA61FT	42.8	0.155	0.678	8.75%	2	6	0.9263	80.0, 54.4, 37.0, 25.2, 17.1, 11.7, 7.93, 5.39	1.47	YES			SLS-P55

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61FT-B2	DF	AA61FT	13.0	0.047	0.592	9.27%	5	3	0.8332	80.0, 54.4, 37.0, 25.2, 17.1, 11.7, 7.93, 5.39	1.47	YES			SLS-P57
AA61FT-B3	DF	AA61FT	28.8	0.105	0.354	8.67%	5	3	0.9265	80.0, 54.4, 37.0, 25.2, 17.1, 11.7, 7.93, 5.39	1.47	YES			SLS-P59
FRAME															
FAL.3T3.GT.A1.21.10.04	RF	AA61GT	34.4	0.125	0.217	3.30%	1	0	0.9835	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50-100%		FAL.3T3.SLS.21.10.04
FAL.3T3.GT.B1.25.11.04	DF	AA61GT	38.2	0.139	0.344	4.78%	4	2	0.9738	100, 75.2, 56.5, 42.5, 32.0, 24.0, 18.1, 13.6	1.33	YES		ppt in 2X C1-C2;	FAL.3T3.SLS.25.11.04
FAL.3T3.GT.B2.26.11.04	DF	AA61GT	35.7	0.130	0.167	1.60%	4	3	0.6701	100, 75.2, 56.5, 42.5, 32.0, 24.0, 18.1, 13.6	1.33	YES		ppt in 2X C1-C2;	FAL.3T3.SLS.26.11.04
FAL.3T3.GT.B3.02.12.04	DF	AA61GT	77.1	0.280	0.179	5.82%	0	1	0.2009	100, 75.2, 56.5, 42.5, 32.0, 24.0, 18.1, 13.6	1.33	NO	no points between 0-50%	most values above 125%	FAL.3T3.SLS.02.12.04 (RB)
FAL.3T3.GT.B4.09.12.04	DF	AA61GT	39.5	0.144	0.286	6.43%	2	2	0.9799	100, 75.2, 56.5, 42.5, 32.0, 24.0, 18.1, 13.6	1.33	YES			FAL.3T3.SLS.09.12.04
POTASSIUM I CHLORIDE															
IIVS															
A1	RF	AA61FF	611	8.196	0.457	25.09%	1	1	0.8205	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF			SLS-A1
B1	DF	AA61FF	4150	55.667	0.394	5.13%	2	6	0.9627	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES			SLS-B1
B2	DF	AA61FF	3660	49.095	0.536	1.54%	2	5	0.9837	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	YES			SLS-B2
B3	DF	AA61FF	3230	43.327	0.561	1.06%	2	5	0.9387	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	NO	PC failed		SLS-B3
B4	DF	AA61FF	3320	44.534	0.442	4.82%	2	4	0.9856	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	YES			SLS-B4
ECBC															
AA61KM-A1	RF	AA61KM	2160	28.974	0.424	3.92%	0	0	0.8877	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	no points between 10 - 90%; range finder		SLS-P1
AA61KM-B1	DF	AA61KM	3140	42.119	0.607	0.88%	1	4	0.8821	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P6
AA61KM-B2	DF	AA61KM	4060	54.460	0.552	4.78%	1	1	0.9805	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P8
AA61KM-B3	DF	AA61KM	3160	42.388	0.526	0.98%	1	3	0.9435	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P10
AA61KM-B4	DF	AA61KM	3080	41.315	0.676	1.49%	1	4	0.9563	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P13
FRAME															
FAL.3T3.A1.MY.200603	RF	AA61MY	1290	17.304	0.745	1.93%	0	1	0.9580	10000,1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	no points between 10 - 50%; range finder		FAL.3T3.SLS2.A1.200603
FAL.3T3.MY.A2.27.06.03	RF	AA61MY	9440	126.626	0.511	2.94%	0	2	0.7401	6000, 4080, 2780, 1890, 1280, 874, 595, 405	1.47	NO	no points between 10 - 50%; low r2; range finder		FAL.3T3.SLS.A2.27.06.03
FAL.3T3.MY.B1.04.07.03	DF	AA61MY	4470	59.960	0.551	2.97%	0	4	0.9514	20000, 13600, 9260, 6300, 4280, 2910, 1980, 1350	1.47	NO	PC failed; no points between 10 - 50%		FAL.3T3.SLS.04.07.03

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.B2.MY.11.07.03	DF	AA61MY	4350	58.350	0.583	0.25%	1	4	0.9622	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	YES			FAL.3T3.SLS.11.07.03
FAL.3T3.B3.MY.18.07.03	DF	AA61MY	4760	63.850	0.499	0.50%	2	2	0.9202	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	YES			FAL.3T3.SLS.18.07.03
FAL.3T3.B4.MY.25.07.03	DF	AA61MY	4740	63.581	0.478	6.48%	1	2	0.9631	10000, 7519, 5633, 4251, 3196, 2403, 1807, 1350	1.33	YES			FAL.3T3.SLS.25.07.03
FAL.3T3.B5.MY.070803	DF	AA61MY	3440	46.144	1.263	6.60	3	5	0.9364	10000, 6802, 4627, 3148, 2141, 1456, 991, 674	1.47	NO	PC failed		FAL.3T3.SLS.070803
FAL.3T3.B6.MY.080803	DF	AA61MY	1160	15.560	0.432	11.91	5	2	0.6458	10000, 6802, 4627, 3148, 2141, 1456, 991, 674	1.47	NO	PC failed; low r2		FAL.3T3.SLS.080803
FAL.3T3.MY.B7.120903	DF	AA61MY	1920	25.755	0.629	1.58	4	2	0.9144	10000, 7519, 5633, 4251, 3196, 2403, 1807, 1358	1.33	YES			FAL.3T3.SLS.120903
FAL.3T3.MY.B8.180903	DF	AA61MY	3450	46.278	0.367	6.74	3	5	0.8706	10000, 7519, 5633, 4251, 3196, 2403, 1807, 1358	1.33	YES			FAL.3T3.SLS.180903
POTASSIUM CYANIDE															
IIVS															
A1	RF	AA61KW	25.5	0.392	0.116	99.22%	1	5	0.9238	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	VC1 ODs < VC2 ODs;VC1 removed from subsequent analysis; volatility issues.	SLS-A5
B1	DF	AA61KW	19.8	0.304	0.403	7.47%	3	3	0.9494	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES		plate sealer used	SLS-B4
B2	DF	AA61KW	18.9	0.291	0.366	0.25%	5	3	0.9756	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES			SLS-B7
B3	DF	AA61KW	17.9	0.275	0.408	5.75%	5	3	0.9767	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES			SLS-B8
ECBC															
AA61MN-A1	RF	AA61MN	421	6.461	0.085	0.69%	1	6	0.9516	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1	SLS-P49
AA61MN-B1 (sealer)	DF	AA61MN	NA	NA	0.125	6.06%	7	0	NA	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	NO	no points between 50-100%; PC failed	ppt in 1X C1-C5	SLS-P52
AA61MN-B2 (sealer)	DF	AA61MN	NA	NA	0.434	3.69%	0	8	NA	200, 136, 92.6, 63.0, 42.8, 29.1, 19.8, 13.5	1.47	NO	no points between 0 - 50%		SLS-P62
AA61MN-B3 (sealer)	DF	AA61MN	19.6	0.301	0.325	1.90%	3	5	0.9619	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P64
AA61MN-B4 (sealer)	DF	AA61MN	13.9	0.213	0.435	9.17%	3	5	0.9485	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		ppt in 2X C1-C2; ppt in 1X C1-C5	SLS-P66
AA61MN-B5 (sealer)	DF	AA61MN	12.5	0.192	0.446	0.73%	3	5	0.8689	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P68
FRAME															
FAL.3T3.GP.A1.21.10.04	RF	AA61GP	153	2.357	0.029	97.12%	0	0	0.9807	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0-100%	ppt in 1X C1	FAL.3T3.SLS.21.10.04
FAL.3T3.GP.B1.11.11.04	DF	AA61GP	219	3.360	0.203	10.87%	8	0	0.8961	1000, 826, 683, 565, 467, 386, 319, 263	1.21	NO	no points between 50-100%	ppt in 1X C1-C8; viability not above 50% for any conc	FAL.3T3.SLS.10.11.04
FAL.3T3.GP.B2.26.11.04	DF	AA61GP	253	3.884	0.184	5.76%	2	6	0.3284	500, 413, 342,282, 233,193,159, 132	1.21	YES			FAL.3T3.SLS.26.11.04
FAL.3T3.GP.B3.09.12.04	DF	AA61GP	172	2.638	0.195	22.57%	6	1	0.6436	500, 413, 342,282, 233,193,159, 132	1.21	NO	% VC difference >15		FAL.3T3.SLS.09.12.04
FAL.3T3.GP.B4.10.12.04	DF	AA61GP	106	1.634	0.236	5.84%	4	2	0.5610	500, 376, 283, 213, 160, 120, 90.3, 67.9	1.33	YES			FAL.3T3.SLS.10.12.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.GP.B5.15.12.04	DF	AA61GP	117	1.804	0.126	2.18%	4	4	0.6827	500, 376, 283, 213, 160, 120, 90.3, 67.9	1.33	YES			FAL.3T3.SLS.15.12.04
PROCAINAMIDE HCL															
IIVS															
A1	RF	AA61ML	406	1.492	0.421	5.01%	0	1	0.9614	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A4
B1	DF	AA61ML	453	1.666	0.485	3.11%	1	1	0.9213	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES			SLS-B4
B2	DF	AA61ML	485	1.786	0.400	0.77%	1	0	0.8992	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	NO	no points between 50-100%	outliers removed bySD	SLS-B7
B3	DF	AA61ML	528	1.944	0.453	4.01%	1	1	0.8702	1000, 714, 510, 364, 260, 186, 133, 94.9	1.4	YES			SLS-B8
B4	DF	AA61ML	511	1.878	0.457	3.83%	3	1	0.9248	1000, 833, 694, 579, 482, 402, 335, 279	1.2	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
ECBC															
AA61KC-A1	RF	AA61KC	363	1.336	0.365	3.67%	0	1	0.9503	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P13
AA61KC-B1	DF	AA61KC	406	1.495	0.499	11.41%	3	3	0.9929	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			SLS-P37
AA61KC-B2	DF	AA61KC	412	1.516	0.392	0.00%	4	1	0.9682	800, 661, 546, 452, 373, 308, 255, 211	1.21	YES			SLS-P40
AA61KC-B3	DF	AA61KC	383	1.409	0.528	4.19%	3	1	0.9813	800, 661, 546, 452, 373, 308, 255, 211	1.21	YES			SLS-P41
FRAME															
FAL.3T3.GV.A1.10.09.04	RF	AA61GV	550	2.022	0.582	11.63%	2	0	0.8758	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50 - 100%		FAL.3T3.SLS.10.09.04
FAL.3T3.GV.B1.16.09.04	DF	AA61GV	423	1.555	0.367	5.86%	4	0	0.8061	1000, 752, 565, 425, 320, 240, 181, 136	1.33	NO	no points between 50 - 100%	outlier removed by SD	FAL.3T3.SLS.16.09.04
FAL.3T3.GV.B2.23.09.04	DF	AA61GV	433	1.591	0.405	3.52%	1	1	0.4667	500, 413, 342, 282, 233, 193, 159, 132	1.21	YES			FAL.3T3.SLS.23.09.04
FAL.3T3.GV.B3.14.10.04	DF	AA61GV	426	1.566	0.340	7.26%	3	1	0.5102	750, 620, 512, 423, 350, 289, 239, 197	1.21	YES		C5-C8 show % viabilities >144%; outlier removed bySD	FAL.3T3.SLS.14.10.04
FAL.3T3.GV.B4.04.11.04	DF	AA61GV	435	1.599	0.238	1.58%	3	1	0.4580	750, 620, 512, 423, 350, 289, 239, 197	1.21	YES		4 concentrations with values >150%	FAL.3T3.SLS.04.11.04
2-PROPANOL															
IIVS															
A1	RF	AA61GC	NA	NA	0.486	1.76%	0	8	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
A1 with plate cover	RF	AA61GC	4380	72.879	0.421	6.01%	1	6	0.8257	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61GC	11000	183.028	0.082	89.61%	3	1	0.8759	100000, 62500, 39063, 24414, 15259, 9537, 5960, 3725	1.6	NO	% VC difference > 15		SLS-B1
B1 with plate cover	DF	AA61GC	6280	104.493	0.216	15.53%	4	1	0.9691	100000, 62500, 39063, 24414, 15259, 9537, 5960, 3725	1.6	NO	% VC difference > 15		SLS-B1
B2	DF	AA61GC	9620	160.067	0.098	87.70%	3	1	0.9420	100000, 62500, 39063, 24414, 15259, 9537, 5960, 3725	1.6	NO	% VC difference > 15		SLS-B2
B2 with plate cover	DF	AA61GC	3160	52.579	0.404	2.68%	5	0	0.9710	100000, 62500, 39063, 24414, 15259, 9537, 5960, 3725	1.6	NO	no points between 50 - 99.9%		SLS-B2

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B3	DF	AA61GC	11200	186.356	0.223	61.18%	4	2	0.8927	50000, 33333, 22222, 14815, 9877, 6584, 4390, 2926	1.5	NO	% VC difference > 15; PC failed		SLS-B3
B3 with plate cover	DF	AA61GC	4280	71.215	0.525	6.06%	5	1	0.9764	50000, 33333, 22222, 14815, 9877, 6584, 4390, 2926	1.5	NO	PC failed		SLS-B3
B4	DF	AA61GC	16600	276.206	0.230	22.95%	0	6	0.6865	20500, 14643, 10459, 7471, 5336, 3812, 2723, 1945	1.4	NO	% VC difference > 15; no points between 0.1 - 50%; low r2		SLS-B4
B4 with plate cover	DF	AA61GC	4690	78.037	0.418	15.64%	4	3	0.9516	20500, 14643, 10459, 7471, 5336, 3812, 2723, 1945	1.4	NO	% VC difference > 15		SLS-B4
B5 with plate cover	DF	AA61GC	3940	65.557	0.432	3.99%	5	3	0.9607	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES			SLS-B5
B6 with plate cover	DF	AA61GC	4260	70.882	0.344	2.04%	5	3	0.9911	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES			SLS-B6
B7 with plate cover	DF	AA61GC	5860	97.504	0.344	9.77%	4	4	0.6186	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	NO	low r2; study director also rejected due to excessive well to well variability		SLS-B7
B8 with plate cover	DF	AA61GC	4130	68.719	0.452	0.86%	5	3	0.9399	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES			SLS-B8
B8 with DYNEX plate cover - for research only	DF	AA61GC	3210	53.411	0.347	1.34%	6	2	0.9369	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	NO	for research; gives lower OD values than the EXCEL plate sealers		SLS-B8
ECBC															
AA61JL-A1	RF	AA61JL	NA	NA	0.405	7.48%	0	0	NA	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	no points between 10 - 90; range finder	high volatility	SLS-P1
AA61JL-A2	RF	AA61JL	NA	NA	0.19	62.97%	1	2	NA	100000, 10000, 1000, 100, 10, 1.0, 0.1, 0.01	10	RF	% VC difference > 15; range finder	high volatility	SLS-P3
AA61JL-B1	DF	AA61JL	NA	NA	0.133	75.92%	3	2	NA	50000, 34014, 23139, 15740, 10707, 7284, 4955, 3370	1.47	NO	PC failed; % VC difference > 15	high volatility	SLS-P9
AA61JL-B2	DF	AA61JL	NA	NA	0.119	75.18%	4	1	NA	50000, 34014, 23139, 15740, 10707, 7284, 4955, 3370	1.47	NO	PC failed; % VC difference > 15	high volatility	SLS-P11
AA61JL-B3 sealer	DF	AA61JL	NA	NA	0.256	30.03	4	1	NA	50000, 34014, 23139, 15740, 10707, 7284, 4955, 3370	1.47	NO	% VC difference > 15	high volatility	SLS-P17
AA61JL-B4 sealer	DF	AA61JL	NA	NA	0.446	19.53	7	1	NA	34014, 23139, 15740, 10707, 7284, 4955, 3370, 2293	1.47	NO	% VC difference > 15	high volatility	SLS-P19
AA61JL-B6	DF	AA61JL	NA	NA	0.204	46.32	0	4	NA	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	NO	% VC difference > 15; no points between 0 -50 %		SLS-P20
AA61JL-B5 sealer	DF	AA61JL	NA	NA	0.117	67.16	5	2	NA	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	NO	% VC difference > 15		SLS-P20
AA61JL-B7 sealer	DF	AA61JL	NA	NA	0.475	15.59	5	3	NA	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	NO	% VC difference > 15: no r2		SLS-P21
AA61JL-B8 sealer	DF	AA61JL	NA	NA	0.373	31.51	5	2	NA	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	NO	% VC difference > 15		SLS-P21

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61JL-B9 sealer	DF	AA61JL	2440	40.599	0.324	0.26	5	3	0.9415	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	YES			SLS-P22
AA61JL-B10 sealer	DF	AA61JL	2780	46.256	0.214	11.21	5	3	0.9572	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	YES			SLS-P23
AA61JL-B11 sealer	DF	AA61JL	2710	45.092	0.171	16.20	5	3	0.9661	15000, 10204, 6942, 4722, 3212, 2185, 1487, 1011	1.47	NO	% VC difference > 15		SLS-P24
FRAME															
A1NG190603	RF	AA61NG	> 10,000	NA	0.965	0.22%	0	8	0.0127	10000,1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	PC failed; no points between 10 -50%; low r2; range finder		A1SLS190603
F_L.3T3.NG.A2.26.06.03	RF	AA61NG	11700	194.676	0.251	42.34%	0	2	0.7469	100000,10000,1000, 100, 10, 1.0, 0.1, 0.01	10	RF	VC difference greater than 15%; no points between 10 - 50; r2 too low; range finder		FAL.3T3.SLS.A2.26.06.03
FAL.3T3.NG.B1.03.07.03	DF	AA61NG	92500	1539.101	0.404	12.52%	0	2	0.5706	50000, 23256, 10817, 3031, 2340, 1088, 506, 235	2.15	NO	no points between 10 - 50; r2 too low		FAL.3T3.SLS.B1.03.07.03
FAL.3T3.B2.NG.10.07.03	DF	AA61NG	NA	NA	0.157	56.97%	NA	NA	NA	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	NO	% VC difference > 15; range finder	high volatility	FAL.3T3.SLS.10.07.03
FAL.3T3.NG.B3.120903	DF	AA61NG	34900	580.699	0.251	42.34	0	2	0.7468	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	No points between 0 & 50%viability; low r2; %VC difference > 15		FAL.3T3.SLS.120903
FAL.3T3.NG.B5.180903 plate sealer	DF	AA61NG	3900	64.892	0.417	3.46	4	1	0.9517	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	YES			FAL.3T3.SLS.180903
FAL.3T3.NG.B5.180903 mineral oil	DF	AA61NG	5940	98.835	0.366	8.45	5	2	0.9380	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	Mineral oil		FAL.3T3.SLS.180903
FAL.3T3.NG.B6.190903 plate sealer	DF	AA61NG	4570	76.040	0.258	17.26	5	1	0.8993	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	%VC difference > 15		FAL.3T3.SLS.190903
FAL.3T3.NG.B6.190903 mineral oil	DF	AA61NG	4740	78.869	0.384	7.46	5	2	0.9301	50000, 34014, 23139, 15741, 10708, 7284, 4955, 3371	1.47	NO	Mineral oil		FAL.3T3.SLS.190903
FAL.3T3.NG.B7.25.09.03 plate sealer	DF	AA61NG	4130	68.719	0.347	10.58	3	4	0.9244	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			FAL.3T3.SLS.250903
FAL.3T3.NG.B8.25.09.03 mineral oil	DF	AA61NG	4220	70.216	0.361	7.83	4	4	0.9513	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	NO	Mineral oil		FAL.3T3.SLS.250903
FAL.3T3.NG.B8-03-10-03 plate sealer	DF	AA61NG	3880	64.559	0.510	2.39	5	3	0.9519	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			FAL.3T3.SLS.031003

PROPRANOLOL

IIVS															
A1 Preliminary	RF	AA61GU	19.3	0.065	0.320	5.15%	0	1	0.9764	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A1
B1	DF	AA61GU	21.1	0.071	0.384	7.68%	1	1	0.9906	1000, 559.5, 313.0, 175.0, 98.0, 54.5, 30.6, 17.0 [IIVS retested; used wrong dilution scheme]	1.79	YES			SLS-B1
B2	DF	AA61GU	19.7	0.067	0.386	4.75%	0	1	0.9834	100, 56.2, 31.6, 17.8, 10.0, 5.63, 3.16, 1.78	1.78	NO	No points between 10 and 50%		SLS-B2

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B3	DF	AA61GU	13.6	0.046	0.484	4.31%	1	4	0.9443	100, 56.2, 31.6, 17.8, 10.0, 5.63, 3.16, 1.78	1.78	YES			SLS-B3
B4	DF	AA61GU	18.3	0.062	0.444	0.43%	1	3	0.9816	100, 56.2, 31.6, 17.8, 10.0, 5.63, 3.16, 1.78	1.78	NO	PC failed		SLS-B4
B5	DF	AA61GU	18.2	0.062	0.319	0.94%	1	2	0.9927	100, 56.2, 31.6, 17.8, 10.0, 5.63, 3.16, 1.78	1.78	YES			SLS-B5
ECBC															
ECBC-3T3-lb-01 AA61KH-A1	RF	AA61KH	17.5	0.059	0.279	6.15%	0	1	0.8598	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P1
ECBC-3T3-lb-02 AA61KH-B1	DF	AA61KH	11.4	0.039	0.204	1.02%	2	2	0.9384	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	PC failed		SLS-P3
ECBC-3T3-lb-03 AA61KH-B2	DF	AA61KH	16.2	0.055	0.249	4.55%	1	2	0.9601	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	PC failed		SLS-P4
ECBC-3T3-lb-04 AA61KH-B3	DF	AA61KH	12.2	0.041	0.476	16.18%	2	4	0.8629	50, 34, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	NO	VC difference > 15%		SLS-P5
ECBC-3T3-lb-05 AA61KH-B4	DF	AA61KH	11.3	0.038	0.297	4.17%	2	4	0.9493	50, 34, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			SLS-P7
ECBC-3T3-lb-06 AA61KH-B5	DF	AA61KH	8.90	0.030	0.474	9.70%	2	3	0.8932	50, 34, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			SLS-P9
ECBC-3T3-lb-07 AA61KH-B6	DF	AA61KH	18.7	0.063	0.306	3.70%	2	2	0.9475	50, 34, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			SLS-P12
ECBC-3T3-lb-08 AA61KH-B7	DF	AA61KH	15.6	0.053	0.311	11.73%	2	2	0.9549	50, 34, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			SLS-P13
FRAME															
A1 1b3T3RF01FALNM	RF	AA61NM	57.7	0.195	0.413	12.84%	0	0	0.9454	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		1b3T3CRTFALSLS 12/4/02
A2 1b3T3RF02FALNM	RF	AA61NM	0.022	0.000	0.479	8.47%	1	3	0.9694	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		1b3T3CRTFALSLS 12/10/02
1b3T3DF02FALNM	DF	AA61NM	19.8	0.067	0.350	6.58%	1	3	0.8123	35, 23.81, 16.20, 11.02, 7.50, 5.10, 3.47, 2.36	1.47	NO	PC failed		1b3T3CRTFALSLS 12/17/02
1b3T3DF02FALNM	DF	AA61NM	23.1	0.078	0.477	10.31%	1	1	0.8691	35, 23.81, 16.20, 11.02, 7.50, 5.10, 3.47, 2.36	1.47	YES		NR crystals in plate	1b3T3CRTFALSLS 1/7/03
1b3T3DF02FALNM	DF	AA61NM	23.9	0.081	0.220	13.61%	0	2	0.8821	35, 23.81, 16.20, 11.02, 7.50, 5.10, 3.47, 2.36	1.47	NO	NR crystals in plate; stopped after 1 h; no point between 10 & 50% viability; PC failed		1b3T3CRTFALSLS 1/8/03
1b3T3DF05FALNM	DF	AA61NM	13.8	0.047	0.449	8.47%	1	3	0.9401	35.000, 23.810, 16.197, 11.018, 7.495, 5.099, 3.469, 2.360	1.47	YES			1b3T3CRTFALSLS 1/14/03
1b3T3DF06FALNM	DF	AA61NM	33.3	0.113	0.300	11.67%	1	2	0.8052	35, 23.81, 16.20, 11.02, 7.50, 5.10, 3.47, 2.36	1.47	NO	PC failed		1b3T3CRTFALSLS 1/15/03
1b3T3DF07FALNM	DF	AA61NM	8.80	0.030	0.538	9.69%	1	5	0.9020	35, 23.81, 16.20, 11.02, 7.50, 5.10, 3.47, 2.36	1.47	YES			1b3T3CRTFALSLS 1/21/03
1b3T3DF08FALNM A2650	DF	AA61NM	15.2	0.051	0.223	5.91%	1	4	0.8979	35, 23.81, 16.20, 11.02, 7.50, 5.10, 3.47, 2.36	1.47	NO	NR crystals in plate; stopped after 1 h; PC failed		1b3T3CRTFALSLS 1/28/03
1b3T3DF09FALNM	DF	AA61NM	22.2	0.075	0.582	4.98%	1	0	0.9438	35.000, 23.810, 16.197, 11.018, 7.495, 5.099, 3.469, 2.360	1.47	NO	No points between 50 & 90% viability		1b3T3CRTFALSLS 2/4/03
1b3T3DF10FALNM	DF	AA61NM	8.36	0.028	0.426	12.59%	4	3	0.8917	35.000, 23.810, 16.197, 11.018, 7.495, 5.099, 3.469, 2.360	1.47	YES			1b3T3CRTFALSLS 2/5/03
1b3T3DF11FALNM	DF	AA61NM	18.5	0.063	0.227	13.72%	1	4	0.6461	35, 23.81, 16.20, 11.02, 7.50, 5.10, 3.47, 2.36	1.47	NO	r2< 0.8	Nonmonotonic curve.	1b3T3CRTFALSLS 2/26/03

PROPYLPARABEN

IIVS															
A1	RF	AA61PX	19.4	0.108	0.451	0.06%	1	2	0.9659	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-A2

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B1	DF	AA61PX	19.2	0.106	0.445	3.97%	3	4	0.9395	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		C8 removed from PRISM by SD due to the upswing of the response curve at that conc.	SLS-B5
B2	DF	AA61PX	17.0	0.094	0.354	9.68%	4	4	0.9707	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES			SLS-B9
B3	DF	AA61PX	15.0	0.083	0.368	7.51%	4	4	0.9675	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES			SLS-B10
ECBC															
AA61PK-A1	RF	AA61PK	22.9	0.127	0.231	2.13%	1	2	0.9271	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-P7
AA61PK-B1	DF	AA61PK	18.2	0.101	0.561	6.97%	4	4	0.9538	215, 100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01	2.15	YES			SLS-P26
AA61PK-B2	DF	AA61PK	19.8	0.110	0.543	6.49%	4	4	0.9827	215, 100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01	2.15	YES			SLS-P28
AA61PK-B3	DF	AA61PK	21.8	0.121	0.367	17.33%	3	5	0.9431	215, 100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01	2.15	NO	% VC difference > 15		SLS-P30
AA61PK-B4	DF	AA61PK	24.6	0.137	0.341	7.63%	2	5	0.9812	215, 100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01	2.15	YES			SLS-P32
FRAME															
FAL.3T3.HT.A1.01/04/04	RF	AA61HT	73.5	0.408	0.229	5.88%	2	1	0.8795	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2	FAL.3T3.SLS.01/04/04
FAL.3T3.HT.B1.29/04/04	DF	AA61HT	41.3	0.229	0.193	7.55%	1	4	0.7787	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		NR crystals; high background	FAL.3T3.SLS.29/04/04
FAL.3T3.HT.B2.07/05/04	DF	AA61HT	45.3	0.251	0.278	8.04%	4	2	0.9762	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.3T3.SLS.07/05/04
FAL.3T3.HT.B3.20/05/04	DF	AA61HT	68.7	0.381	0.332	10.50%	3	3	0.9633	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.3T3.SLS.20/05/04
SODIUM ARSENITE															
IIVS															
A1	RF	AA61MV	0.454	0.003	0.368	14.66%	2	3	0.9583	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A5
B1	DF	AA61MV	0.745	0.006	0.418	11.53%	3	3	0.9754	3.00, 2.00, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES		SD removed 7 data points from PRISM analysis; considered them outliers even though EXCEL macros did not identify as such	SLS-B4
B2	DF	AA61MV	0.755	0.006	0.414	5.16%	3	4	0.9674	3.00, 2.00, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES			SLS-B7
B3	DF	AA61MV	0.548	0.004	0.464	4.40%	4	4	0.9506	3.00, 2.00, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES			SLS-B8
ECBC															
AA61KA-A1	RF	AA61KA	0.483	0.004	0.506	2.78%	3	3	0.9940	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-16
AA61KA-B1	DF	AA61KA	0.482	0.004	0.565	4.68%	4	4	0.9795	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			SLS-P41
AA61KA-B2	DF	AA61KA	0.528	0.004	0.739	1.30%	2	4	0.9661	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			SLS-P43
AA61KA-B3	DF	AA61KA	0.477	0.004	0.617	1.99%	2	4	0.9795	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			SLS-P43
FRAME															
FAL.3T3.GS.A1.21.10.04	RF	AA61GS	1.11	0.009	0.254	4.69%	0	3	0.9858	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0-50%	ppt in 2X C1	FAL.3T3.SLS.21.10.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.GS.B1.11.11.04	DF	AA61GS	0.678	0.005	0.731	1.75%	8	0	0.9745	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	no points between 50-100%	ppt in 1X C1-C8	FAL.3T3.SLS.10.11.04
FAL.3T3.GS.B2.25.11.04	DF	AA61GS	0.872	0.007	0.381	2.01%	3	1	0.9740	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			FAL.3T3.SLS.25.11.04
FAL.3T3.GS.B3.26.11.04	DF	AA61GS	1.07	0.008	0.299	7.88%	1	2	0.9795	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES		outliers removed bySD	FAL.3T3.SLS.26.11.04
FAL.3T3.GS.B4.02.12.04	DF	AA61GS	2.38	0.018	0.232	12.64%	2	2	0.9073	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			FAL.3T3.SLS.02.12.04 (SW)
SODIUM CHLORIDE															
IIVS															
A1	RF	AA61PE	3400	58.249	0.474	1.86%	1	6	0.9680	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A3
B1	DF	AA61PE	5160	88.367	0.496	1.02%	2	6	0.9548	10000, 7143, 5102, 3644, 2603, 1859, 1328, 949	1.4	YES			SLS-B4
B2	DF	AA61PE	5120	87.557	0.391	6.63%	3	3	0.9651	20000, 13333, 8889, 5926, 3951, 2634, 1756, 1171	1.5	YES			SLS-B7
B3	DF	AA61PE	4350	74.352	0.450	5.91%	2	4	0.9484	20000, 13333, 8889, 5926, 3951, 2634, 1756, 1171	1.5	YES			SLS-B8
ECBC															
AA61JW-A1	RF	AA61JW	4140	70.842	0.365	0.96%	1	6	0.9393	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-11
AA61JW-B1	DF	AA61JW	5050	86.355	0.538	7.42%	2	6	0.9446	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-27
AA61JW-B2	DF	AA61JW	4720	80.777	0.449	8.39%	2	6	0.9401	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-29
AA61JW-B3	DF	AA61JW	4600	78.757	0.519	5.06%	2	6	0.9369	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P31
FRAME															
FAL.3T3.FM.A1.21.05.04	RF	AA61FM	3540	60.574	0.396	0.86%	1	4	0.9371	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.21.05.04
FAL.3T3.FM.B1.04.06.04	DF	AA61FM	3010	51.557	0.452	18.08%	2	3	0.8138	20000, 9302, 4327, 2012, 936, 435, 202, 94.2	2.15	NO	% VC difference > 15		FAL.3T3.SLS.04.06.04
FAL.3T3.FM.B2.17.06.04	DF	AA61FM	4500	76.964	0.538	0.15%	2	6	0.9728	20000, 9302, 4327, 2012, 936, 435, 202, 94.2	2.15	YES			FAL.3T3.SLS.17.06.04
FAL.3T3.FM.B3.08.07.04	DF	AA61FM	4010	68.595	0.322	7.57%	2	4	0.9618	20000, 9302, 4327, 2012, 936, 435, 202, 94.2	2.15	YES			FAL.3T3.SLS.08.07.04
FAL.3T3.FM.B4.09.07.04	DF	AA61FM	4520	77.320	0.384	3.06%	2	3	0.7556	20000, 9302, 4327, 2012, 936, 435, 202, 94.2	2.15	YES			FAL.3T3.SLS.09.07.04
FAL.3T3.FM.B5.16.07.04	DF	AA61FM	5470	93.603	0.399	4.36%	1	3	0.9361	20000, 9302, 4327, 2012, 936, 435, 202, 94.2	2.15	YES			FAL.3T3.SLS.16.07.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
SODIUM DICHROMATE DIHYDRATE															
IIVS															
A1	RF	AA61FP	0.642	0.002	0.380	5.57%	1	1	0.9860	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A4
B1	DF	AA61FP	0.548	0.002	0.502	2.42%	5	3	0.9910	3.00, 2.00, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES			SLS-B4
B2	DF	AA61FP	0.527	0.002	0.435	0.54%	4	4	0.9751	2.47, 1.65, 1.10, 0.733, 0.489, 0.326, 0.217, 0.145	1.5	YES			SLS-B7
B3	DF	AA61FP	0.455	0.002	0.449	3.30%	5	3	0.9931	3.00, 2.00, 1.33, 0.889, 0.593, 0.395, 0.263, 0.176	1.5	YES			SLS-B8
ECBC															
AA61NT-A1	RF	AA61NT	0.561	0.002	0.291	2.92%	2	1	0.9850	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder		SLS-P12
AA61NT-B1	DF	AA61NT	0.555	0.002	0.438	5.78%	4	4	0.9835	6.00, 2.79, 1.30, 0.604, 0.281, 0.131, 0.061, 0.028	2.15	YES			SLS-P32
AA61NT-B2	DF	AA61NT	0.550	0.002	0.409	9.10%	4	4	0.9713	6.00, 2.79, 1.30, 0.604, 0.281, 0.131, 0.061, 0.028	2.15	YES			SLS-P34
AA61NT-B3	DF	AA61NT	0.703	0.002	0.654	1.90%	3	5	0.9871	6.00, 2.79, 1.30, 0.604, 0.281, 0.131, 0.061, 0.028	2.15	YES			SLS-P36
FRAME															
FAL.3T3.HK.A1.10.09.04	RF	AA61HK	0.871	0.003	0.496	11.79%	5	0	0.9710	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50 - 100%		FAL.3T3.SLS.10.09.04
FAL.3T3.HK.B1.16.09.04	DF	AA61HK	NA	NA	0.343	4.69%	0	0	NA	10.0, 4.7, 2.2, 1.0, 0.5, 0.2, 0.101, 0.047	2.15	NO	no points between 0 - 100%		FAL.3T3.SLS.16.09.04
FAL.3T3.HK.B2.23.09.04	DF	AA61HK	0.388	0.001	0.367	3.41%	2	1	0.9713	1.00, 0.680, 0.463, 0.315, 0.214, 0.146, 0.099, 0.067	1.47	YES			FAL.3T3.SLS.23.09.04
FAL.3T3.HK.B3.14.10.0404	DF	AA61HK	0.864	0.003	0.340	3.67%	1	7	0.9167	1.00, 0.752, 0.565, 0.425, 0.320, 0.240, 0.181, 0.136	1.33	YES			FAL.3T3.SLS.14.10.04
FAL.3T3.HK.B4.04.11.04	DF	AA61HK	0.719	0.002	0.265	8.67%	2	3	0.7857	1.00, 0.752, 0.565, 0.425, 0.320, 0.240, 0.181, 0.136	1.33	YES			FAL.3T3.SLS.04.11.04
SODIUM I FLUORIDE															
IIVS															
A1	RF	AA61HF	59.2	1.410	0.526	0.64%	1	3	0.9854	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61HF	86.7	2.065	0.391	0.28%	2	4	0.9788	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B1
B2	DF	AA61HF	75.5	1.798	0.512	5.46%	3	3	0.9857	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B2
B3	DF	AA61HF	71.4	1.700	0.541	9.13%	2	2	0.9894	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	NO	PC failed		SLS-B3
B4	DF	AA61HF	83.8	1.996	0.465	2.42%	3	3	0.9676	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B4
ECBC															
AA61MG-A1	RF	AA61MG	61.7	1.469	0.361	7.05%	0	0	0.9569	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	PC failed; no points between 10 - 90%; range finder		SLS-P2

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61MG-B1	DF	AA61MG	59.7	1.422	0.597	4.34%	1	2	0.9567	200, 136.1, 92.6, 63.0, 42.8, 29.1, 19.8, 13.5	1.47	YES			SLS-P6
AA61MG-B2	DF	AA61MG	56.8	1.353	0.566	1.90%	3	4	0.9553	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES			SLS-P7
AA61MG-B3	DF	AA61MG	67.5	1.608	0.522	6.32%	3	2	0.9336	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES			SLS-P10
FRAME															
FAL.3T3.A1.RH.200603	RF	AA61RH	208	4.954	0.716	0.48%	1	0	0.9733	10000,1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	no points between 50 - 90%; range finder		FAL.3T3.SLS2.A1.200603
FAL.3T3.B1.RH.27.06.03	DF	AA61RH	102	2.429	0.425	2.23%	2	1	0.9119	150, 102.0, 69.4, 47.2, 32.1, 21.8, 14.9, 10.1	1.47	YES			FAL.3T3.SLS.A2.27.06.03
FAL.3T3.B2.RH.04.07.03	DF	AA61RH	85.9	2.046	0.568	0.12%	2	1	0.9438	300, 204, 139, 94.4, 64.2, 43.7, 29.7, 20.2	1.47	NO	PC failed		FAL.3T3.SLS.04.07.03
FAL.3T3.B3.RH.11.07.03	DF	AA61RH	76.0	1.810	0.575	3.23%	2	1	0.9762	300, 204, 139, 94.4, 64.2, 43.7, 29.7, 20.2	1.47	YES			FAL.3T3.SLS.11.07.03
FAL.3T3.B4.RH.18.07.03	DF	AA61RH	110	2.620	0.552	4.70%	2	1	0.9301	300, 204, 139, 94.4, 64.2, 43.7, 29.7, 20.2	1.47	YES			FAL.3T3.SLS.18.07.03
SODIUM HYPOCHLORITE															
IIVS															
A1	RF	AA61RD	310	4.171	0.414	28.60%	1	4	0.9878	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	VC1 ODs < VC2 ODs; VC1 removed from subsequent analysis; volatility issues.	SLS-A1
B1	DF	AA61RD	NA	NA	0.464	1.53%	0	5	NA	1000, 667, 444, 296, 198, 132, 87.8, 58.5	1.5	NO	no points between 0 - 50%		SLS-B4
B1 (should be B2)	DF	AA61RD	1110	14.866	0.425	2.24%	2	1	0.9708	10000, 7143, 5102, 3644, 2603, 1859, 1328, 949	1.4	YES		plate sealer used	SLS-B6
B3	DF	AA61RD	1600	21.537	0.446	5.64%	3	2	0.9810	10000, 7143, 5102, 3644, 2603, 1859, 1328, 949	1.4	YES		plates read 15-16 hr late; orignial reading used wrong OD wavelength; plate sealer used	SLS-B11
B4	DF	AA61RD	2170	29.187	0.404	9.58%	2	6	0.8825	4000, 2857, 2041, 1458, 1041, 744, 531, 379	1.4	YES			SLS-B12
B5	DF	AA61RD	3140	42.188	0.431	0.64%	1	3	0.9519	4000, 2857, 2041, 1458, 1041, 744, 531, 379	1.4	YES		plate sealer used	SLS-B15
ECBC															
AA61HE-A1	RF	AA61HE	NA	NA	0.241	44.19%	1	1	0.0000	10000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P3
AA61HE-A2	RF	AA61HE	600	8.057	0.409	0.71%	1	1	0.6930	1000, 100,10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P17
AA61HE-B1	DF	AA61HE	728	9.777	0.418	6.56%	2	6	0.9476	1000, 826, 683, 565, 467, 386, 319, 263	1.21	YES			SLS-P19
AA61HE-B2	DF	AA61HE	802	10.769	0.550	3.94%	3	5	0.8389	1210, 1000, 826, 683, 565, 467, 386, 319	1.21	YES			SLS-P22
AA61HE-B3	DF	AA61HE	940	12.624	0.603	3.31%	2	5	0.9363	1210, 1000, 826, 683, 565, 467, 386, 319	1.21	YES			SLS-P23
FRAME															
FAL.3T3.LU.A1.09/01/04	RF	AA61LU	1060	14.295	0.483	0.62%	0	1	0.9323	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.3T3.SLS.09/01/04
FAL3T3.LU.A2.16.01.04	DF	AA61LU	391	5.250	0.897	4.13%	3	5	0.7288	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			FAL.3T3.SLS.16/01/04
FAL3T3.LU.B1.23.01.04	DF	AA61LU	1090	14.696	0.505	7.27%	1	2	0.9546	5000, 2325.6, 1081.7, 503.1, 234.0, 108.8, 50.6, 23.5	2.15	YES			FAL3T3.23-01-04
FAL3T3.LU.B2.30.01.04	DF	AA61LU	935	12.566	0.401	11.07%	3	2	0.9787	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	YES		steep toxicity curve; will adjust concentrations for B3 to 2500 ug/ml (1.47 dil)	FAL.3T3.SLS.29/01/04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL3T3.LU.B3.06-02-04	DF	AA6 LU	923	12.393	0.361	18.60%	1	3	0.9557	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	NO	%VC difference >15; possible volatility problem	VC1 ODs lower than VC2 ODs	FAL.3T3.SLS.06/02/04
SODIUM OXALATE															
IIVS															
A1	RF	AA61GX	24.9	0.186	0.341	1.34%	1	4	0.9800	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	ppt in 1X C1 and 2X C1	SLS-A5
B1	DF	AA61GX	19.7	0.147	0.435	3.04%	5	3	0.9762	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES		ppt in 2X C1; ppt in 1X C1-C3	SLS-B6
B2	DF	AA61GX	37.9	0.283	0.472	1.09%	3	5	0.9774	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES		ppt in 2X C1-C4; plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B3	DF	AA61GX	80.2	0.598	0.349	13.14%	1	4	0.9617	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES		ppt in 2X C1-C2; ppt in 1X C1-C4	SLS-B12
B4	DF	AA61GX	60.1	0.449	0.509	1.26%	2	6	0.9495	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.5	YES		ppt in 2X C1	SLS-B15
ECBC															
AA61LZ-A1	RF	AA61LZ	55.3	0.413	0.544	0.17%	1	4	0.9689	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1; ppt in 1X in C1-C2	SLS-15
AA61LZ-B1	DF	AA61LZ	49.9	0.372	0.455	3.70%	3	5	0.9871	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 2X C1-C2; ppt in 1X C1-C5	SLS-P65
AA61LZ-B2	DF	AA61LZ	54.0	0.403	0.527	6.96%	3	5	0.9578	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 2X C1-C2; ppt in 1X in C1-C5	SLS-P66
AA61LZ-B3	DF	AA61LZ	22.2	0.166	0.450	3.43%	3	3	0.9836	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 2X C1-C2; ppt in 1X C1-C6	SLS-P68
FRAME															
FAL.3T3.RC.A1.21.10.04	RF	AA61RC	74.6	0.557	0.291	1.60%	3	0	0.9198	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50-100%	ppt in 2X C1; ppt in 1X in C1-C3	FAL.3T3.SLS.21.10.04
FAL.3T3.RC.B1.11.11.04	DF	AA61RC	28.8	0.215	0.471	13.15%	5	0	0.8505	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	NO	no points between 50-100%	ppt in 1X C1-C8	FAL.3T3.SLS.10.11.04
FAL.3T3.RC.B2.25.11.04	DF	AA61RC	34.5	0.258	0.369	4.78%	1	1	0.8807	250, 116, 54.1, 25.2, 11.7,5.44, 2.53, 1.18	2.15	YES		ppt in 2X C1; ppt in 1X in C1-C4	FAL.3T3.SLS.25.11.04
FAL.3T3.RC.B3.26.11.04	DF	AA61RC	37.3	0.279	0.309	3.13%	1	1	0.9655	250, 116, 54.1, 25.2, 11.7,5.44, 2.53, 1.18	2.15	YES		ppt in 2X C1; ppt in 1X in C1-C4;	FAL.3T3.SLS.26.11.04
FAL.3T3.RC.B4.02.12.04	DF	AA61RC	235	1.753	0.282	2.19%	1	0	0.2212	250, 116, 54.1, 25.2, 11.7,5.44, 2.53, 1.18	2.15	NO	no points between 50-100%	C7 gives > 200% viability; ppt in 2X C1 and 1X C1-C2	FAL.3T3.SLS.02.12.04 (RB)
FAL.3T3.RC.B5.09.12.04	DF	AA61RC	21.1	0.157	0.380	8.96%	2	2	0.8788	250, 116, 54.1, 25.2, 11.7,5.44, 2.53, 1.18	2.15	YES		ppt in 2X C1; ppt in 1X in C1-C4	FAL.3T3.SLS.09.12.04
SODIUM SELENATE															
IIVS															
A1	RF	AA61FS	39.2	0.208	0.540	3.31%	1	2	0.9909	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1
B1	DF	AA61FS	42.3	0.224	0.407	0.61%	5	3	0.9884	300, 200, 133, 89, 59.3, 39.5, 26.3, 17.6	1.5	YES			SLS-B1
B2	DF	AA61FS	35.2	0.186	0.507	2.69%	6	2	0.9874	300, 200, 133, 89, 59.3, 39.5, 26.3, 17.6	1.5	YES			SLS-B2
B3	DF	AA61FS	40.0	0.212	0.504	9.65%	5	2	0.9879	300, 200, 133, 89, 59.3, 39.5, 26.3, 17.6	1.5	NO	PC failed		SLS-B3
B4	DF	AA61FS	32.1	0.170	0.458	0.02%	5	2	0.9884	300, 200, 133, 89, 59.3, 39.5, 26.3, 17.6	1.5	YES			SLS-B4
ECBC															
AA61LF-A1	RF	AA61LF	6.04	0.032	0.438	0.99%	1	2	0.9663	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	PC failed; range finder		SLS-P2
AA61LF-B1	DF	AA61LF	13.6	0.072	0.537	2.38%	3	2	0.9271	100, 68.1, 46.3, 31.5, 21.4, 14.6, 9.9, 6.8	1.47	YES			SLS-P6

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61LF-B2	DF	AA61LF	13.8	0.073	0.597	3.18%	4	2	0.9754	100, 68.1, 46.3, 31.5, 21.4, 14.6, 9.9, 6.8	1.47	YES			SLS-P8
AA61LF-B3	DF	AA61LF	10.8	0.057	0.569	3.10%	3	2	0.9626	100, 68.1, 46.3, 31.5, 21.4, 14.6, 9.9, 6.8	1.47	YES			SLS-P10
FRAME															
FAL.3T3.A1.NS.200603	RF	AA61NS	221	1.170	0.670	1.17%	0	0	0.9739	10000,1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	no points between 10 - 90%; range finder		FAL.3T3.SLS2.A1.200603
FAL.3T3.B1.NS.27.06.03	DF	AA61NS	62.4	0.330	0.497	3.76%	1	1	0.8042	120, 81.6, 55.5, 37.8, 25.7, 17.5, 11.9, 8.1	1.47	YES			FAL.3T3.SLS.A2.27.06.03
FAL.3T3.B2.NS.04.07.03	DF	AA61NS	52.6	0.278	0.525	2.86%	2	1	0.9189	200, 136, 92.6, 63, 42.8, 29.2, 19.8, 13.5	1.47	NO	PC failed		FAL.3T3.SLS.04.07.03
FAL.3T3.B3.NS.11.07.03	DF	AA61NS	57.7	0.305	0.555	5.84%	2	1	0.9734	200, 136, 92.6, 63, 42.8, 29.2, 19.8, 13.5	1.47	YES			FAL.3T3.SLS.11.07.03
FAL.3T3.B4.NS.17.07.03	DF	AA61NS	42.4	0.224	0.666	2.83%	2	1	0.9758	200, 136, 92.6, 63, 42.8, 29.2, 19.8, 13.5	1.47	YES			FAL.3T3.SLS.17.07.03
STRYCHNINE															
IIVS															
A1	RF	AA61JY	77.8	0.233	0.337	1.56%	1	0	0.8728	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A5
B1	DF	AA61JY	89.7	0.268	0.489	1.52%	1	3	0.8961	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES			SLS-B4
B2	DF	AA61JY	80.2	0.240	0.355	6.46%	1	2	0.8383	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES			SLS-B7
B3	DF	AA61JY	80.7	0.241	0.434	7.93%	1	2	0.9277	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES		ppt in 2X C1; slight film of powder on medium surface	SLS-B8
ECBC															
AA61NR-A1	RF	AA61NR	NA	NA	0.317	12.60%	1	4	NA	500, 50.0, 5.0, 0.5, 0.05, 0.005, 0.0005, 0.00005	10	RF	range finder	ppt in 2X C1	SLS-P49
AA61NR-B1	DF	AA61NR	NA	NA	0.431	6.47%	8	0	NA	800, 661, 546, 452, 373, 308, 255, 211	1.21	NO	no points between 50 - 100%	ppt in 2X C1-C7; dilution is 1.21 but no points have greater than 50% viability	SLS-P65
AA61NR-B2	DF	AA61NR	452	1.351	0.526	5.34%	2	6	0.8969	800, 544, 370, 252, 171, 117, 79.3, 53.9	1.47	YES		ppt in 2X C1-C4; ppt in 1X C1	SLS-P66
AA61NR-B3	DF	AA61NR	418	1.249	0.461	0.27%	2	5	0.9559	800, 544, 370, 252, 171, 117, 79.3, 53.9	1.47	YES		ppt in 2X C1-C2	SLS-P68
AA61NR-B4	DF	AA61NR	298	0.891	0.410	5.55%	1	6	0.8163	800, 544, 370, 252, 171, 117, 79.3, 53.9	1.47	YES		ppt in 2X C1; ppt in 1X C1	SLS-P70
FRAME															
FAL.3T3.FY.A1.21.10.04	RF	AA61FY	133	0.397	0.362	10.70%	1	0	0.5214	250, 25, 2.5, 0.25, 0.025, 0.0025, 0.00025, 0.000025	10	RF	range finder; no points between 50-100%		FAL.3T3.SLS.21.10.04
FAL.3T3.FY.B1.25.11.04	DF	AA61FY	108	0.322	0.436	8.15%	5	2	0.8455	250, 207, 171, 141, 117, 96.4, 79.7, 65.8	1.21	YES			FAL.3T3.SLS.25.11.04
FAL.3T3.FY.B2.26.11.04	DF	AA61FY	118	0.352	0.289	2.16%	5	2	0.9110	250, 207, 171, 141, 117, 96.4, 79.7, 65.8	1.21	YES		steep toxicity curve	FAL.3T3.SLS.26.11.04
FAL.3T3.FY.B3.02.12.04	DF	AA61FY	NA	NA	0.258	2.30%	0	0	NA	250, 207, 171, 141, 117, 96.4, 79.7, 65.8	1.21	NO	no points between 0-100%	no toxicity values less than 140% viability	FAL.3T3.SLS.02.12.04 (SW)
FAL.3T3.FY.B4.09.12.04	DF	AA61FY	147	0.440	0.350	0.00%	4	3	0.7540	250, 207, 171, 141, 117, 96.4, 79.7, 65.8	1.21	YES			FAL.3T3.SLS.09.12.04
THALLIUM I SULFATE															
IIVS															
A1	RF	AA61KJ	7.74	0.015	0.407	4.94%	2	3	0.9809	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A1
B1	DF	AA61KJ	5.31	0.011	0.466	2.22%	6	2	0.9348	50.0, 31.3, 19.5, 12.2, 7.63, 4.77, 2.98, 1.86	1.6	YES			SLS-B4

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B2	DF	AA61KJ	8.29	0.02	0.357	10.53%	4	4	0.9392	50.0, 31.3, 19.5, 12.2, 7.63, 4.77, 2.98, 1.86	1.6	YES		outlier removed bySD	SLS-B7
B3	DF	AA61KJ	5.22	0.01	0.454	0.66%	5	3	0.9603	50.0, 31.3, 19.5, 12.2, 7.63, 4.77, 2.98, 1.86	1.6	YES			SLS-B8
ECBC															
AA61PB-A1	RF	AA61PB	5.41	0.011	0.362	9.63%	3	5	0.9706	500, 50.0, 5.0, 0.5, 0.05, 0.005, 0.0005, 0.00005	10	RF	range finder		SLS-P49
AA61PB-B1	DF	AA61PB	NA	NA	0.509	7.59%	6	2	NA	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	PC failed		SLS-P53
AA61PB-B2	DF	AA61PB	3.46	0.007	0.703	7.58%	5	3	0.9831	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P54
AA61PB-B3	DF	AA61PB	2.12	0.004	0.539	11.54%	6	2	0.9629	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P57
AA61PB-B4	DF	AA61PB	2.86	0.006	0.399	3.57%	3	5	0.9627	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P59
FRAME															
FAL.3T3.GB.A1.09/01/04	RF	AA61GB	0.015	0.000	0.664	4.29%	1	3	0.9201	0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001, 0.0000001, 0.00000001	10	RF	range finder		FAL.3T3.SLS.09/01/04
FAL3T3.GB.A2.16.01.04	DF	AA61GB	2.01	0.004	0.861	8.61%	7	1	0.8562	250.0, 116.0, 54.1, 25.2, 11.8, 5.4, 2.5, 1.2	2.15	YES			FAL.3T3.SLS.16/01/04
FAL3T3.GB.B1.23.01.04	DF	AA61GB	13.6	0.027	0.552	1.48%	3	3	0.9318	250, 79.1, 25.0, 7.9, 2.5, 0.8, 0.25, 0.08	3.16	YES		difficult to get above 250 ug/ml; unlikely to reach 100% toxicity	FAL3T3.23-01-04
FAL3T3.GB.B2.30.01.04	DF	AA61GB	27.1	0.054	0.422	2.70%	3	3	0.9382	500, 158.7, 50.4, 16.0, 5.1, 1.6, 0.5, 0.2	3.15	YES		slow increase in toxicity; reached 90% toxicity;	FAL.3T3.SLS.29/01/04
FAL3T3.GB.B3.06-02-04	DF	AA61 GB	10.9	0.022	0.412	3.80%	3	5	0.9648	500, 158.7, 50.4, 16.0, 5.1, 1.6, 0.5, 0.2	3.15	YES			FAL.3T3.SLS.06/02/04

TRICHLOROACETIC ACID

IIVS															
A1	RF	AA61MR	637	3.897	0.387	5.74%	2	1	0.9378	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1	SLS-A4
B1	DF	AA61MR	861	5.269	0.510	3.85%	3	5	0.9807	3000, 2000, 1333, 889, 593, 395, 263, 176	1.5	YES		outlier removed bySD	SLS-B4
B2	DF	AA61MR	873	5.343	0.351	6.44%	3	5	0.9556	3000, 2000, 1333, 889, 593, 395, 263, 176	1.5	YES			SLS-B7
B3	DF	AA61MR	670	4.100	0.423	0.22%	4	4	0.9652	3000, 2000, 1333, 889, 593, 395, 263, 176	1.5	YES			SLS-B8
ECBC															
AA61KT-A1	RF	AA61KT	977	5.981	0.403	6.66%	2	2	0.9703	10000,1000, 100, 10, 1,0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1	SLS-P13
AA61KT-B1	DF	AA61KT	859	5.257	0.408	5.82%	4	3	0.9878	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES		ppt in 1X C1	SLS-P33
AA61KT-B2	DF	AA61KT	NA	NA	0.585	1.42%	1	0	NA	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	NO	no points between 50 - 100%; closest point is 100.0%	SD rejected test; ppt in 1X C1	SLS-P35
AA61KT-B3	DF	AA61KT	767	4.696	0.491	0.48%	4	4	0.9890	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES		ppt in 1X C1	SLS-P37
AA61KT-B4	DF	AA61KT	661	4.043	0.403	0.04%	4	4	0.9878	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES		ppt in 1X C1	SLS-P40
FRAME															
FAL.3T3.GH.A1.10.09.04	RF	AA61GH	1380	8.428	0.459	10.90%	1	2	0.9027	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1	FAL.3T3.SLS.10.09.04
FAL.3T3.GH.1.16.09.04	DF	AA61GH	1240	7.564	0.394	2.96%	2	3	0.9170	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	YES			FAL.3T3.SLS.16.09.04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.GH.B2.15.10.04	DF	AA61GH	1140	6.962	0.302	14.14%	2	3	0.9396	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	YES			FAL.3T3.SLS.15.10.04
FAL.3T3.GH.B3.28.10.04	DF	AA61GH	1280	7.830	0.188	9.65%	2	2	0.9091	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	YES			FAL.3T3.SLS.28.10.04
1,1,1-TRICHLOROETHANE															
IIVS															
A1	RF	AA61KG	5900	44.240	0.312	5.85%	1	6	0.6051	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A5
B1	DF	AA61KG	9710	72.746	0.446	10.58%	1	0	0.9158	10000, 7692, 5917, 4552, 3501, 2693, 2072, 1594	1.3	NO	no points between 50 - 100%	ppt in 2X C1	SLS-B6
B2	DF	AA61KG	9840	73.758	0.474	5.24%	1	6	0.7420	10000, 8333, 6944, 5787, 4823, 4019, 3349, 2791	1.2	YES		ppt in 2X C1; plates read 15-16 hr late; orignial reading used wrong OD wavelength	SLS-B11
B3	DF	AA61KG	10000	75.303	0.355	0.14%	0	4	0.8872	10000, 8333, 6944, 5787, 4823, 4019, 3349, 2791	1.2	YES	no points between 0 - 50%;	ppt in 2X C1; passes because of 1.2 dilution factor	SLS-B12
B4	DF	AA61KG	9640	72.246	0.490	2.52%	1	2	0.9252	10000, 8333, 6944, 5787, 4823, 4019, 3349, 2791	1.2	YES		ppt in 2X C1	SLS-B15
ECBC															
AA61JV-A1	RF	AA61JV	NA	NA	0.565	0.77%	0	4	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-15
AA61JV-B1 (sealer)	DF	AA61JV	NA	NA	0.621	5.88%	0	8	NA	30000, 24793, 20490, 16934, 13995, 11566, 9559, 7900	1.21	NO	PC failed; no points between 0 - 50%	dilution factor is 1.21; no points between 0-50%; test would pass due to dilution factor; ppt in 2X C4	SLS-P61
AA61JV-B2 (sealer)	DF	AA61JV	41100	308.185	0.353	2.97%	3	5	0.6525	50000, 41322, 34151, 28224, 23325, 19277, 15932, 13167	1.21	YES		ppt in 2X C1-C5; chemical made pipets sticky and corrosive to the reservoir	SLS-P64
AA61JV-B3 (sealer)	DF	AA61JV	NA	NA	0.448	5.01%	?	?	NA	50000, 41322, 34151, 28224, 23325, 19277, 15932, 13167	1.21	NO	can't properly determine points between 0 - 100%	"roller coaster" toxicity curve; chemical physically intereacted with plastic pipets; ppt in 2X C1-C8 (oily)	SLS-P73
FRAME															
FAL.3T3.PN.A1.21.10.04	RF	AA61PN	NA	NA	0.315	6.33%	0	0	0.0000	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0-100%		FAL.3T3.SLS.21.10.04
FAL.3T3.PN.B1.04.11.04	DF	AA61PN	18400	137.661	0.285	9.94%	1	2	0.6655	25000, 17007, 11569, 7870, 5354, 3642, 2478, 1686	1.47	YES		ppt in 1X C1	FAL.3T3.SLS.04.11.04
FAL.3T3.PN.B2.19.11.04	DF	AA61PN	20600	154.458	0.278	4.29%	2	0	0.7843	25000, 20661, 17075, 14112, 11663, 9639, 7966, 6583	1.21	YES	no points between 50-100%	test passes because lowest dilution factor used (1.21); ppt in 2X C1-C2	FAL.3T3.SLS.19.11.04
FAL.3T3.PN.B3.25.11.04	DF	AA61PN	22000	165.125	0.365	1.64%	1	2	0.6250	25000, 20661, 17075, 14112, 11663, 9639, 7966, 6583	1.21	YES		ppt in 2X C1; ppt in 1X C1; C8 concentration shows high toxicity	FAL.3T3.SLS.25.11.04
FAL.3T3.PN.B4.26.11.04	DF	AA61PN	24000	179.809	0.331	2.57%	2	4	0.1704	25000, 20661, 17075, 14112, 11663, 9639, 7966, 6583	1.21	YES		ppt in 2X C1-C4;	FAL.3T3.SLS.26.11.04
TRIETHYLENEMELAMINE															
IIVS															
A1	RF	AA61MT	0.214	0.0010	0.338	10.51%	2	4	0.9591	10.0, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder	ppt in 2000ug/ml stock in DMSO	SLS-A2

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B1	DF	AA61MT	0.223	0.0011	0.497	2.36%	4	4	0.9169	1.00, 0.625, 0.391, 0.244, 0.153, 0.095, 0.060, 0.037	1.6	YES			SLS-B5
B2	DF	AA61MT	0.127	0.0006	0.377	3.14%	5	3	0.9339	2.00, 1.11, 0.617, 0.343, 0.191, 0.106, 0.059, 0.033	1.8	YES			SLS-B9
B3	DF	AA61MT	0.156	0.0008	0.321	8.67%	5	3	0.9469	2.00, 1.11, 0.617, 0.343, 0.191, 0.106, 0.059, 0.033	1.8	YES			SLS-B10
ECBC															
AA61GE-A1 revised by	RF	AA61GE	0.2	0.0010	0.256	6.24%	2	5	0.9389	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-P9
AA61GE-B1	DF	AA61GE	0.117	0.0006	0.424	19.49%	5	3	0.9178	4.00, 1.86, 0.685, 0.402, 0.187, 0.087, 0.40, 0.019	2.15	NO	% VC difference > 15	ppt in 2X C1	SLS-P19
AA61GE-B2	DF	AA61GE	0.0766	0.0004	0.375	6.15%	6	2	0.9339	4.00, 1.86, 0.685, 0.402, 0.187, 0.087, 0.40, 0.019	2.15	YES			SLS-P21
AA61GE-B3	DF	AA61GE	0.0951	0.0005	0.599	3.52%	2	6	0.9594	4.00, 1.86, 0.685, 0.402, 0.187, 0.087, 0.40, 0.019	2.15	YES			SLS-P24
AA61GE-B4	DF	AA61GE	0.0861	0.0004	0.563	11.19%	2	6	0.9512	4.00, 1.86, 0.685, 0.402, 0.187, 0.087, 0.40, 0.019	2.15	YES			SLS-P26
FRAME															
FAL.3T3.LB.A1.01/04/04	RF	AA61LB	2.83	0.0138	0.270	4.91%	1	1	0.7626	10.0, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001,	10	RF	range finder		FAL.3T3.SLS.01/04/04
FAL.3T3.LB.B1.29/04/04	DF	AA61LB	1.44	0.0071	0.289	3.08%	3	3	0.8508	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.506, 0.235	2.15	YES		NR crystals; high background	FAL.3T3.SLS.29/04/04
FAL.3T3.LB.B2.07/05/04	DF	AA61LB	1.72	0.0084	0.269	3.01%	7	1	0.9859	25.0, 17.0, 11.6, 7.9, 5.4, 3.6, 2.5, 1.7	1.47	YES			FAL.3T3.SLS.07/05/04
FAL.3T3.LB.B3.20/05/04	DF	AA61LB	1.19	0.0058	0.336	5.70%	4	3	0.9404	25.0, 11.6, 5.4, 2.5, 1.2, 0.5, 0.3, 0.1	2.15	YES			FAL.3T3.SLS.20/05/04

TRIPHENYLTIN HYDROXIDE

IIVS															
A1	RF	AA61JR	0.013	0.00004	0.456	8.54%	0	1	0.9726	10.0, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-A2
B1	DF	AA61JR	0.0206	0.00006	0.434	2.34%	3	4	0.9576	0.100, 0.0625, 0.0391, 0.0244, 0.0153, 0.00954, 0.00596, 0.00373	1.6	YES			SLS-B5
B2	DF	AA61JR	0.00547	0.00001	0.371	8.48%	3	1	0.9569	0.100, 0.0625, 0.0391, 0.0244, 0.0153, 0.00954, 0.00596, 0.00373	1.6	YES			SLS-B9
B3	DF	AA61JR	0.0184	0.00005	0.367	0.57%	3	4	0.9073	0.100, 0.0625, 0.0391, 0.0244, 0.0153, 0.00954, 0.00596, 0.00373	1.6	YES			SLS-B10
ECBC															
AA61LL-A1	RF	AA61LL	0.0132	0.00004	0.297	4.91%	1	2	0.9825	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-P7
AA61LL-B1	DF	AA61LL	0.0258	0.00007	0.569	0.10%	2	6	0.9539	0.100, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			SLS-P24

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61LL-B2	DF	AA61LL	0.0296	0.00008	0.519	1.61%	2	5	0.9359	0.100, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			SLS-P26
AA61LL-B3	DF	AA61LL	0.0212	0.00006	0.486	8.28%	2	6	0.9428	0.100, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			SLS-P28
FRAME															
FAL.3T3.GG.A1.01/04/04	RF	AA61GG	0.0143	0.00004	0.267	9.46%	3	5	0.9563	10.0, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001,	10	RF	range finder		FAL.3T3.SLS.01/04/04
FAL.3T3.GG.B1.29/04/04	DF	AA61GG	0.00286	0.00001	0.239	5.43%	1	1	0.9869	0.100, 0.047, 0.022, 0.010, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			FAL.3T3.SLS.29/04/04
FAL.3T3.GG.B2.07/05/04	DF	AA61GG	0.0314	0.00009	0.340	1.06%	1	1	0.7735	0.100, 0.0233, 0.0108, 0.0050, 0.0023, 0.0011, 0.0005, 0.0002	2.15	YES			FAL.3T3.SLS.07/05/04
FAL.3T3.GG.B3.20/05/04	DF	AA61GG	0.0438	0.00012	0.367	1.82%	2	6	0.8325	0.100, 0.047, 0.022, 0.010, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			FAL.3T3.SLS.20/05/04
VALPROIC ACID															
IIVS															
A1	RF	AA61MZ	665	4.614	0.415	7.61%	1	2	0.8257	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	small immiscible droplets initially coated insides of dilution tube in the highest 2X solution	SLS-A2
B1	DF	AA61MZ	574	3.981	0.353	10.44%	3	4	0.6749	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	YES		ppt in 2X C1-C2; test article adherred to glass pipettes upon transference to the 8-well reservoir	SLS-B6
B2	DF	AA61MZ	NA	NA	0.372	15.70%	0	4	NA	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	NO	no points between 0-50%; %VC difference >15; no toxicity detected	ppt in 2X C1	SLS-B9
B3	DF	AA61MZ	NA	NA	0.354	4.99%	0	6	NA	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	NO	no points between 0-50%	ppt in 2X C1-C4	SLS-B10
B4	DF	AA61MZ	NA	NA	0.366	1.91%	0	3	NA	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	NO	no points between 0-50%	ppt in 2X C1-C3	SLS-18
ECBC															
AA61JJ-A1	RF	AA61JJ	723	5.012	0.252	3.67%	1	3	0.8319	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P9
AA61JJ-B1	DF	AA61JJ	624	4.325	0.537	3.43%	4	2	0.9027	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES		highest 2X solution clear, oily, & orange; DMSO < 0.5%; no diff. in JJ-B2 & JJ-B3 when compared to JJ-B1 (no ppt)	SLS-P26
AA61JJ-B2	DF	AA61JJ	519	3.598	0.433	11.56%	3	4	0.8624	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES		ppt in 2X C1 - C5; oily; no diff. in JJ-B2 & JJ-B3 when compared to JJ-B1 (no ppt)	SLS-P28
AA61JJ-B3	DF	AA61JJ	499	3.460	0.379	5.14%	4	4	0.9240	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES		ppt in 2X C1 - C5; no diff. in JJ-B2 & JJ-B3 when compared to JJ-B1 (no ppt)	SLS-P30
FRAME															
FAL.3T3.GK.A1.01/04/04	RF	AA61GK	NA	NA	0.280	5.02%	0	1	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.3T3.SLS.01/04/04
FAL.3T3.GK.B1.29/04/04	DF	AA61GK	1660	11.535	0.228	7.54%	1	2	0.7855	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.3T3.SLS.29/04/04
FAL.3T3.GK.B2.07/05/04	DF	AA61GK	1760	12.219	0.284	7.69%	1	2	0.4313	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.3T3.SLS.07/05/04

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD^2	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.3T3.GK.B3.20/05/04	DF	AA61GK	2000	13.837	0.337	0.94%	1	2	0.5501	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.3T3.SLS.20/05/04
VERAPAMIL HCL															
IIVS															
A1	RF	AA61NH	38.1	0.078	0.266	2.04%	0	0	0.5147	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	solvent controls treated with 1% DMSO, rather than 0.5%.	SLS-A4
B1	DF	AA61NH	35.9	0.073	0.480	1.13%	1	2	0.9635	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES			SLS-B5
B2	DF	AA61NH	43.7	0.089	0.352	7.48%	2	2	0.9750	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES			SLS-B9
B3	DF	AA61NH	37.1	0.075	0.359	12.81%	1	5	0.9378	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES			SLS-B10
ECBC															
AA61LY-A1	RF	AA61LY	15.1	0.031	0.287	1.45%	0	6	0.9401	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-P14
AA61LY-B1	DF	AA61LY	26.7	0.054	0.347	12.36%	3	4	0.9375	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			SLS-P34
AA61LY-B2	DF	AA61LY	38.3	0.078	0.523	5.85%	2	4	0.9789	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES			SLS-P36
AA61LY-B3	DF	AA61LY	31.6	0.064	0.444	15.05%	3	4	0.9643	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	YES		potential volatility problem	SLS-P38
FRAME															
FAL.3T3.MC.A1.10.09.04	RF	AA61MC	62.8	0.128	0.369	12.62%	2	0	0.9133	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50 - 100%	ppt in 2X C1 and 1X C1	FAL.3T3.SLS.10.09.04
FAL.3T3.MC.B1.16.09.04	DF	AA61MC	48.1	0.098	0.277	7.34%	0	1	0.9557	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	NO	no points between 0 - 50%	ppt in 2X C1	FAL.3T3.SLS.16.09.04
FAL.3T3.MC.B2.23.09.04	DF	AA61MC	23.1	0.047	0.201	2.68%	3	0	0.8298	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	NO	no points between 50 - 100%	1.21 dilution factor doesn't affect outcome since no values > 50% viability; ppt in 2X C1; outlier removed by SD	FAL.3T3.SLS.23.09.04
FAL.3T3.MC.B3.14.10.04.04	DF	AA61MC	32.7	0.067	0.268	12.64%	2	1	0.9323	50.0, 34.0, 23.1, 15.7, 10.7, 7.28, 4.96, 3.37	1.47	YES		ppt in 2X C1	FAL.3T3.SLS.14.10.04
FAL.3T3.MC.B4.21.10.04	DF	AA61MC	36.1	0.073	0.169	0.30%	1	1	0.1575	50.0, 34.0, 23.1, 15.7, 10.7, 7.28, 4.96, 3.37	1.47	YES		ppt in 2X C1; very high viability values for C3-C7	FAL.3T3.SLS.21.10.04
FAL.3T3.MC.B5.04.11.04	DF	AA61MC	34.9	0.071	0.199	8.03%	2	1	0.6920	75.0, 51.0, 34.7, 23.6, 16.1, 10.9, 7.43, 5.06	1.47	YES			FAL.3T3.SLS.04.11.04
XYLENE															
IIVS															
A1	RF	AA61MA	NA	NA	0.415	0.04%	0	1	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-A3
B1	DF	AA61MA	728	6.855	0.371	3.21%	5	3	0.9121	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	YES			SLS-B6
B2	DF	AA61MA	809	7.621	0.371	4.51%	5	3	0.9567	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	YES		ppt in 2X C1	SLS-B9
B3	DF	AA61MA	635	5.984	0.311	4.85%	6	2	0.9597	2500, 1923, 1479, 1138, 875, 673, 518, 398	1.3	YES			SLS-B10
ECBC															
AA61GM-A1	RF	AA61GM	NA	NA	0.232	5.68%	0	5	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-12
AA61GM-B1	DF	AA61GM	NA	NA	0.754	7.45%	0	8	NA	3000, 2479, 2049, 1693, 1400, 1157, 956, 790	1.21	NO	PC failed; no points between 0 - 50%	test could pass due to dilution factor	SLS-P61
AA61GM-B2	DF	AA61GM	NA	NA	0.624	4.21%	0	7	NA	4000, 3306, 2732, 2258, 1866, 1542, 1275, 1053	1.21	NO	no points between 0 - 50%	test could pass due to dilution factor	SLS-P63

3T3 NRU Reference Substance Data

Experiment ID 3T3 Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61GM-B3	DF	AA61GM	NA	NA	0.553	15.44%	2	6	NA	4000, 3306, 2732, 2258, 1866, 1542, 1275, 1053	1.21	NO	can't properly determine points between 0 - 100%	roller coaster toxicity curve; ppt in 2X C1-C8 (oily)	SLS-P73
FRAME															
FAL.3T3.JG.A1.28.05.04	RF	AA61JG	NA	NA	0.327	3.31%	0	3	0.6108	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.3T3.SLS.28.05.04
FAL.3T3.JG.B1.04.06.04	DF	AA61JG	NA	NA	0.250	0.50%	0	0	NA	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 0 - 100%	ppt in 2X C1-C3;	FAL.3T3.SLS.04.06.04
FAL.3T3.JG.B2.17.06.04	DF	AA61JG	NA	NA	0.448	0.74%	0	NA	NA	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 0 - 100%	ppt in 2X C1-C3; toxicity did not reach 50%	FAL.3T3.SLS.17.06.04
FAL.3T3.JG.B2.24.06.04 (should be B3)	DF	AA61JG	NA	NA	0.396	9.40%	0	5	0.1548	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 0 - 50%	no toxicity detected; SD ends testing	FAL.3T3.SLS.24.06.04

Abbreviations: ppt=Precipitate; SD=Study Director; RF=Range Finder; DF=Definitive Test; PC=Positive Control; C1 - C8=Concentration series applied to the the cells. C1 is the highest concentration and C8 is lowest; NA=Not Available; RC=Registry of Cytotoxicity; 2X=Two times the concentration applied to the cells; VC=Vehicle Control; R2=Coefficient of Determination; OD=Optical Density; ID=Identification. Substance ID was the code assigned by the chemical distributor (BioReliance Corp.). Experiment ID and PC ID are test identification numbers assigned by the cytotoxicity testing laboratory.

[1] Range finder or definitive test
[2] Mean OD value for all VC wells in test plate
[3] Difference of right and left VC column of wells in the test plate
[4] % Viability values between 0 and 50% viability; test acceptance criterion. Phase Ib used the range of 10 -50%.
[5] % Viability values between 50 and 100% viability; test acceptance criterion. Phase Ib used the range of 50 - 90%.
[6] Calculated value from the Prism® software
[7] Reference substance concentrations applied to the cells
[8] Step-wise dilution factor used to determine reference substance exposure concentrations
[9] Determination for whether test meets or doesn't meet test acceptance criteria; not applied to RF tests
Shaded boxes identify values that do not meet the specific test acceptance criteria

Appendix I2

NHK NRU Reference Substance Data

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NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
ACETAMINOPHEN															
IIVS															
A1	RF	AA61HU	1450	9.560	0.525	0.11%	0	1	0.5444	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 - 50%		SLS-A5-N040401A
B1	DF	AA61HU	541	3.576	0.678	1.54%	5	3	0.9557	2500, 1786, 1276, 911, 651, 465, 332, 237	1.4	YES			SLS-B12-N041022B
B2	DF	AA61HU	661	4.370	0.622	9.36%	5	3	0.9738	2500, 1786, 1276, 911, 651, 465, 332, 237	1.4	YES			SLS-B13-N041029B
B3	DF	AA61HU	512	3.384	0.777	0.82%	5	3	0.9526	2500, 1786, 1276, 911, 651, 465, 332, 237	1.4	YES			SLS-B14-N041030A
ECBC															
AA61LR-A1	RF	AA61LR	196	1.299	0.972	0.43%	1	6	0.8186	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P19
AA61LR-B1	DF	AA61LR	467	3.086	0.731	1.13%	3	5	0.9694	4000, 1861, 865, 403, 187, 87.1, 40.5, 18.8	2.15	YES			SLS-P41
AA61LR-B2	DF	AA61LR	586	3.877	0.704	2.81%	3	4	0.9642	4000, 1861, 865, 403, 187, 87.1, 40.5, 18.8	2.15	YES			SLS-P43
AA61LR-B3	DF	AA61LR	621	4.106	1.019	4.94%	3	4	0.9495	4000, 1861, 865, 403, 187, 87.1, 40.5, 18.8	2.15	YES			SLS-P45
FRAME															
FAL.NHK.PY.A1.24.09.04	RF	AA61PY	137	0.907	0.578	8.76%	1	3	0.6981	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.PY.B1.01.10.04	DF	AA61PY	1130	7.489	1.026	8.47%	2	5	0.9753	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	PC failed		FAL.NHK.SLS.01.10.04
FAL.NHK.PY.B2.07.10.04	DF	AA61PY	421	2.783	0.575	3.20%	4	3	0.6590	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		C1 shows high toxicity; should this point be removed & new calc. be made?	FAL.NHK.SLS.07.10.03
FAL.NHK.PY.B3.05.11.04	DF	AA61PY	541	3.576	0.418	10.47%	3	1	0.9335	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		outlier removed by SD	FAL.NHK.SLS.05.11.04
FAL.NHK.PY.B4.10.11.04	DF	AA61PY	380	2.514	1.156	1.74%	3	5	0.7537	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.10.11.04
ACETONITRILE															
IIVS															
A1	RF	AA61GF	43700	1063.376	0.479	4.37%	0	4	0.5946	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0 - 50%		SLS-A1-N040317B
B1	DF	AA61GF	6810	165.839	0.494	99.86%	3	2	0.9841	200000, 111111, 61728, 34294, 19052, 10584, 5880, 3267	1.8	NO	%VC difference >15	Left VC was removed from calc. due to volatility	SLS-B8-N040819A
B2	DF	AA61GF	9730	236.966	0.624	3.54%	3	2	0.9960	200000, 111111, 61728, 34294, 19052, 10584, 5880, 3267	1.8	YES		plate seal used; SD removed top dose from analysis since only 4 wells of 8 were treated	SLS-B10-N040903A
B3	DF	AA61GF	9230	224.743	0.693	4.62%	3	2	0.9964	200000, 111111, 61728, 34294, 19052, 10584, 5880, 3267	1.8	YES		plate seal used	SLS-B11-N040904H
B4	DF	AA61GF	8910	217.114	0.605	5.04%	3	3	0.9878	40000, 25000,15625, 9766, 6104, 3815, 2384, 1490	1.6	YES			SLS-B12-N041022B
ECBC															
AA61PH-A1	RF	AA61PH	NA	NA	0.635	1.57%	0	5	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	no toxicity detected	SLS-P1
AA61PH-A2	RF	AA61PH	NA	NA	0.231	97.27%	3	1	NA	200000, 20000, 2000, 200, 20, 2, 0.2, 0.02	10	RF	range finder	probable volatility problem	SLS-P3
AA61PH-B1	DF	AA61PH	22600	551.679	0.911	13.28%	1	3	0.8640	50000, 23256, 10817, 5031, 2340, 1088, 506, 235	2.15	YES			SLS-P7

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61PH-B2	DF	AA61PH	31800	775.688	0.865	21.14%	1	5	0.8532	50000, 34014, 23139, 15740, 10708, 7284, 4955, 3371	1.47	NO	%VC difference > 15	possible volatility problem	SLS-P9
AA61PH-B3(sealer)	DF	AA61PH	7110	173.255	0.561	4.36%	6	2	0.9839	50000, 34014, 23139, 15740, 10708, 7284, 4955, 3371	1.47	YES			SLS-P17
AA61PH-B4(sealer)	DF	AA61PH	7050	171.667	0.643	1.06%	5	2	0.9812	50000, 34014, 23139, 15740, 10708, 7284, 4955, 3371	1.47	YES			SLS-P18
AA61PH-B5	DF	AA61PH	6710	163.564	0.484	0.05%	5	2	0.9783	40000, 27211, 18511, 12592, 8566, 5827, 3964, 2697	1.47	YES			SLS-P24
FRAME															
FAL.NHK.PL.A1.18.02.04	RF	AA61PL	NA	NA	0.107	11.79%	0	0	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	no values calculated by PRISM; % viability are "nonsense" values	FAL.NHK.SLS.18.02.04
FAL.NHK.PL.26.02.04	RF	AA61PL	8220	200.303	0.138	32.31%	1	0	0.4136	100000, 10000, 1000, 100, 10, 1, 0.1, 0.01	10	RF	range finder	chem. needs to be tested at high conc. but have volatility problems even w/plate sealer	FAL.NHK.SLS/NB.26.02.03
FAL.NHK.PL.B1.25.03.04	DF	AA61PL	8790	214.135	0.502	3.22%	1	2	0.9338	25000, 7937, 2520, 800, 254, 80.6, 25.6, 8.12	3.15	YES		did SD use plate film cover?	FAL.NHK.SLS.25.03.03
FAL.NHK.PL.B3.26.03.04	DF	AA61PL	7480	182.258	0.549	4.16%	2	0	0.8428	25000, 7911, 2504, 792, 251, 79.3, 25.1, 7.9	3.16	NO	no points between 50-100%	wrong solvent reported but correct one used (correction by SD); pts between 50 - 100% but several > 100%	FAL.NHK.SLS.26.03.04
FAL.NHK.PL.B4.25.04.04	DF	AA61PL	12400	302.473	0.860	5.09%	1	1	0.9371	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	YES			FAL.NHK.SLS.25.04.04
FAL.NHK.PL.B5.28.04.04	DF	AA61PL	8020	195.293	0.909	6.73%	0	1	0.8109	25000, 7937, 2520, 800, 254, 80.6, 25.6, 8.12	3.15	NO	no points between 0-50%	wells D3,D4,E3,E4 data removed by SD after NICEATM recomm. to review potential outliers; revised data eliminates point between 0-50% and test	FAL.NHK.SLS.28.04.03
FAL.NHK.PL.B5.19.08.04(rb) should be B6	DF	AA61PL	10800	262.233	0.266	7.45%	2	0	0.5395	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	NO	PC failed; no points between 50-100%		FAL.NHK.SLS-RB.19.08.04
FAL.NHK.PL.B6.20.08.04 should be B7	DF	AA61PL	9270	225.781	0.824	2.53%	2	2	0.9559	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	YES			FAL.NHK.SLS.20.08.04

ACETYLSALICYLIC ACID

IIVS															
A1	RF	AA61HM	552	3.064	0.748	3.52%	1	4	0.9540	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A3-N040331A
B1	DF	AA61HM	509	2.826	0.653	1.76%	5	3	0.9836	2000, 1429, 1020, 729, 521, 372, 266, 190	1.4	YES			SLS-B8-N040819A
B2	DF	AA61HM	596	3.306	0.599	5.27%	4	4	0.9664	2000, 1429, 1020, 729, 521, 372, 266, 190	1.4	YES			SLS-B9-N040820A
B3	DF	AA61HM	438	2.428	0.607	3.62%	5	3	0.9107	2000, 1429, 1020, 729, 521, 372, 266, 190	1.4	YES			SLS-B10-N040903A
ECBC															
AA61ME-A1	RF	AA61ME	631	3.501	0.916	2.80%	1	7	0.9492	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C2 and 1X C1	SLS-P14
AA61ME-B1	DF	AA61ME	614	3.406	0.765	3.36%	3	5	0.9409	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	YES			SLS-P53
AA61ME-B2	DF	AA61ME	653	3.624	0.791	2.60%	3	5	0.9719	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	YES			SLS-P54
AA61ME-B3	DF	AA61ME	627	3.477	0.983	0.71%	3	5	0.9596	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	YES			SLS-P56
FRAME															

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.JA.A1.14.05.04	RF	AA61JA	340	1.889	0.764	4.39%	1	2	0.9410	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.14.05.03
FAL.NHK.JA.B1.08.10.04	DF	AA61JA	719	3.993	0.722	0.54%	2	3	0.9913	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.08.10.03
FAL.NHK.JA.B2.22.10.04	DF	AA61JA	778	4.318	0.715	2.72%	3	5	0.9753	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.NHK.SLS.22.10.04 (MO)
FAL.NHK.JA.B3.28.10.04	DF	AA61JA	586	3.253	0.635	3.07%	4	4	0.9817	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.NHK.SLS.28.10.04
AMINOPTERIN															
IIVS															
A2	RF	AA61JD	1480	3.360	0.809	5.29%	0	6	0.7064	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A2
B1	DF	AA61JD	561	1.274	0.476	6.77%	2	6	0.9289	1000, 714, 510, 364, 260, 186, 133, 94.9	1.40	YES		evidence of precipitate at highest dose	SLS-B1
B2	DF	AA61JD	661	1.501	0.328	4.35%	2	6	0.9353	1000, 714, 510, 364, 260, 186, 133, 94.9	1.40	YES		evidence of precipitate at highest dose	SLS-B2
B3	DF	AA61JD	986	2.239	0.34	6.44%	0	5	0.9305	1000, 714, 510, 364, 260, 186, 133, 94.9	1.40	NO	No points 0-50%	evidence of precipitate at highest dose	SLS-B3
ECBC															
AA61MB-A1	RF	AA61MB	627	1.424	0.566	1.64%	1	3	0.8101	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	range finder	SLS-P4
AA61MB-B1	DF	AA61MB	962	2.184	1.042	1.45%	1	7	0.7701	1000, 680.3, 462.8, 314.8, 214.2, 145.7, 99.1, 67.4	1.47	NO	low r2		SLS-P8
AA61MB-B2	DF	AA61MB	718	1.630	0.914	0.84%	3	5	0.8326	1200, 991.7, 819.6, 677.4, 559.8, 462.7, 382.4, 316.0	1.21	YES			SLS-P10
AA61MB-B3	DF	AA61MB	1080	2.452	0.778	2.61%	1	7	0.7956	1200, 991.7, 819.6, 677.4, 559.8, 462.7, 382.4, 316	1.21	YES			SLS-P12
AA61MB-B4	DF	AA61MB	944	2.143	0.904	5%	3	5	0.7754	1200, 991.7, 819.6, 677.4, 559.8, 462.7, 382.4, 316.0	1.21	YES			SLS-P20
FRAME															
FAL.NHK.PU.30.07.03	RF	AA61PU	NA	NA	1.355	3.29%	0	8	0.0373	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	solution is yellow and may bind to the cells thus affecting NRU	FAL.NHK.SLS.30.07.03
FAL.NHK.PU.B1.07.08.03	DF	AA61PU	516	1.172	0.245	10.54%	2	6	0.2733	1000, 680, 463, 314.8, 214.1, 145.6, 99.1, 67.4	1.47	NO	low r2	biphasic response	FAL.NHK.SLS.07.08.03
FAL.NHK.PU.B2.13.08.03	DF	AA61PU	NA	NA	0.722	30.35%	0	7	NA	1000, 680, 463, 314.8, 214.1, 145.6, 99.1, 67.4	1.47	NO	PC failed; no points between 0 - 50%; no r2; %VC difference > 15	SD rejects this assay; can't explain the variability of cell growth in the wells	FAL.NHK.SLS.13.08.03
FAL.NHK.PU.B3.23.08.03	DF	AA61PU	366	0.831	0.408	5.58%	3	5	0.8213	1000, 680, 463, 314.8, 214.1, 145.6, 99.1, 67.4	1.47	NO	PC failed		FAL.NHK.SLS.230803
FAL.NHK.PU.B4.28.08.05	DF	AA61PU	593	1.346	0.470	8.87%	2	6	0.7804	1000, 680, 463, 314.8, 214.1, 145.6, 99.1, 67.4	1.47	YES		challenging chemical; SMT accepts this test	FAL.NHK.SLS.280803
FAL.NHK.PU.B5.05.09.03	DF	AA61PU	515	1.169	0.217	7.60%	2	6	0.7145	1000, 680, 463, 314.8, 214.1, 145.6, 99.1, 67.4	1.47	YES		challenging chemical; SMT accepts this test	FAL.NHK.SLS.050903
FAL.NHK.PU.B6.01.10.03	DF	AA61PU	NA	NA	1.373	5.40%	0	8	0.0149	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	no points between 50 - 100%; low r2		FAL.NHK.SLS.01.10.03
FAL.NHK.PU.B6.19.10.03	DF	AA61PU	157	0.356	0.170	1.73%	0	7	0.4794	100, 46.5, 21.6, 10.1, 4.7, 2.2, 1.0, 0.47	2.15	NO	low r2; no points between 0-50%	SD worked with wrong dilution range; wanted to start at 1000	FAL.NHK.SLS.19.10.03
FAL.NHK.PU.B7.23.10.03	DF	AA61PU	526	1.194	0.236	3.75%	2	6	0.6618	1000, 680, 463, 314.8, 214.1, 145.6, 99.1, 67.4	1.47	YES		challenging chemical; SMT accepts this test	FAL.NHK.SLS.23.10.03
FAL.NHK.PU.B8.24.10.03	DF	AA61PU	9950	22.591	0.869	1.69%	1	7	0.2607	1000, 680, 463, 314.8, 214.1, 145.6, 99.1, 67.4	1.47	NO	low r2		FAL.NHK.SLS.24.10.03
FAL.NHK.PU.B9.07.11.03	DF	AA61PU	5400	12.260	0.385	2.23%	1	7	0.1515	2000, 930, 433, 201, 94, 44, 20.2, 9.4	2.15	NO	low r2		FAL.NHK.SLS.07.11.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
5-AMINOSALICYLIC ACID															
IIVS															
A1	RF	AA61GZ	93.1	0.608	0.631	0.67%	1	0	0.8972	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	SD did not use data from the highest dose in Hill analyses due to the effects of the ppts; ppt in 2X C1 & 1X C1	SLS-A3-N040331A
B1	DF	AA61GZ	41.7	0.272	0.548	2.71%	6	2	0.9682	500, 313, 195, 122, 76.3, 47.7, 29.8, 18.6	1.6	YES			SLS-B1-N040423A
B2	DF	AA61GZ	47.3	0.309	0.557	3.54%	5	2	0.9749	500, 313, 195, 122, 76.3, 47.7, 29.8, 18.6	1.6	YES			SLS-B2-N040424A
B3	DF	AA61GZ	57.3	0.374	0.438	9.57%	3	3	0.9328	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9 ,7.45	1.6	YES		flattening of the curve at 35% viability	SLS-B3-N040506A
ECBC															
AA61KD-A1	RF	AA61KD	NA	NA	0.856	3.85%	1	4	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1	SLS-P12
AA61KD-B1	DF	AA61KD	34.8	0.228	0.529	0.76%	4	1	0.9692	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			SLS-P32
AA61KD-B2	DF	AA61KD	32.4	0.212	0.539	0.94%	5	2	0.9214	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	YES			SLS-P34
AA61KD-B3	DF	AA61KD	22.5	0.147	0.401	3.53%	6	2	0.9529	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	YES			SLS-P36
FRAME															
FAL.NHK.PA.A1.14.05.04	RF	AA61PA	35.6	0.232	0.784	2.17%	2	0	0.8834	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1; NR taken up by C1 ppt	FAL.NHK.SLS.14.05.03
FAL.NHK.PA.B1.19.08.04 rb	DF	AA61PA	62.1	0.406	0.234	1.25%	6	2	0.7433	500, 340, 231, 157, 108, 72.8, 50.0, 33.7	1.47	NO	PC failed		FAL.NHK.SLS-RB.19.08.04
FAL.NHK.PA-NB.B2.25.08.04	DF	AA61PA	127	0.830	0.988	1.33%	2	3	0.8882	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.NHK.SLS.25.08.04
FAL.NHK.PA.17.09.04	DF	AA61PA	54.3	0.355	0.705	2.54%	2	1	0.8385	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		outlier removed by SD; ppt in C1; interference with NRU in C1-C3 conc.; SD consider removing C1-C3 data from PRISM analyses?	FAL.NHK.SLS.17.09.04
FAL.NHK.PA.B4.30.09.04	DF	AA61PA	53.3	0.348	0.753	2.27%	3	2	0.9753	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		toxicity curve begins to rise at high concentrations; maybe affecting NRU; outlier removed by SD	FAL.NHK.SLS.30.09.03
AMITRIPTYLINE HCL															
IIVS															
A1	RF	AA61RF	10.3	0.033	0.516	5.22%	0	1	0.9945	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 - 50%		SLS-A1-N040317B
B1	DF	AA61RF	10.1	0.032	0.543	3.51%	2	3	0.9878	100, 55.6, 30.9, 17.1, 9.53, 5.29, 2.94, 1.63	1.8	YES			SLS-B1-N040423A
B2	DF	AA61RF	10.6	0.034	0.636	2.41%	2	3	0.9899	100, 55.6, 30.9, 17.1, 9.53, 5.29, 2.94, 1.63	1.8	YES			SLS-B2-N040424A
B3	DF	AA61RF	12.1	0.039	0.496	1.03%	2	2	0.9713	100, 55.6, 30.9, 17.1, 9.53, 5.29, 2.94, 1.63	1.8	YES			SLS-B3-N040506A
ECBC															
AA61PR-A1	RF	AA61PR	7.64	0.024	0.518	3.91%	2	3	0.9625	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-P4
AA61PR-B1	DF	AA61PR	12.4	0.040	0.647	4.74%	2	3	0.9678	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P21
AA61PR-B2	DF	AA61PR	13.0	0.042	0.921	1.85%	3	3	0.9817	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P23
AA61PR-B3	DF	AA61PR	6.94	0.022	0.648	2.47%	3	4	0.9710	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P24
FRAME															

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.LE.A1.13.02.03	RF	AA61LE	6.52	0.021	0.114	4.66%	2	2	0.8453	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	SD rejected due to bacterial contamination in some plates in test series; ppt in 2X C1	FAL.NHK.SLS.13.02.03
FAL.NHK.LE.A2.20.02.03	DF	AA61LE	3.08	0.010	0.213	0.12%	3	3	0.9449	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.NHK.SLS.20.02.03
FAL.NHK.LE.B1.27.02.04new	DF	AA61LE	13.6	0.043	0.548	1.40%	3	4	0.9200	50, 34.0, 23.1, 15.7, 10.7, 7.28, 4.96, 3.37	1.47	YES		file corrected by SD	FAL.NHK.SLS.27.02.03
FAL.NHK.LE.B3.19.03.04	DF	AA61LE	6.04	0.019	0.528	4.71%	3	5	0.9296	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.51, 0.24	2.15	YES			FAL.NHK.SLS.19.03.03
ARSENIC III TRIOXIDE															
IIVS															
Preliminary	RF	AA61FX	5.16	0.026	0.585	3.78%	1	0	0.9828	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		Preliminary
B1	DF	AA61FX	26.4	0.133	0.487	0.24%	2	2	0.9238	100, 46.4, 21.6, 10, 4.64, 2.16, 1.00, 0.46	2.15	YES			SLS-B1
B2	DF	AA61FX	22.5	0.114	0.633	7.02%	2	1	0.9682	100, 46.4, 21.6, 10, 4.64, 2.16, 1.00, 0.46	2.15	YES			SLS-B2
B3	DF	AA61FX	22.5	0.114	0.817	7.11%	2	0	0.9900	100, 46.4, 21.6, 10, 4.64, 2.16, 1.00, 0.46	2.15	NO	No points between 50 & 90%		SLS-B3
B4	DF	AA61FX	13.9	0.070	0.826	6.84%	1	1	0.9850	100, 46.4, 21.6, 10, 4.64, 2.16, 1.00, 0.46	2.15	YES			SLS-B4
ECBC															
ECBC-NHK-Ib-01 AA61KU-A1	RF	AA61KU	32.2	0.163	0.811	7.13%	0	1	-0.8980	25, 2.5, 0.25, 0.025, 0.0025,0.00025, 0.000025, 0.0000025	10	RF	range finder		SLS-P2
ECBC-NHK-Ib-02 AA61KU-B1	DF	AA61KU	4.51	0.023	0.978	2.63%	3	1	0.9577	50, 34, 23.1, 15.7, 10.7, 7.3, 5.0, 3.4	1.47	YES			SLS-P3
ECBC-NHK-Ib-03 AA61KU-B2	DF	AA61KU	7.76	0.039	1.200	2.58%	3	1	0.9757	25, 17.0, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	YES			SLS-P4
ECBC-NHK-Ib-04 AA61KU-B3	DF	AA61KU	8.11	0.041	1.080	5.57%	3	2	0.8912	25, 17.0, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	YES			SLS-P5
ECBC-NHK-Ib-05 AA61KU-B4	DF	AA61KU	10.7	0.054	1.086	3.26%	2	1	0.9369	25, 17.0, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	YES			SLS-P7
FRAME															
A1 1b/NHKRF1/FAL/NC	RF	AA61NC	1.49	0.008	0.160	0.52%	1	1	0.6560	12.5, 2.5, 0.5, 0.1, 0.02, 0.004, 0.00080, 0.00016	5	RF	range finder		A1 1b/NHKCTR1/FAL/SLS
A2 1b/NHKRF2/FAL/NC	RF	AA61NC	3.01	0.015	0.685	10.17%	4	4	0.5164	12.5, 8.5, 5.78, 3.93, 2.67, 1.82, 1.23, 0.84	1.47	NO	low r2		A2 1b/NHKCTR2/FAL/SLS
A3 1b/NHK/DF2/FAL/NC	DF	AA61NC	0.00016	0.000	0.051	18.01%	0	0	-0.9880	10, 6.8, 4.6, 3.14, 2.13, 1.45, 0.98, 0.67	1.47	NO	VC difference > 15%; no points between 10 & 90%; R^2 < 0.8; PC failed	NR crystal problems; used different medium; % viability values are negative; PRISM curve below 0	A3 1b/NHK/CTR4/FAL/
A4 1b/NHK/DF3/FAL/NC	DF	AA61NC	0.502	0.003	0.144	1.97%	5	0	0.7012	10, 6.8, 4.6, 3.14, 2.13, 1.45, 0.98, 0.67	1.47	NO	No point between 50 & 90%; R^2 < 0.8	NR crystal problems; used medium not normally used	A4 1b/NHK/CTR5/FAL
A5 1b/NHK/DF4/FAL/NC	DF	AA61NC	NA	NA	-0.003	83.48%	0	0	NC	10, 6.8, 4.6, 3.14, 2.13, 1.45, 0.98, 0.67	1.47	NO	VC difference > 15%; no points between 10& 90%; no R^2 or ICx; PC failed	NR crystal problems; used different medium; OD values of test wells no different than background ODs; negative values for VC	A5 1b/NHK/CTR6/FAL
A6 1b/NHK/DF5/FAL/NC	DF	AA61NC	2.95	0.015	1.145	11.51%	2	3	0.8929	10, 6.8, 4.6, 3.14, 2.13, 1.45, 0.98, 0.67	1.47	YES			A6 1b/NHK/CTR7/FAL
A8 1b/NHK/DF7/FAL/NC	DF	AA61NC	6.26	0.032	0.740	2.23%	1	2	0.8855	15, 10.2, 6.93, 4.72, 3.21, 2.18, 1.48, 1.01	1.47	YES			A8 1b/NHK/CTR9/FAL

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
A9 1b/NHK/DF8/FAL/NC	DF	AA61NC	6.25	0.032	0.798	9.28%	1	6	0.7381	15, 10.2, 6.93, 4.72, 3.21, 2.18, 1.48, 1.01	1.47	NO	R^2 < 0.8; PC failed		A9 1b/NHK/CTR10/FAL
A10 1b/NHK/DF9/FAL/NC	DF	AA61NC	1.29	0.007	1.108	3.81%	4	1	0.8550	15, 10.2, 6.93, 4.72, 3.21, 2.18, 1.48, 1.01	1.47	YES		no outliers	A10 1b/NHK/CTR11/FAL
A11 1b/NHK/DF10/FAL/SLS//NC	DF	AA61NC	1.54	0.008	1.439	0.51%	4	1	0.8443	15, 10.2, 6.93, 4.72, 3.21, 2.18, 1.48, 1.01	1.47	YES		removed outliers from VCs	A11 1b/NHK/CTR12/FAL
A12 1b/NHK/DF11/FAL/NC	DF	AA61NC	1.88	0.010	0.459	1.00%	5	2	0.8901	15, 10.2, 6.93, 4.72, 3.21, 2.18, 1.48, 1.01	1.47	YES			A12 1b/NHK/CTR13/FAL/SLS
1b/NHK/DF4/FAL/NC	DF	AA61NC	1.36	0.007	0.755	1.17%	4	1	0.8346	15, 10.2, 6.93, 4.72, 3.21, 2.18, 1.48, 1.01	1.47	YES			1b/NHK/CTR14/FAL/SLS

ATROPINE SULFATE

IIVS															
A1	RF	AA61NE	91.6	0.132	0.544	0.93%	2	1	0.9667	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A4-N040331N
B1	DF	AA61NE	106	0.152	0.578	5.65%	5	3	0.9599	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B4-N040513C
B2	DF	AA61NE	64.6	0.093	0.492	0.17%	5	3	0.9862	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B5-N040514B
B3	DF	AA61NE	78.9	0.114	0.705	3.13%	5	3	0.9915	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		outlier removed by SD	SLS-B6-N040716A
ECBC															
AA61KX-A1	RF	AA61KX	57.5	0.083	0.549	2.70%	3	2	0.9435	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P16
AA61KX-B1	DF	AA61KX	79.4	0.114	0.798	3.96%	4	4	0.9761	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES			SLS-P30
AA61KX-B2	DF	AA61KX	97.5	0.140	0.673	1.08%	3	5	0.9491	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES			SLS-P40
AA61KX-B3	DF	AA61KX	79.4	0.114	0.675	2.42%	4	2	0.9655	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES			SLS-P42
FRAME															
FAL.NHK.FU.A1.28.07.04	RF	AA61FU	33.3	0.048	0.059	10.09%	3	3	0.7561	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.28.07.04
FAL.NHK.FU.B1.11.08.04	DF	AA61FU	202	0.291	0.809	8.32%	3	3	0.9333	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS.11.08.04
FAL.NHK.FU-NB.B2.25.08.04	DF	AA61FU	80.7	0.116	1.010	3.32%	6	2	0.9459	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	YES			FAL.NHK.SLS.25.08.04
FAL.NHK.FU.B3.27.08.04	DF	AA61FU	30.4	0.044	0.526	4.53%	5	1	0.9696	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	YES			FAL.NHK.SLS.27.08.04

BORIC ACID

IIVS															
A1	RF	AA61LD	724	11.717	0.536	2.15%	1	1	0.9101	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A4-N040331N
B1	DF	AA61LD	455	7.359	0.583	4.16%	4	4	0.9594	2500, 1563, 977, 610, 381, 238, 149, 93	1.6	YES		ppt in 1X C1	SLS-B8-N040819A
B2	DF	AA61LD	460	7.444	0.541	3.17%	4	4	0.9778	2500, 1563, 977, 610, 381, 238, 149, 93	1.6	YES		ppt in 1X C1	SLS-B9-N040820A
B3	DF	AA61LD	476	7.705	0.553	4.25%	4	4	0.9713	2500, 1563, 977, 610, 381, 238, 149, 93	1.6	YES			SLS-B10-N040903A
ECBC															
AA61JH-A1	RF	AA61JH	449	7.258	0.449	0.45%	2	2	0.9280	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P17
AA61JH-B1	DF	AA61JH	598	9.678	0.690	6.95%	4	4	0.9413	6000, 2791, 1298, 604, 281, 131, 60.7, 28.3	2.15	YES			SLS-P32
AA61JH-B2	DF	AA61JH	371	5.995	0.736	3.27%	4	3	0.9757	6000, 2791, 1298, 604, 281, 131, 60.7, 28.3	2.15	YES			SLS-P35
AA61JH-B3	DF	AA61JH	350	5.660	0.438	3.54%	4	4	0.9848	6000, 2791, 1298, 604, 281, 131, 60.7, 28.3	2.15	YES			SLS-P37
FRAME															

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.GR.A1.28.07.04	RF	AA61GR	1020	16.474	0.055	0.90%	1	1	0.6145	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.28.07.04
FAL.NHK.GR.B1.11.08.04	DF	AA61GR	592	9.568	0.739	0.12%	4	4	0.9157	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS.11.08.04
FAL.NHK.GR-NB.B2.25.08.04	DF	AA61GR	851	13.766	0.943	0.07%	4	4	0.9741	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS.25.08.04
FAL.NHK.GR.B3.27.08.04	DF	AA61GR	107	1.733	0.534	8.67%	6	2	0.9607	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS.27.08.04
BUSULFAN															
IIVS															
A1	RF	AA61RL	1150	4.683	0.500	10.83%	0	3	0.5430	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; no points between 0 - 50%		SLS-A1-N040317B
B1	DF	AA61RL	274	1.113	0.732	7.46%	2	4	0.9237	750, 417, 231, 129, 71.4, 39.7, 22.1, 12.3	1.8	YES			SLS-B12-N041022B
B2	DF	AA61RL	317	1.287	0.598	3.83%	2	5	0.9721	500, 333, 222, 148, 98.8, 65.8, 43.9, 29.3	1.5	YES			SLS-B113-N041029B
B3	DF	AA61RL	348	1.414	0.792	2.36%	2	6	0.9429	500, 333, 222, 148, 98.8, 65.8, 43.9, 29.3	1.5	YES			SLS-B14-N041030A
ECBC															
AA61LH-A1	RF	AA61LH	NA	NA	0.624	3.53%	0	7	NA	100, 10, 1, 0.1, 0.01, 0.001, 0.0001,0.00001	10	RF	range finder		SLS-P4
AA61LH-B1	DF	AA61LH	217	0.882	1.103	1.81%	1	7	0.6962	800, 372, 173, 80.5, 37.4, 17.4, 8.10, 3.77	2.15	YES		ppt in 2X C1	SLS-P47
AA61LH-B2	DF	AA61LH	211	0.856	0.792	1.88%	2	6	0.8550	800, 372, 173, 80.5, 37.4, 17.4, 8.10, 3.77	2.15	YES		ppt in 2X C1	SLS-P48
AA61LH-B3	DF	AA61LH	332	1.347	1.344	2.99%	1	7	0.6216	800, 372, 173, 80.5, 37.4, 17.4, 8.10, 3.77	2.15	YES		ppt in 2X C1	SLS-P51
FRAME															
FAL.NHK.JE.A1.13.02.03	RF	AA61JE	29.8	0.121	0.152	15.63%	1	2	0.7100	250, 25, 2.5, 0.25, 0.025, 0.0025, 0.00025	10	RF	range finder	SD rejected due to bacterial contamination in some of the plates in this test series	FAL.NHK.SLS.13.02.03
FAL.NHK.JE.A2.20.02.03	DF	AA61JE	171	0.694	0.195	6.46%	2	3	0.6939	250, 116.3, 54.1, 25.2, 11.7, 5.4, 2.5, 1.2	2.15	YES		DF since conc. series is different from A1 RF	FAL.NHK.SLS.20.02.03
FAL.NHK.JE.B1.27.02.04	DF	AA61JE	142	0.575	0.622	3.35%	2	6	0.8940	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.27.02.03
FAL.NHK.JE.B2.19.03.03	DF	AA61JE	490	1.988	0.573	1.40%	1	6	0.8387	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.19.03.03
CADMIUM II CHLORIDE															
IIVS															
A2	RF	AA61NK	2.05	0.011	0.841	4.19	2	2	0.9692	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A2
B1	DF	AA61NK	1.84	0.010	0.444	6.37	5	3	0.9906	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.47	YES			SLS-B1
B2	DF	AA61NK	1.72	0.009	0.344	6.83	3	3	0.9819	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.47	YES			SLS-B2
B3	DF	AA61NK	2.02	0.011	0.338	4.78	2	2	0.9738	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.47	YES			SLS-B3
ECBC															
AA61KR-A1	RF	AA61KR	1.75	0.010	0.492	0.22	3	3	0.9218	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P4
AA61KR-B1	DF	AA61KR	2.31	0.013	0.918	6.16	4	3	0.9738	100, 46.5, 21.6, 10.1, 4.7, 2.2, 1.0, 0.47	2.15	YES			SLS-P8
AA61KR-B3	DF	AA61KR	3.29	0.018	0.749	0.44	2	2	0.9446	8.00, 5.44, 3.70, 2.52, 1.71, 1.17, 0.793, 0.539	1.47	YES			SLS-P12
AA61KR-B5	DF	AA61KR	1.16	0.006	0.143	12.96	2	3	0.8299	8.00, 5.44, 3.70, 2.52, 1.71, 1.17, 0.793, 0.539	1.47	YES			SLS-P15

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61KR-B6	DF	AA61KR	2.57	0.014	0.867	2.57	3	3	0.9730	8.00, 5.44, 3.70, 2.52, 1.71, 1.17, 0.793, 0.539	1.47	YES			SLS-P16
AA61KR-B7	DF	AA61KR	1.66	0.009	0.507	6.37	3	4	0.9495	8.00, 5.44, 3.70, 2.52, 1.71, 1.17, 0.793, 0.539	1.47	YES			SLS-P18
FRAME															
FAL.NHK.JP.A1.30.07.03	RF	AA61JP	1.71	0.009	1.263	6.60	3	5	0.9364	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.30.07.03
FAL.NHK.JP.B1.07.08.03	DF	AA61JP	0.722	0.004	0.253	4.61	4	0	0.9034	12.0, 8.2, 5.6, 3.2, 2.6, 1.8, 1.2, 0.8	1.47	NO	No points between 50 & 100% viability		FAL.NHK.SLS.07.08.03
FAL.NHK.JP.B2.13.08.03	DF	AA61JP	NA	NA	0.219	9.58	0	3	NA	3.0, 2.04, 1.39, 0.94, 0.64, 0.44, 0.3, 0.2	1.47	NO	PC failed; no points between 0 - 50%; no r2;	SD rejects this assay; can't explain the variability of cell growth in the wells	FAL.NHK.SLS.13.08.03
FAL.NHK.JP.B3.23.08.03	DF	AA61JP	2.19	0.012	0.384	4.86	2	6	0.9507	5.0, 3.401, 2.314, 1.574, 1.071, 0.728, 0.496, 0.337	1.47	NO	PC failed		FAL.NHK.SLS.230803
FAL.NHK.JP.B4.28.08.03	DF	AA61JP	2.96	0.016	0.504	7.31	1	1	0.8321	5.0, 3.401, 2.314, 1.574, 1.071, 0.728, 0.496, 0.337	1.47	YES			FAL.NHK.SLS.280803
FAL.NHK.JP.B5.05.09.03	DF	AA61JP	0.553	0.003	0.180	4.62	3	2	0.8972	5.0, 3.401, 2.314, 1.574, 1.071, 0.728, 0.496, 0.337	1.47	YES			FAL.NHK.SLS.050903
FAL.NHK.JP.B6.01.10.03	DF	AA61JP	2.46	0.013	1.289	6.38	2	6	0.4951	5.0, 3.401, 2.314, 1.574, 1.071, 0.728, 0.496, 0.337	1.47	NO	low r2		FAL.NHK.SLS.01.10.03
FAL.NHK.JP.B6.15.10.03 (should be B7?)	DF	AA61JP	2.12	0.012	0.482	1.44	2	4	0.9753	5.0, 3.401, 2.314, 1.574, 1.071, 0.728, 0.496, 0.337	1.47	YES			FAL.NHK.SLS.15.10.03

CAFFEINE

IIVS															
A1	RF	AA61JM	390	2.008	0.440	7.52%	2	3	0.9708	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1-N040317B
B1	DF	AA61JM	565	2.909	0.489	3.92%	3	4	0.9805	10000, 4545, 2066, 939, 427, 194, 88.2, 40.1	2.2	YES			SLS-B1-N040423A
B2	DF	AA61JM	578	2.977	0.554	4.28%	4	4	0.9817	10000, 4545, 2066, 939, 427, 194, 88.2, 40.1	2.2	YES		two phase dose response curve	SLS-B2-N040424A
B3	DF	AA61JM	579	2.984	0.456	2.91%	3	3	0.9762	10000, 4545, 2066, 939, 427, 194, 88.2, 40.1	2.2	YES		ppt in 1X C2	SLS-B3-N040506A
ECBC															
AA61NU-A1	RF	AA61NU	221	1.137	0.469	5.83%	2	3	0.9546	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P3
AA61NU-B1	DF	AA61NU	1070	5.492	1.065	6.83%	1	7	0.9140	2000, 930, 433,201,93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P7
AA61NU-B2	DF	AA61NU	824	4.244	1.076	0.91%	4	4	0.9433	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P9
AA61NU-B3	DF	AA61NU	558	2.876	0.777	7.01%	4	4	0.9590	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P11
FRAME															
FAL.NHK.GW.A1.13.02.03	RF	AA61GW	340	1.753	0.189	12.28%	2	2	0.8133	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.13.02.03
FAL.NHK.GW.A2.13.02.03	DF	AA61GW	553	2.849	0.247	2.26%	3	4	0.9267	10000, 3175, 1008, 320, 102, 32.2, 10.2, 3.25	3.15	YES		DF because conc. series is different from A1 RF	FAL.NHK.SLS.20.02.03
FAL.NHK.GW.B1.27.02.04	DF	AA61GW	794	4.090	0.456	0.75%	2	2	0.9523	10000, 3175, 1008, 320, 102, 32.2, 10.2, 3.25	3.15	YES			FAL.NHK.SLS.27.02.03
FAL.NHK.GW.B3.18.03.04	DF	AA61GW	427	2.197	0.522	9.68%	3	5	0.9542	10000, 3175, 1008, 320, 102, 32.2, 10.2, 3.25	3.15	YES			FAL.NHK.SLS.18.03.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
CARBAMAZEPINE															
IIVS															
A1	RF	AA61NB	NA	NA	0.575	4.51%	0	1	NA	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder; no points between 0 - 50%		SLS-A5-N040401A
B1	DF	AA61NB	67.3	0.285	0.698	0.74%	1	7	0.9759	75.0, 46.9, 29.3, 18.3, 11.4, 7.15, 4.47, 2.79	1.6	YES			SLS-B12-N041022B
B2	DF	AA61NB	88.3	0.374	0.609	1.12%	0	5	0.8732	75.0, 46.9, 29.3, 18.3, 11.4, 7.15, 4.47, 2.79	1.6	NO	no points between 0 - 50%		SLS-B113-N041029B
B3	DF	AA61NB	57.8	0.245	0.726	1.01%	1	5	0.9378	75.0, 46.9, 29.3, 18.3, 11.4, 7.15, 4.47, 2.79	1.6	YES			SLS-B14-N041030A
B4	DF	AA61NB	66.5	0.282	0.691	8.74%	3	5	0.9237	200, 125, 78.1, 48.8, 30.5, 19.1, 11.9, 7.45	1.6	YES			SLS-B15-N041110A
ECBC															
AA61LX-A1	RF	AA61LX	40.7	0.17240	0.827	3.59%	1	4	0.9327	200, 20, 2, 0.2, 0.02, 0.002, 0.0002, 0.00002	10	RF	range finder		SLS-P19
AA61LX-B1	DF	AA61LX	56.5	0.239	0.669	1.51%	3	4	0.9784	400, 186, 86.5, 40.2, 18.7, 8.71, 4.05, 1.88	2.15	YES		ppt in 1X C1	SLS-P41
AA61LX-B2	DF	AA61LX	71.9	0.304	0.693	3.27%	3	3	0.9477	400, 186, 86.5, 40.2, 18.7, 8.71, 4.05, 1.88	2.15	YES		ppt in 2X C1 and 1X C1	SLS-P43
AA61LX-B3	DF	AA61LX	70.0	0.296	1.100	2.84%	2	5	0.9566	400, 186, 86.5, 40.2, 18.7, 8.71, 4.05, 1.88	2.15	YES		ppt in 2X C1 and 1X C1	SLS-P45
FRAME															
FAL.NHK.HD.A1.24.09.04	RF	AA61HD	594	2.515	0.292	5.56%	1	2	-0.5440	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.HD.B1.01.10.04	DF	AA61HD	187	0.78983	1.037	6.43%	2	5	0.9721	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	PC failed		FAL.NHK.SLS.01.10.04
FAL.NHK.HD.B2.07.10.04	DF	AA61HD	58.2	0.24634	0.631	2.15%	4	4	0.9855	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		ppt In 1X C1-C2	FAL.NHK.SLS.07.10.03
FAL.NHK.HD.B3.05.11.04	DF	AA61HD	71.3	0.30167	0.521	2.51%	4	4	0.9236	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		ppt In 1X C1-C2	FAL.NHK.SLS.05.11.04
FAL.NHK.HD.B4.10.11.04	DF	AA61HD	628	2.65789	1.114	4.71%	3	5	0.9316	1000, 8870, 756, 658, 572, 497, 432, 376	1.15	YES		ppt In 1X C1-C2; ppt in 2X C1-C2	FAL.NHK.SLS.10.11.04
CARBON TETRACHLORIDE															
IIVS															
A1	RF	AA61JK	NA	NA	0.627	0.48%	0	0	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 - 100%		SLS-A2-N040320B
B1	DF	AA61JK	1540	10.023	0.679	3.90%	0	2	0.7803	2500, 1389, 772, 429, 238, 132, 73.5, 40.8	1.8	NO	no points between 0 - 50%	SD removed highest dose from Hill analyses due to ppt and upswing in response curve; ppt in 2X C1-C8	SLS-B12-N041022B
B2	DF	AA61JK	NA	NA	0.634	6.32%	0	2	NA	2500, 1389, 772, 429, 238, 132, 73.5, 40.8	1.8	NO	no points between 0 - 50%	ppt in 2X C1-C4	SLS-B113-N041029B
B3	DF	AA61JK	NA	NA	0.755	0.42%	0	1	NA	2500, 1389, 772, 429, 238, 132, 73.5, 40.8	1.8	NO	no points between 0 - 50%	ppt in 2X C1-C4	SLS-B14-N041030A
ECBC															
AA61NZ-A1	RF	AA61NZ	NA	NA	0.844	3.30%	0	3	NA	3000, 300, 30, 3, 0.3, 0.03, 0.003, 0.0003	10	RF	range finder; no points between 0 - 50%		SLS-P13
AA61NZ-B1	DF	AA61NZ	NA	NA	0.642	0.54%	0	4	NA	4500, 3719, 3074, 2540, 2099, 1735, 1434, 1185	1.21	NO	no points between 0 - 50%	ppt in 2X C1- C5	SLS-P52
AA61NZ-B2	DF	AA61NZ	NA	NA	0.770	0.36%	NA	N/A	NA	7000, 5785, 4781, 3951, 3266, 2699, 2230, 1843	1.21	NO	SD rejects	ppt in 2X C1-C5; chemical globules in 1X C1-C4; plate columns C6 and C7 show no cells were plated	SLS-P56
AA61NZ-B3	DF	AA61NZ	NA	NA	0.668	1.36%	6	1	NA	7000, 5785, 4781, 3951, 3266, 2699, 2230, 1843	1.21	NO	can't properly determine points between 0 - 100%	"roller coaster" toxicity curve; ppt in 2X C1-C8; outliers removed by SD	SLS-P59
FRAME															

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.HC.A1.25.04.04	RF	AA61HC	NA	NA	0.920	2.74%			NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder ; no points between 0 - 100%		FAL.NHK.SLS.25.04.04
FAL.NHK.HC.B1.11.06.04	DF	AA61HC	NA	NA	1.044	2.28%		8	NA	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	PC failed; no points between 0 - 50%		FAL.NHK.SLS.11.06.04
FAL.NHK.HC.B2.25.06.04	DF	AA61HC	1380	8.953	1.023	7.07%		2	0.8467	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 0 - 50%		FAL.NHK.SLS.25.06.04
FAL.NHK.HC.B3.19.08.04 nb	DF	AA61HC	NA	NA	0.419	8.26%		7	0.0000	2500, 2066, 1708, 1411, 1166, 964, 797, 658	1.21	NO	curve unacceptable; no points between 0 - 50% would be acceptable due to	no toxicity detectedd	FAL.NHK.SLS-NB.19.08.04
FAL.NHK.HC.B4.20.08.04	DF	AA61HC	NA	NA	0.739	2.93%		1	0.0000	2500, 2066, 1708, 1411, 1166, 964, 797, 658	1.21	NO	curve unacceptable; no points between 0 - 50% would be acceptable due to 1.21 dilution	no toxicity detected; outliers removed by SD	FAL.NHK.SLS.20.08.04
CHLORAL HYDRATE															
IIVS															
A1	RF	AA61FJ	104	0.626	0.650	59.25%	2	1	0.9885	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; %VC difference >0	volatility problem; VC1 OD values much lower than VC2; VC1 removed from subsequent analysis by SD	SLS-A2-N040320B
B1	DF	AA61FJ	114	0.686	0.601	3.48%	5	3	0.9882	5000, 2273, 1033, 470, 213, 97.0, 44.1, 20.0	2.2	YES			SLS-B1-N040423A
B2	DF	AA61FJ	111	0.674	0.513	0.29%	5	3	0.9904	5000, 2273, 1033, 470, 213, 97.0, 44.1, 20.0	2.2	YES		used plate sealer	SLS-B2-N040424A
B3	DF	AA61FJ	111	0.672	0.517	6.49%	3	3	0.9917	5000, 2273, 1033, 470, 213, 97.0, 44.1, 20.0	2.2	YES			SLS-B3-N040506A
ECBC															
AA61KB-A1	RF	AA61KB	NA	NA	0.268	59.01%	1	0	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	probable volatility problem	SLS-P6
AA61KB-B1	DF	AA61KB	170	1.027	0.553	2.62%	3	5	0.9314	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			SLS-P20
AA61KB-B2	DF	AA61KB	148	0.892	0.825	2.87%	4	4	0.9619	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			SLS-P22
AA61KB-B3	DF	AA61KB	103	0.62153	0.394	3.13%	4	4	0.9671	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			SLS-P24
FRAME															
FAL.NHK.LK.A1.25.03.04	RF	AA61LK	103	0.620	0.412	65.79%	2	1	0.3337	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; %VC difference > 15	possible volatility problem	FAL.NHK.SLS.25.03.03
FAL.NHK.LK.B1.25.04.04	DF	AA61LK	NA	NA	0.039	12.80%	2	1	NA	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	wrong desorb solution used in NRU; SD rejects this test		FAL.NHK.SLS.25.04.04
FAL.NHK.LK.B2.28.04.04	DF	AA61LK	142	0.860	0.825	0.16%	3	5	0.9864	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7,	2.15	YES			FAL.NHK.SLS.28.04.03
FAL.NHK.LK.B2.11.06.04 (should be B3)	DF	AA61LK	135	0.816	0.797	3.73%	3	3	0.9586	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7,	2.15	NO	PC failed		FAL.NHK.SLS.11.06.04
FAL.NHK.LK.B4.23.06.04	DF	AA61LK	215	1.299	0.970	1.58%	3	3	0.9863	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.23.06.04
FAL.NHK.LK.B5.25.06.04	DF	AA61LK	119	0.722	0.927	2.14%	3	3	0.9801	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7,	2.15	YES			FAL.NHK.SLS.25.06.04
CHLORAMPHENICOL															
IIVS															
A2	RF	AA61GJ	355	1.099	0.801	5.41%	0	2	0.6374	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A2
B1	DF	AA61GJ	296	0.916	0.487	7.17%	2	6	0.9691	560, 311, 173, 96, 53.3, 29.6, 16.5, 9.15	1.80	YES			SLS-B1

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B2	DF	AA61GJ	351	1.086	0.358	5.44%	1	6	0.9165	560, 311, 173, 96, 53.3, 29.6, 16.5, 9.15	1.80	YES			SLS-B2
B3	DF	AA61GJ	453	1.402	0.377	0.99%	1	5	0.93	560, 311, 173, 96, 53.3, 29.6, 16.5, 9.15	1.80	YES			SLS-B3
ECBC															
AA61JS-A1	RF	AA61JS	239	0.740	0.706	3.80%	1	7	0.8464	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P4
AA61JS-B1	DF	AA61JS	252	0.780	1.175	3.03%	2	5	0.9626	2000, 930.2, 432.7, 201.2, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P8
AA61JS-B2	DF	AA61JS	222	0.687	0.975	0.22%	3	5	0.9452	2000, 930.2, 432.7, 201.2, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P10
AA61JS-B3	DF	AA61JS	481	1.488	0.767	0.14%	2	6	0.9349	2000, 930.2, 432.7, 201.2, 93.6, 43.5, 20.2, 9.4	2.15	YES			SLS-P12
FRAME															
FAL.NHK.MU.A1.30.07.03	RF	AA61MU	232	0.718	1.246	1.87%	1	6	0.8736	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.30.07.03
FAL.NHK.MU.B1.07.08.03	DF	AA61MU	160	0.495	0.187	55.29%	5	2	0.0978	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	NO	VC difference > 15%; low r2		FAL.NHK.SLS.07.08.03
FAL.NHK.MU.B2.15.08.03	DF	AA61MU	873	2.702	0.394	6.64%	1	2	0.6646	2500, 1163, 541, 252, 117, 54, 25, 12	2.15	NO	low r2		FAL.NHK.SLS.15.08.03
FAL.NHK.MU.B3.23.08.03	DF	AA61MU	587	1.816	0.329	2.15%	2	3	0.8892	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	NO	PC failed		FAL.NHK.SLS.230803
FAL.NHK.MU.B4.28.08.03	DF	AA61MU	476	1.473	0.472	15.82%	1	5	0.8489	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	NO	% VC difference >15		FAL.NHK.SLS.280803
FAL.NHK.MU.B5.05.09.03	DF	AA61MU	473	1.464	0.171	10.94%	2	4	0.8686	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	YES			FAL.NHK.SLS.050903
FAL.NHK.MU.B6.01.10.03	DF	AA61MU	173	0.535	1.304	7.20%	2	6	0.5745	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	NO	low r2		FAL.NHK.SLS.01.10.03
FAL.NHK.MU.B6.15.10.03 (should be B7?)	DF	AA61MU	625	1.934	0.485	0.38%	2	5	0.9212	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	YES			FAL.NHK.SLS.15.10.03
FAL.NHK.MU.B7.19.10.03	DF	AA61MU	916	2.835	0.164	2.34%	1	2	0.7152	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	NO	low r2		FAL.NHK.SLS.19.10.03
FAL.NHK.MU.B8.23.10.03	DF	AA61MU	362	1.120	0.249	8.70%	2	5	0.8807	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	YES			FAL.NHK.SLS.23.10.03
FAL.NHK.MU.B9.24.10.03	DF	AA61MU	194	0.600	0.861	4.38%	3	4	0.8814	2500, 1162, 541, 251, 171, 79.6, 54.1, 25.2	2.15	YES			FAL.NHK.SLS.24.10.03
CITRIC ACID															
IIVS															
A1	RF	AA61MH	298	1.551	0.413	4.09%	2	1	0.9217	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A1-N040317B
B1	DF	AA61MH	447	2.325	0.547	4.83%	4	4	0.9681	10000, 4545, 2066, 939, 427, 194, 88.2, 40.1	2.2	YES			SLS-B1-N040423A
B2	DF	AA61MH	407	2.121	0.562	0.18%	2	4	0.9655	10000, 4545, 2066, 939, 427, 194, 88.2, 40.1	2.2	YES			SLS-B2-N040424A
B3	DF	AA61MH	444	2.309	0.477	2.95%	2	5	0.9609	3000, 1667, 926, 514, 286, 159, 88.2, 49.0	1.8	YES		ppt in 1X C1-C2	SLS-B3-N040506A
ECBC															
AA61HH-A1	RF	AA61HH	295	1.54	0.511	3.95%	2	1	0.9327	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P1
AA61HH-B1	DF	AA61HH	557	2.900	1.160	3.05%	2	6	0.9595	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			SLS-P7
AA61HH-B2	DF	AA61HH	589	3.065	1.191	1.62%	2	6	0.9588	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			SLS-P9
AA61HH-B3	DF	AA61HH	433	2.252	0.740	2.11%	2	6	0.9690	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			SLS-P11
FRAME															
FA.NH.HV.A1.11.02.04 (should be RB)	RF	AA61RB	406	2.111	1.459	3.77%	2	6	0.9700	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	pH and color of 2X matches citric acid for 3T3	FAL.NHK.SLS.11.02.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.RB.A2.18.02.04	DF	AA61RB	362	1.886	0.210	4.13%	6	0	0.7857	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	NO	PC failed; no points between 50-100%	this is a definitive test since conc. series is different from A1 range finder	FAL.NHK.SLS.18.02.04
FAL.NHK.RB.B1.26.02.04	DF	AA61RB	348	1.809	0.183	5.10%	3	5	0.9225	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS/MO.26.02.03
FAL.NHK.RB.B2.27.02.04	DF	AA61RB	361	1.881	0.415	5.54%	4	3	0.9577	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES		ppt detected in C1-C3 at end of test	FAL.NHK.SLS.27.02.04
FAL.NHK.RB.B3.18.03.04	DF	AA61RB	288	1.501	0.361	12.24%	4	3	0.9324	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	YES			FAL.NHK.SLS.18.03.03
FAL.NHK.RB.B4.19.03.04	DF	AA61RB	251	1.308	0.510	2.65%	4	4	0.9369	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	YES			FAL.NHK.SLS.19.03.03
COLCHICINE															
IIVS															
A2	RF	AA61FL	3.94	0.010	0.705	0.78%	4	3	0.4952	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A2
B1	DF	AA61FL	0.00184	0.0000046	0.384	4.49%	8	0	0.6346	1.0, 0.56, 0.31, 0.17, 0.095, 0.053, 0.029, 0.016	1.8	NO	No points 50-100%; low R2		SLS-B1
B2	DF	AA61FL	0.000675	0.0000017	0.289	9.86%	8	0	0.5984	1.0, 0.56, 0.31, 0.17, 0.095, 0.053, 0.029, 0.016	1.8	NO	No points 50-100%; low R2		SLS-B2
B3	DF	AA61FL	0.0000306	0.0000001	0.335	7.90%	8	0	0.3037	1.0, 0.56, 0.31, 0.17, 0.095, 0.053, 0.029, 0.016	1.8	NO	No points 50-100%; low R2		SLS-B3
B4	DF	AA61FL	0.0215	0.0000538	0.2	4.67%	5	0	0.7647	1.0, 0.313, 0.098, 0.031, 0.0095, 0.0030, 0.00093, 0.00029	3.19	NO	No points 50-100%; low R2		SLS-B4
B7	DF	AA61FL	0.000733	0.0000018	0.624	0.50%	6	2	0.06259	0.03, 0.02, 0.013, 0.0089, 0.0059, 0.0040, 0.0026, 0.0018	1.5	NO	Low R2		SLS-B7
B8* Hill function w/unconstrained bottom	DF	AA61FL	0.00507	0.0000127	0.677	4.22%	1	5	0.4741	0.1, 0.056, 0.031, 0.017, 0.0095, 0.0053, 0.0029,0.0016	1.8	NO	PC failed	slow NHK growth; media problems	SLS-B8
B9* Hill function w/unconstrained bottom	DF	AA61FL	0.00506	0.0000127	0.598	3.21%	0	6	0.5162	0.1, 0.056, 0.031, 0.017, 0.0095, 0.0053, 0.0029,0.0016	1.8	NO	PC failed; no points between 0 - 50%	slow NHK growth; media problems	SLS-B9
B10* Hill function w/unconstrained bottom	DF	AA61FL	NA	NA	0.44	22.49%	0	7	0.6108	0.1, 0.056, 0.031, 0.017, 0.0095, 0.0053, 0.0029,0.0016	1.8	NO	PC failed; no points between 0 - 50%; low r2; %VC difference > 15	slow NHK growth; media problems	SLS-B10
B11* Hill function w/unconstrained bottom	DF	AA61FL	0.00609	0.0000152	0.436	4.74%	5	1	0.8455	0.1, 0.056, 0.031, 0.017, 0.0095, 0.0053, 0.0029,0.0016	1.8	NO	PC failed	slow NHK growth; media problems	SLS-B11
B12* Hill function w/unconstrained bottom	DF	AA61FL	0.00927	0.0000232	0.727	5.52%	3	3	0.7899	0.045, 0.030, 0.020, 0.0133, 0.0089, 0.0059, 0.0040, 0.0026	1.5	YES		morning (a.m.) harvest; SMT accepts this test	SLS-B12
B13* Hill function w/unconstrained bottom	DF	AA61FL	0.00892	0.0000223	0.237	1.66%	5	1	0.9513	0.045, 0.030, 0.020, 0.0133, 0.0089, 0.0059, 0.0040, 0.0026	1.5	YES		afternoon (p.m.) harvest	SLS-B13
B14* Hill function w/unconstrained bottom	DF	AA61FL	0.00617	0.0000154	0.351	8.77%	5	1	0.9223	0.045, 0.030, 0.020, 0.0133, 0.0089, 0.0059, 0.0040, 0.0026	1.5	YES			SLS-B14
B15* Hill function w/unconstrained bottom	DF	AA61FL	0.00571	0.0000143	0.276	4.29%	5	2	0.873	0.045, 0.030, 0.020, 0.0133, 0.0089, 0.0059, 0.0040, 0.0026	1.5	NO	PC failed		SLS-B15
ECBC															
AA61JZ-A1	RF	AA61JZ	NA	NA	0.326	23.32%	5	2	0.0097	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	low r2; couldn't calc. ICx values; range finder	range finder	SLS-P3

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61JZ-A2	RF	AA61JZ	NA	NA	0.202	3.41%	6	2	NA	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	NO	no r2 nor ICx values could be calculated	range finder	SLS-P5
AA61JZ-B1	DF	AA61JZ	557	1.394	0.770	0.63%	4	4	0.9016	10000, 4651.2, 2163.3, 1006.2, 468, 217.7, 101.2, 47.1	2.15	NO	PC failed		SLS-P11
AA61JZ-B2	DF	AA61JZ	817	2.045	0.099	1.01%	3	4	0.9437	10000, 4651.2, 2163.3, 1006.2, 468, 217.7, 101.2, 47.1	2.15	NO	PC failed		SLS-P13
AA61JZ-B3	DF	AA61JZ	0.017	0.00004	0.089	9.22%	1	2	0.4165	0.02140, 0.00995, 0.00463, 0.00215, 0.001, 0.00046, 0.00022, 0.0001	2.15	NO	PC failed; low r2		SLS-P13
AA61JZ-B4	DF	AA61JZ	0.012	0.00003	0.089	9.29%	2	3	0.5530	0.0200, 0.0136, 0.0093, 0.0063, 0.0043, 0.0029, 0.0020, 0.0014	1.47	NO	low r2		SLS-P15
AA61JZ-B5	DF	AA61JZ	0.003	0.00001	0.884	5.21%	5	3	0.8528	0.0200, 0.0136, 0.0093, 0.0063, 0.0043, 0.0029, 0.0020, 0.0014	1.47	YES			SLS-P16
AA61JZ-B6	DF	AA61JZ	0.011	0.00003	0.494	4.09%	3	2	0.7228	0.0200, 0.0136, 0.0093, 0.0063, 0.0043, 0.0029, 0.0020, 0.0014	1.47	YES			SLS-P18
AA61JZ-B7	DF	AA61JZ	0.009	0.00002	0.687	1.01%	4	3	0.7162	0.0200, 0.0136, 0.0093, 0.0063, 0.0043, 0.0029, 0.0020, 0.0014	1.47	YES			SLS-P19
FRAME															
FAL.NHK.NW.A1.010803	RF	AA61NW	0.198	0.00050	0.305	17.20%	5	3	0.6953	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001		RF	range finder	SD says toxicity biphasic; chemical may be volatile	FAL.NHK.SLS.010803
FAL.NHK.NW.B1.080803	DF	AA61NW	0.024	0.00006	0.713	705.50%	7	1	0.6233	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001		NO	RF format	high background; biphasic response; determined ICx values with only 3 points	FAL.NHK.SLS.07.08.03
FAL.NHK.NW.B2.15.08.03	DF	AA61NW	1.00	0.00250	0.510	4.47%	6	1	0.5677	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001		NO	RF format; low r2	biphasic response	FAL.NHK.SLS.15.08.03
FAL.NHK.NW.B3.19.10.03	DF	AA61NW	0.008	0.00002	0.312	8.59%	4	2	0.8637	0.100, 0.047, 0.022, 0.01006, 0.00468, 0.00218, 0.00101, 0.00047		YES			FAL.NHK.SLS.19.10.03
FAL.NHK.NW.B4.23.10.03	DF	AA61NW	0.007	0.00002	0.340	0.96%	4	1	0.9166	0.100, 0.047, 0.022, 0.01006, 0.00468, 0.00218, 0.00101, 0.00047		YES			FAL.NHK.SLS.23.10.03
FAL.NHK.NW.B5.24.10.03	DF	AA61NW	0.008	0.00002	0.974	0.55%	4	4	0.8869	0.100, 0.047, 0.022, 0.01006, 0.00468, 0.00218, 0.00101, 0.00047		YES			FAL.NHK.SLS.24.10.03

CUPRIC SULFATE PENTAHYDRATE

IIVS															
A1	RF	AA61LA	NA	NA	0.643	3.80%	0	2	NA	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; no points between 0 - 50%		SLS-A2-N040320B
B1	DF	AA61LA	213	0.854	0.646	5.61%	3	3	0.9907	750, 536, 383, 273, 195, 139, 99.6, 71.1	1.4	YES		ppt in 2X C1 (homogeneous blue suspension); ppt in 1X C1-C8	SLS-B12-N041022B
B2	DF	AA61LA	199	0.797	0.583	1.02%	3	3	0.9957	750, 536, 383, 273, 195, 139, 99.6, 71.1	1.4	YES		ppt in 2X C1; ppt in 1X C1-C8	SLS-B113-N041029B
B3	DF	AA61LA	208	0.833	0.675	1.17%	3	3	0.9811	750, 536, 383, 273, 195, 139, 99.6, 71.1	1.4	YES		ppt in 2X C1; ppt in 1X C1-C8	SLS-B14-N041030A
ECBC															
AA61HX-A1	RF	AA61HX	NA	NA	0.487	1.42%	0	1	NA	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-P6
AA61HX-B1	DF	AA61HX	195	0.783	0.880	2.81%	6	1	0.9370	500, 413, 342, 282, 233, 193, 159, 132	1.21	YES			SLS-P47
AA61HX-B2	DF	AA61HX	168	0.672	0.675	3.43%	6	2	0.9871	500, 413, 342, 282, 233, 193, 159, 132	1.21	YES			SLS-P48

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61HX-B3	DF	AA61HX	206	0.823	1.320	1.52%	5	3	0.9814	500, 413, 342, 282, 233, 193, 159, 132	1.21	YES			SLS-P50
FRAME															
FAL.NHK.LP.A1.20.10 .04	RF	AA61LP	8.41	0.034	0.998	4.10%	3	0	0.9793	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder ; no points between 50 - 100%	outlier removed by SD	FAL.NHK.SLS.20.10.04
FAL.NHK.LP.B1.29.10.04	DF	AA61LP	NA	NA	0.545	7.44%	0	1	NA	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	no points between 0 - 50%		FAL.NHK.SLS.29.10.04
FAL.NHK.LP.B2.10.11.04	DF	AA61LP	189	0.756	1.026	0.20%	5	3	0.9474	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		outliers removed by SD	FAL.NHK.SLS.10.11.04
FAL.NHK.LP.B3.12.11.04	DF	AA61LP	186	0.746	0.696	6.80%	2	1	0.9794	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.NHK.SLS.12.11.04
FAL.NHK.LP.B4.17.11.04	DF	AA61LP	209	0.837	0.999	3.03%	2	1	0.9822	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.NHK.SLS.17.11.04
CYCLOHEXIMIDE															
IIVS															
A1	RF	AA61GL	0.0589	0.0002	0.518	2.80%	5	1	0.9832	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A4-N040331N
B1	DF	AA61GL	0.0753	0.0003	0.534	1.79%	4	3	0.9783	1.00, 0.455, 0.207, 0.094, 0.043, 0.019, 0.0088, 0.0040	2.2	YES			SLS-B4-N040513C
B2	DF	AA61GL	0.0566	0.0002	0.499	1.72%	4	4	0.9931	1.00, 0.455, 0.207, 0.094, 0.043, 0.019, 0.0088, 0.0040	2.2	YES			SLS-B5-N040514B
B3	DF	AA61GL	0.0822	0.0003	0.712	3.28%	4	2	0.9858	1.00, 0.455, 0.207, 0.094, 0.043, 0.019, 0.0088, 0.0040	2.2	YES			SLS-B6-N040716A
ECBC															
AA61KK-A1	RF	AA61KK	0.0441	0.0002	0.456	2.74%	6	1	0.9660	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P17
AA61KK-B1	DF	AA61KK	0.0558	0.0002	0.737	3.19%	4	4	0.9741	1.00, 0.465, 0.216, 0.101, 0.047, 0.022, 0.010, 0.0005	2.15	YES			SLS-P33
AA61KK-B2	DF	AA61KK	0.0634	0.0002	0.823	3.39%	4	4	0.9764	1.00, 0.465, 0.216, 0.101, 0.047, 0.022, 0.010, 0.005	2.15	YES			SLS-P35
AA61KK-B3	DF	AA61KK	0.0401	0.0001	0.418	6.74%	5	3	0.9655	1.00, 0.465, 0.216, 0.101, 0.047, 0.022, 0.010, 0.005	2.15	YES			SLS-P36
FRAME															
FAL.NHK.PF.A1.28.07.04	RF	AA61PF	0.0873	0.0003	0.042	0.79%	4	2	0.8106	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.28.07.04
FAL.NHK.PF.B1.12.08.04	DF	AA61PF	0.432	0.0015	0.862	1.46%	6	2	0.9511	100, 31.6, 10.0, 3.17, 1.00, 0.317, 0.100, 0.0318	3.16	NO	PC failed		FAL.NHK.SLS.12.08.04
FAL.NHK.PF-NB.B2.25.08.04	DF	AA61PF	0.0675	0.0002	1.104	1.57%	7	1	0.9690	100, 31.6, 10.0, 3.17, 1.00, 0.317, 0.100, 0.0318	3.16	YES			FAL.NHK.SLS.25.08.04
FAL.NHK.PF.B3.20.10 .04	DF	AA61PF	0.2285	0.0010	1.179	5.59%	5	3	0.9771	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			FAL.NHK.SLS.20.10.04
FAL.NHK.PF.B4.29.10.04	DF	AA61PF	NA	0.0000	0.507	2.36%	8	0	0.9378	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	NO	no points between 50 - 100%	toxicity curve doesn't go above 20% viability	FAL.NHK.SLS.29.10.04
FAL.NHK.PF.B5.05.11.04	DF	AA61PF	NA	NA	0.475	3.35%	6	0	NA	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	NO	no points between 50 - 100%		FAL.NHK.SLS.05.11.04
FAL.NHK.PF.B6.12.11.04	DF	AA61PF	0.0647	0.0002	0.725	2.10%	4	4	0.9513	1.00, 0.47, 0.22, 0.10, 0.05, 0.02, 0.010, 0.0047	2.15	YES			FAL.NHK.SLS.12.11.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
DIBUTYL PHTHALATE															
IIVS															
A1	RF	AA61FD	25.2	0.090	0.684	8.39%	2	1	0.9676	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1-C2; ppt in 2X C1-C2	SLS-A3-N040331A
B1	DF	AA61FD	23.2	0.083	0.562	2.55%	5	3	0.9704	1000, 455, 207, 93.9, 42.7, 19.4, 8.82, 4.01	2.2	YES		ppt in 1X C1-C4; ppt in 2X C1-C5	SLS-B1-N040423A
B2	DF	AA61FD	22.3	0.080	0.613	1.33%	3	3	0.9866	1000, 455, 207, 93.9, 42.7, 19.4, 8.82, 4.01	2.2	YES		ppt in 1X C1-C5; ppt in 2X C1-C5	SLS-B2-N040424A
B3	DF	AA61FD	20.6	0.074	0.515	7.46%	4	4	0.9634	200, 111, 61.7, 34.3, 19.1, 10.6, 5.88, 3.27	1.8	YES		ppt in 1X C1-C4; ppt in 2X C1-C4	SLS-B3-N040506A
ECBC															
AA61JX-A1	RF	AA61JX	26.8	0.096	0.892	1.40%	2	2	0.9594	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C2	SLS-P15
AA61JX-B1	DF	AA61JX	34.0	0.122	0.957	0.03%	3	5	0.9281	200, 93.0, 43.3, 20.1, 9.4, 4.4, 2.0, 0.9	2.15	YES			SLS-P46
AA61JX-B2	DF	AA61JX	19.6	0.071	0.698	0.13%	3	5	0.9518	200, 93.0, 43.3, 20.1, 9.4, 4.4, 2.0, 0.9	2.15	YES		ppt in 2X C2; 1X C1-C3 has small chunks-possibly chemical crystals	SLS-P49
AA61JX-B3	DF	AA61JX	31.2	0.112	1.251	5.20%	3	4	0.9461	200, 93.0, 43.3, 20.1, 9.4, 4.4, 2.0, 0.9	2.15	YES		chunks of chemical in 1X C1-C3; ppt in 2X C4	SLS-P51
FRAME															
FAL.NHK.MK.A1.14.05.04	RF	AA61MK	152	0.546	0.692	8.77%	1	1	0.7744	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1	FAL.NHK.SLS.14.05.03
FAL.NHK.MK.B1.19.08.04 nb	DF	AA61MK	NA	NA	0.342	2.58%	8	0	0.0000	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	NO	no points between 50 - 100%	ppt in 1X C1-C8	FAL.NHK.SLS-NB.19.08.04
FAL.NHK.MK-RB.B2.25.08.04	DF	AA61MK	17.5	0.063	0.972	4.85%	4	4	0.9053	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.NHK.SLS-RB.20.08.04
FAL.NHK.MK.B3.07.10.04	DF	AA61MK	39.7	0.143	0.602	7.72%	4	4	0.9531	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 1X C1-C5	FAL.NHK.SLS.07.10.03
FAL.NHK.MK.B4.20.10 .04	DF	AA61MK	84.9	0.305	1.289	5.24%	3	3	0.9716	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 1X C1-C4	FAL.NHK.SLS.20.10.04
DICHLORVOS															
IIVS															
A1	RF	AA61NP	12.6	0.057	0.702	59.99%	2	1	0.9650	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; % VC difference > 15	volatility problem; VC1 OD values much lower than VC2; VC1 removed from subsequent analysis by SD	SLS-A3-N040331A
B1	DF	AA61NP	12.1	0.055	0.599	10.60%	5	3	0.9934	500, 227,103, 47.0, 21.3, 9.70, 4.41, 2.00	2.2	YES			SLS-B1-N040423A
B2	DF	AA61NP	11.9	0.054	0.627	7.89%	4	3	0.9912	500, 227,103, 47.0, 21.3, 9.70, 4.41, 2.00	2.2	YES		used plate sealer	SLS-B2-N040424A
B3	DF	AA61NP	12.7	0.057	0.581	1.03%	4	2	0.9802	200, 90.9, 41.3, 18.8, 8.54, 3.88, 1.76, 0.802	2.2	YES		used plate sealer	SLS-B3-N040506A
ECBC															
AA61PZ-A1	RF	AA61PZ	NA	NA	0.532	72.53%	1	2	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P15
AA61PZ-B1(sealer)	DF	AA61PZ	8.44	0.038	0.631	6.94%	4	4	0.9304	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P32
AA61PZ-B2 (sealer)	DF	AA61PZ	10.9	0.049	0.860	3.50%	3	5	0.9861	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P34
AA61PZ-B3 (sealer)	DF	AA61PZ	6.35	0.029	0.381	4.51%	4	4	0.9428	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P36
FRAME															
FAL.NHK.HS.A1.14.05.04	RF	AA61HS	9.55	0.043	0.391	72.35%	3	0	0.4969	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; %VC difference > 0; no points between 50 - 100%	volatility problem	FAL.NHK.SLS.14.05.03
FAL.NHK.HS.B1.25.06.04	DF	AA61HS	13.2	0.060	1.094	9.37%	2	3	0.9630	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.506, 0.235	2.15	YES			FAL.NHK.SLS.25.06.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.HS.B2.12.08.04	DF	AA61HS	18.9	0.085	0.677	5.08%	2	2	0.6304	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.506, 0.235	2.15	NO	PC failed		FAL.NHK.SLS.12.08.04
FAL.NHK.HS.B3.19.08.04 nb	DF	AA61HS	NA	NA	0.510	1.27%	0	7	0.0466	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.506, 0.235	2.15	NO	no points between 0 - 50%	no toxicity detected; SD removed column of data; odd toxicity curve	FAL.NHK.SLS-NB.19.08.04
FAL.NHK.HS-RB.B4.25.08.04	DF	AA61HS	15.7	0.071	0.773	1.27%	2	1	0.6376	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.506, 0.235	2.15	YES			FAL.NHK.SLS-RB.20.08.04
FAL.NHK.HS.B5.27.08.04	DF	AA61HS	8.35	0.038	0.506	9.96%	2	6	0.8021	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.506, 0.235	2.15	YES			FAL.NHK.SLS.27.08.04
DIETHYL PHTHALATE															
IIVS															
A1	RF	AA61NX	116	0.523	0.556	0.99%	1	1	0.8983	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2	SLS-A4-N040331N
B1	DF	AA61NX	192	0.863	0.570	3.77%	3	4	0.9757	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B4-N040513C
B2	DF	AA61NX	221	0.996	0.505	1.47%	3	3	0.9758	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 2X C1	SLS-B5-N040514B
B3	DF	AA61NX	155	0.695	0.790	6.15%	3	3	0.9904	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B6-N040716A
ECBC															
AA61GA-A1	RF	AA61GA	122	0.551	0.898	5.79%	1	3	0.9642	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-P14
AA61GA-B1	DF	AA61GA	168	0.757	1.039	5.26%	2	4	0.9636	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES			SLS-P27
AA61GA-B2	DF	AA61GA	163	0.732	0.920	1.89%	3	2	0.9498	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES		ppt in 2X C1-C2	SLS-P29
AA61GA-B3	DF	AA61GA	190	0.854	0.776	1.33%	2	3	0.9633	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES		ppt in 2X C1-C2; oily	SLS-P30
FRAME															
FAL.NHK.KZ.A1.28.07.04	RF	AA61KZ	124	0.560	0.079	10.77%	1	1	0.6487	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.28.07.04
FAL.NHK.KZ.B1.11.08.04	DF	AA61KZ	27.7	0.125	0.765	6.15%	1	2	0.9160	2000, 930, 433, 201, 94, 44, 20, 9	2.15	YES		ppt in 2X C1-C4 and 1X C1-C4	FAL.NHK.SLS.11.08.04
FAL.NHK.KZ.B2.08.10.04	DF	AA61KZ	147	0.660	0.737	18.98%	2	5	0.9382	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	NO	% VC difference > 15	volatility issue; incorrect solvent listed in Addendum III; SD corrected	FAL.NHK.SLS.08.10.03
FAL.NHK.KZ.B3.22.10.04	DF	AA61KZ	149	0.670	0.731	9.65%	2	4	0.9568	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.22.10.04 (MO)
FAL.NHK.KZ.B4.28.10.04	DF	AA61KZ	37.9	0.171	0.650	11.96%	4	4	0.9425	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.28.10.04
DIGOXIN															
IIVS															
A1	RF	AA61MF	0.00075	0.0000010	0.695	0.29%	7	0	0.9294	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50 - 100%	ppt in 1X C1 and 2X C1	SLS-A3-N040331A
B1	DF	AA61MF	0.00390	0.0000050	0.575	3.87%	3	1	0.9597	0.020, 0.0091, 0.0041, 0.0019, 0.00085, 0.00039, 0.00018, 0.000080	2.2	YES			SLS-B4-N040513C
B2	DF	AA61MF	0.00374	0.0000048	0.543	0.21%	3	1	0.9615	0.020, 0.0091, 0.0041, 0.0019, 0.00085, 0.00039, 0.00018, 0.000080	2.2	YES		outlier removed by SD	SLS-B5-N040514B
B3	DF	AA61MF	0.00431	0.0000055	0.804	1.90%	2	3	0.9848	0.020, 0.0091, 0.0041, 0.0019, 0.00085, 0.00039, 0.00018, 0.000080	2.2	YES			SLS-B6-N040716A
ECBC															

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61PP-A1	RF	AA61PP	0.00865	0.0000111	1.002	8.88%	5	0	0.9920	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50 - 100%	ppt in 1X C1 and 2X C1	SLS-P13
AA61PP-B1	DF	AA61PP	0.00518	0.0000066	0.864	4.37%	4	4	0.9591	0.100, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			SLS-P33
AA61PP-B2	DF	AA61PP	0.00615	0.0000079	0.890	1.28%	4	4	0.9932	0.100, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			SLS-P35
AA61PP-B3	DF	AA61PP	0.00481	0.0000062	0.477	0.96%	5	2	0.9770	0.100, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			SLS-P37
FRAME															
FAL.NHK.HN.A1.14.05.04	RF	AA61HN	0.00002	0.0000000	0.756	7.58%	5	0	0.9437	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	outlier removed by SD; ppt in 1X C1-C2	FAL.NHK.SLS.14.05.03
FAL.NHK.HN.B1.25.06.04	DF	AA61HN	0.00006	0.0000001	1.205	0.03%	4	3	0.9543	0.0010000, 0.0004651, 0.0002163, 0.0001006, 0.0000468, 0.0000218, 0.0000101, 0.0000047	2.15	YES			FAL.NHK.SLS.25.06.04
FAL.NHK.HN.B2.20.08.04	DF	AA61HN	0.00006	0.0000001	0.845	3.03%	4	3	0.9762	0.0010000, 0.0004651, 0.0002163, 0.0001006, 0.0000468, 0.0000218, 0.0000101, 0.0000047	2.15	YES		row C data removed by SD; most of wells were outliers	FAL.NHK.SLS.20.08.04
FAL.NHK.HN.B3.27.08.04	DF	AA61HN	0.00003	0.0000000	0.404	5.62%	5	3	0.9091	0.0010000, 0.0004651, 0.0002163, 0.0001006, 0.0000468, 0.0000218, 0.0000101, 0.0000047	2.15	YES			FAL.NHK.SLS.27.08.04

DIMETHYLFORMAMIDE

IIVS															
A1	RF	AA61FN	5750	78.720	0.495	3.49%	1	1	0.8849	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A5-N040401A
B1	DF	AA61FN	6180	84.544	0.553	1.90%	3	4	0.9725	15000, 10714, 7653, 5466, 3905, 2789, 1992, 1423	1.4	YES		ppt in 1X C1	SLS-B8-N040819A
B2	DF	AA61FN	6580	89.967	0.543	5.48%	3	3	0.9801	15000, 10714, 7653, 5466, 3905, 2789, 1992, 1423	1.4	YES		ppt in 1X C1	SLS-B9-N040820A
B3	DF	AA61FN	6430	87.919	0.544	0.29%	3	3	0.9823	15000, 10714, 7653, 5466, 3905, 2789, 1992, 1423	1.4	YES			SLS-B10-N040903A
ECBC															
AA61MW-A1	RF	AA61MW	NA	NA	0.773	5.14%	1	0	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50 - 100%		SLS-P19
AA61MW-B1	DF	AA61MW	9350	127.962	0.595	0.67%	2	4	0.9730	30000, 20408, 13883, 9444, 6425, 4371, 2973, 2023	1.47	YES			SLS-P40
AA61MW-B2	DF	AA61MW	9510	130.042	0.722	1.78%	3	4	0.9847	30000, 20408, 13883, 9444, 6425, 4371, 2973, 2023	1.47	YES			SLS-P42
AA61MW-B3	DF	AA61MW	9200	125.916	0.961	1.49%	2	4	0.9788	30000, 20408, 13883, 9444, 6425, 4371, 2973, 2023	1.47	YES			SLS-P44
FRAME															
FAL.NHK.KF.A1.24.09.04	RF	AA61KF	1940	26.551	0.501	2.32%	1	1	0.3487	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.KF.B1.01.10.04	DF	AA61KF	7690	105.216	0.990	2.68%	1	7	0.9741	15000, 6977, 3245, 1509, 702, 327, 152, 70.6	2.15	NO	PC failed		FAL.NHK.SLS.01.10.04
FAL.NHK.KF.B2.10.11.04	DF	AA61KF	7930	108.413	1.031	2.19%	1	4	0.9290	15000, 6977, 3245, 1509, 702, 327, 152, 70.6	2.15	YES		ppt In 2X C1-C5	FAL.NHK.SLS.10.11.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.KF.B3.12.11.04	DF	AA61KF	6040	82.620	0.668	16.78%	1	2	0.8929	15000, 6977, 3245, 1509, 702, 327, 152, 70.6	2.15	NO	%VC difference >15	outliers removed bySD	FAL.NHK.SLS.12.11.04
FAL.NHK.KF.B4.17.11.04	DF	AA61KF	7780	106.435	1.146	1.64%	1	2	0.9281	15000, 6977, 3245, 1509, 702, 327, 152, 70.6	2.15	YES			FAL.NHK.SLS.17.11.04
FAL.NHK.KF.B5.19.11.04	DF	AA61KF	7740	105.946	0.465	5.14%	1	2	0.8514	15000, 6977, 3245, 1509, 702, 327, 152, 70.6	2.15	YES		outliers removed bySD	FAL.NHK.SLS.19.11.04
DIQUAT DIBROMIDE MONOHYDRATE															
IIVS															
A1	RF	AA61GN	5.71	0.016	0.711	0.12%	4	2	0.9904	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A3-N040331A
B1	DF	AA61GN	4.10	0.011	0.570	1.86%	6	2	0.9823	100, 55.6, 30.9, 17.1, 9.53, 5.29, 2.94, 1.63	1.8	YES			SLS-B4-N040513C
B2	DF	AA61GN	3.49	0.010	0.513	5.54%	6	2	0.9793	100, 55.6, 30.9, 17.1, 9.53, 5.29, 2.94, 1.63	1.8	YES			SLS-B5-N040514B
B3	DF	AA61GN	3.92	0.011	0.652	0.15%	4	2	0.9871	100, 55.6, 30.9, 17.1, 9.53, 5.29, 2.94, 1.63	1.8	YES			SLS-B6-N040716A
ECBC															
AA61KS-A1	RF	AA61KS	3.04	0.008	0.862	7.32%	4	4	0.9730	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P14
AA61KS-B1	DF	AA61KS	3.62	0.010	0.671	2.01%	5	3	0.9904	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P33
AA61KS-B2	DF	AA61KS	4.40	0.012	0.570	0.19%	5	2	0.9601	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P34
AA61KS-B3	DF	AA61KS	2.75	0.008	0.361	4.41%	5	3	0.9603	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			SLS-P36
FRAME															
FAL.NHK.NV.A1.14.05.04	RF	AA61NV	3.88	0.011	0.640	4.87%	4	1	0.9854	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.14.05.03
FAL.NHK.NV.B1.12.08.04	DF	AA61NV	7.22	0.020	0.899	3.27%	6	2	0.9571	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	PC failed	row of data removed from analysis by the SD due to low cell growth	FAL.NHK.SLS.12.08.04
FAL.NHK.NV.B2.19.08.04 rb	DF	AA61NV	43.3	0.119	0.271	2.15%	4	1	0.7846	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	PC failed		FAL.NHK.SLS-RB.19.08.04
FAL.NHK.NV.B3.20.08.04	DF	AA61NV	6.09	0.017	0.762	8.68%	6	2	0.9750	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		row C data removed by SD; several wells were outliers	FAL.NHK.SLS.20.08.04
FAL.NHK.NV-RB.B4.25.08.04	DF	AA61NV	11.9	0.033	0.583	7.52%	5	3	0.9780	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.NHK.SLS-RB.20.08.04
FAL.NHK.NV.B5.27.08.04	DF	AA61NV	0.812	0.002	0.493	3.41%	7	0	0.8924	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	no points between 50 - 100%		FAL.NHK.SLS.27.08.04
FAL.NHK.NV.30.09.04	DF	AA61NV	2.97	0.008	0.677	0.21%	5	3	0.9830	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.NHK.SLS.30.09.03
FAL.NHK.NV.B7.07.10.04	DF	AA61NV	6.13	0.017	0.665	1.98%	4	4	0.9794	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.NHK.SLS.07.10.03
DISULFOTON															
IIVS															
A1	RF	AA61FC	140	0.509	0.559	3.49%	1	2	0.5182	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C2	SLS-A4-N040331N

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B1	DF	AA61FC	176	0.641	0.619	10.61%	4	4	0.9647	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES		ppt in 1X C1-C5; ppt in 2X C1-C7; visual observations of the cells are different from the NRU viability results.	SLS-B12-N041022B
B2	DF	AA61FC	133	0.486	0.566	5.12%	4	4	0.9650	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES		ppt in 1X C1-C6; ppt in 2X C1-C6;	SLS-B113-N041029B
B3	DF	AA61FC	250	0.911	0.668	3.22%	3	5	0.9138	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES		ppt in 1X C1-C5; ppt in 2X C1-C6;	SLS-B14-N041030A
ECBC															
AA61NY-A1	RF	AA61NY	NA	NA	0.798	10.85%	1	3	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C2	SLS-P39
AA61NY-B1	DF	AA61NY	139	0.508	0.623	2.86%	2	5	0.8924	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		ppt in 2X C1-C4	SLS-P55
AA61NY-B2a	DF	AA61NY	167	0.610	0.781	1.34%	1	6	0.8173	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES		chem. pieces C1-C4 in 96-well plate; ppt in 2X C1-C2; C1 toxicity < C2; curve rises; SD originally failed test; good toxicity curve when C1 removed by SD	SLS-P56
AA61NY-B3	DF	AA61NY	NA	NA	0.533	0.92%	0	8	NA	300, 204, 139, 94, 64, 44, 30, 20	1.47	NO	no points between 0-50%	no PRISM file generated; globules of chemical in 1X C1-C6; ppt in 2X C1-C4	SLS-P57
AA61NY-B4a	DF	AA61NY	113	0.413	0.128	6.62%	1	6	0.7376	300, 204, 139, 94, 64, 44, 30, 20	1.47	YES		chem. globules in all conc. in test plate; ppt in 2X C1-C5;C1 toxicity< C2 and C3; curve rises; SD originally failed test; good tox. curve when C1 and C2 removed by SD	SLS-P58
FRAME															
FAL.NHK.LC.A1.28.07.04	RF	AA61LC	NA	NA	0.052	15.74%	1	2	-0.3837	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; % VC difference > 15		FAL.NHK.SLS.28.07.04
FAL.NHK.LC.B1.11.08.04	DF	AA61LC	828	3.017	0.764	7.18%	1	5	0.7436	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		ppt in C3	FAL.NHK.SLS.11.08.04
FAL.NHK.LC.B2.17.09.04	DF	AA61LC	1670	6.104	0.685	4.15%	0	7	0.8707	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 0 - 50%	ppt in C1-C4;outliers removed	FAL.NHK.SLS.17.09.04
FAL.NHK.LC.B3.08.10.04	DF	AA61LC	586	2.136	0.681	9.54%	2	6	0.8830	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 2X C1-C4; 1X C1	FAL.NHK.SLS.08.10.03
FAL.NHK.LC.B4.20.10 .04	DF	AA61LC	1010	3.678	1.071	13.87%	2	6	0.9319	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 2X C1; ppt in 1X C1-C8	FAL.NHK.SLS.20.10.04

ENDOSULFAN

IIVS															
A1	RF	AA61HZ	0.817	0.002	0.637	37.84%	2	3	0.9532	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; %VC difference >0	volatility problem; VC1 OD values much lower than VC2; VC1 removed from subsequent analysis by SD	SLS-A2-N040320B
B1	DF	AA61HZ	2.66	0.007	0.690	3.49%	1	3	0.9857	50.0, 27.8, 15.4, 8.57, 4.76, 2.65, 1.47, 0.817	1.8	YES		ppt in 2X C2	SLS-B1-N040423A
B2	DF	AA61HZ	2.10	0.005	0.674	1.76%	3	2	0.9910	50.0, 27.8, 15.4, 8.57, 4.76, 2.65, 1.47, 0.817	1.8	YES		ppt in 2X C2; ppt in 1X C1	SLS-B2-N040424A
B3	DF	AA61HZ	1.80	0.004	0.554	0.89%	3	2	0.9590	20.0, 12.5, 7.81, 4.88, 3.05, 1.91, 1.19, 0.745	1.6	YES			SLS-B3-N040506A
ECBC															
AA61LG-A1	RF	AA61LG	NA	NA	0.612	31.27%	2	1	NA	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; % VC difference > 15	ppt in 2X C1 and C1	SLS-P39
AA61LG-B1(sealer)	DF	AA61LG	4.46	0.011	0.935	2.18%	0	5	0.8732	10.0, 4.65, 2.16, 1.01, 0.47, 0.22, 0.10, 0.05	2.15	NO	no points between 0 - 50%		SLS-P46
AA61LG-B2 (sealer)	DF	AA61LG	4.09	0.010	1.218	0.21%	2	6	0.9121	9.00, 6.12, 4.17, 2.83, 1.93, 1.31, 0.892, 0.607	1.47	YES			SLS-P51

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61LG-B3 (sealer)	DF	AA61LG	3.00	0.007	0.613	0.94%	3	5	0.9278	9.00, 6.12, 4.17, 2.83, 1.93, 1.31, 0.892, 0.607	1.47	YES			SLS-P52
AA61LG-B4 (sealer)	DF	AA61LG	3.24	0.008	0.631	4.02%	3	4	0.9089	9.00, 6.12, 4.17, 2.83, 1.93, 1.31, 0.892, 0.607	1.47	YES			SLS-P54
FRAME															
FAL.NHK.PW.A1.28.04.04	RF	AA61PW	1.79	0.004	0.592	24.69%	1	2	0.4155	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder; %VC difference > 15	possible volatility problem	FAL.NHK.SLS.28.04.03
FAL.NHK.PW.B1.11.06.04	DF	AA61PW	1.05	0.003	0.953	2.52%	5	1	0.6822	10, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	PC failed	incorrect solvent listed; biphasic response	FAL.NHK.SLS.11.06.04
FAL.NHK.PW.B2.25.06.04	DF	AA61PW	2.19	0.005	1.109	6.72%	5	3	0.9113	10, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			FAL.NHK.SLS.25.06.04
FAL.NHK.PW.B3.17.09.04	DF	AA61PW	1.24	0.003	0.820	0.67%	5	2	0.8280	10, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES		outlier removed by SD	FAL.NHK.SLS.17.09.04
FAL.NHK.PW.B4.07.10.04	DF	AA61PW	0.822	0.002	0.731	4.68%	7	1	0.7929	10, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			FAL.NHK.SLS.07.10.03
EPINEPHRINE BITARTRATE															
IIVS															
A1	RF	AA61LT	91.2	0.274	0.637	6.28%	2	1	0.9359	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A2-N040320B
B1	DF	AA61LT	61.1	0.183	0.430	3.51%	5	3	0.9623	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 1X C1	SLS-B1-N040423A
B2	DF	AA61LT	83.8	0.251	0.562	3.01%	2	3	0.9796	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 1X C1-C2	SLS-B2-N040424A
B3	DF	AA61LT	80.0	0.240	0.513	2.26%	2	5	0.9398	200, 143, 102, 72.9, 52.1, 37.2, 26.6, 19.0	1.4	YES			SLS-B3-N040506A
ECBC															
AA61HW-A1	RF	AA61HW	73.5	0.220	0.337	4.12%	2	0	0.6969	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	ppt in 2X C1	SLS-P6
AA61HW-B1	DF	AA61HW	124	0.371	0.897	6.82%	2	2	0.8018	200, 136, 92.6, 63.0, 42.8, 29.1, 19.8, 13.5	1.47	YES			SLS-P26
AA61HW-B2	DF	AA61HW	118	0.354	0.959	3.84%	3	3	0.9373	200, 165, 137, 113, 93.3, 77.1, 63.7, 52.7	1.21	YES			SLS-P29
AA61HW-B3	DF	AA61HW	103	0.308	0.692	0.84%	4	2	0.9411	200, 165, 137, 113, 93.3, 77.1, 63.7, 52.7	1.21	YES			SLS-P31
FRAME															
FAL.NHK.RK.A1.26.03.04	RF	AA61RK	93.5	0.281	0.552	10.97%	3	0	0.7362	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	pts between 50 - 100% but several above 100% ; ppt in C1	FAL.NHK.SLS.26.03.04
FAL.NHK.RK.B1.25.04.04	DF	AA61RK	112	0.337	0.705	1.25%	3	1	0.8428	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		two "outliers" in C4 removed b SD due to low OD	FAL.NHK.SLS.25.04.04
FAL.NHK.RK.B2.28.04.04	DF	AA61RK	77.3	0.232	0.887	5.93%	4	1	0.9755	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7,	2.15	YES		two "outliers" in C4 removed by SD; no NR uptake	FAL.NHK.SLS.28.04.03
FAL.NHK.RK.B3.13.05.04	DF	AA61RK	55.8	0.168	0.606	0.81%	4	3	0.9907	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.13.05.04
ETHANOL															
IIVS															
A1	RF	AA61FH	NA	NA	0.628	2.73%	0	1	0.4299	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0 - 50%		SLS-A2-N040320B
B1	DF	AA61FH	7240	157.247	0.461	100.30%	3	2	0.9851	150000, 83333, 46296, 25720, 14289, 7938, 4410, 2450	1.8	NO	%VC difference >15	Left VC was removed from calculations due to volatility	SLS-B8-N040819A
B2	DF	AA61FH	6430	139.502	0.509	100.04%	2	2	0.9844	150000, 83333, 46296, 25720, 14289, 7938, 4410, 2450	1.8	NO	%VC difference >15	Left VC was removed from calculations due to volatility	SLS-B9-N040820A

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B3	DF	AA61FH	10800	234.197	0.586	1.92%	2	3	0.9760	150000, 83333, 46296, 25720, 14289, 7938, 4410, 2450	1.8	YES			SLS-B11-N040904H
B4	DF	AA61FH	9250	200.716	0.709	2.59%	1	3	0.9781	150000, 83333, 46296, 25720, 14289, 7938, 4410, 2450	1.8	YES			SLS-B10-N040903A
B5	DF	AA61FH	10700	232.050	0.627	1.78%	3	4	0.9858	50000, 31250, 19531, 12207, 7629, 4768, 2980, 1863	1.6	YES			SLS-B12-N041022B
ECBC															
AA61JU-A1	RF	AA61JU	NA	NA	0.436	7.58%	0	1	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P5
AA61JU-B1(sealer)	DF	AA61JU	7940	172.418	0.701	3.02%	6	1	0.9000	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			SLS-P28
AA61JU-B2(sealer)	DF	AA61JU	8710	189.052	0.741	5.60%	5	3	0.9616	50000, 34014, 23139, 15740, 10708, 7284, 4955, 3371	1.47	YES			SLS-P31
AA61JU-B3(sealer)	DF	AA61JU	8220	178.477	0.788	1.41%	3	4	0.9617	30000, 20408, 13883, 9444, 6425, 4371, 2973, 2023	1.47	YES			SLS-P34
FRAME															
FAL.NHK.PC.A1.25.04.04	RF	AA61PC	11800	256.792	0.646	14.49%	0	1	-0.7906	100000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.25.04.04
FAL.NHK.PC.A2.28.04.04	DF	AA61PC	9640	209.210	0.959	3.42%	2	6	0.9428	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	YES			FAL.NHK.SLS.28.04.03
FAL.NHK.PC.B2.11.06.04	DF	AA61PC	11400	247.504	0.753	2.64%	1	3	0.8972	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	NO	PC failed	incorrect solvent listed	FAL.NHK.SLS.11.06.04
FAL.NHK.PC.B3.23.06.04	DF	AA61PC	14200	308.022	0.896	9.81%	1	4	0.8958	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	YES			FAL.NHK.SLS.23.06.04
FAL.NHK.PC.B4.25.06.04	DF	AA61PC	12200	265.816	0.899	4.29%	1	3	0.8875	25000, 11628, 5408, 2516, 1170, 544, 253, 118	2.15	YES			FAL.NHK.SLS.25.06.04
ETHYLENE GLYCOL															
IIVS															
Preliminary	RF	AA61HR	44900	723.027	0.588	4.11%	0	1	0.6185	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		Preliminary
B1	DF	AA61HR	40900	658.615	0.552	1.95%	1	2	0.9752	100000, 56250, 31600, 17800, 10000, 5600, 3160, 1770	1.78	YES			SLS-B1
B2	DF	AA61HR	32200	518.519	0.734	3.50%	1	3	0.9755	100000, 56250, 31600, 17800, 10000, 5600, 3160, 1770	1.78	YES			SLS-B2
B3	DF	AA61HR	43200	695.652	0.798	1.30%	1	1	0.9797	100000, 56250, 31600, 17800, 10000, 5600, 3160, 1770	1.78	YES			SLS-B3
B4	DF	AA61HR	43700	703.704	0.826	4.36%	1	1	0.9780	100000, 56250, 31600, 17800, 10000, 5600, 3160, 1770	1.78	YES			SLS-B4
ECBC															
ECBC-NHK-Ib-01 AA61LM-A1	RF	AA61LM	NA	NA	0.788	1.16%	0	0	-0.5039	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P2
ECBC-NHK-Ib-02 AA61LM-A2	RF	AA61LM	17700	285.024	1.125	7.69%	0	1	0.9617	100000, 10000, 1000, 100, 10, 1, 0.1, 0.01	10	NO	No points between 10 and 50%		SLS-P3
ECBC-NHK-Ib-03 AA61LM-B1	DF	AA61LM	42100	677.939	1.282	1.23%	2	2	0.9764	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	YES			SLS-P4

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
ECBC-NHK-Ib-04 AA61LM-B2 (correction rec'd 4/30/03)	DF	AA61LM	39000	628.019	1.148	5.83%	1	2	0.9491	84869.6, 57656.0, 39168.5, 26609.0, 18076.8, 12280.4, 8342.7, 5667.6	1.47	YES			SLS-P5
ECBC-NHK-Ib-05 AA61LM-B3	DF	AA61LM	44000	708.535	1.119	0.98%	0	2	0.9719	100000, 68000, 46300, 31500, 21400, 14600, 9910, 6740	1.47	NO	No points between 10 and 50%		SLS-P7
ECBC-NHK-Ib-06 AA61LM-B4	DF	AA61LM	32900	529.791	0.910	3.05%	3	3	0.9383	60030, 46200, 35500, 27300, 21000, 16200, 12400, 9570	1.3	YES			SLS-P8
FRAME															
A3 1b/NHK/DF1/FAL/PD	DF	AA61PD	16.1	0.259	0.047	1.95%	5	1	0.3772	100, 68.02, 46.27, 31.47, 21.40, 14.50, 9.90, 6.70	1.47	RF	R^2 < 0.8; PC failed; range finder	NR crystal problems; used medium not normally used	A3 1b/NHK/CTR4/FAL/
A4 1b/NHK/DF2/FAL/PD	DF	AA61PD	4.17	0.067	0.125	25.74%	4	1	0.1465	100, 68.02, 46.27, 31.47, 21.41, 14.56, 9.90, 6.74	1.47	NO	VC difference > 15%; R^2 < 0.8	NR crystal problems; used medium not normally used	A4 1b/NHK/CTR5/FAL
A5 1b/NHK/DF3/FAL/PD	DF	AA61PD	NA	NA	0.140	1.78%	6	1	NA	100, 68.02, 46.27, 31.47, 21.41, 14.56, 9.90, 6.74	1.47	NO	No R^2 or ICx; PC failed	Used different medium; OD values of test wells slightly higher than bkgd. ODs; negative values for VC	A5 1b/NHK/CTR6/FAL
A6 1b/NHK/DF4/FAL/PD	DF	AA61PD	67.1	1.081	0.920	0.29%	1	0	0.5955	100, 68.02, 46.27, 31.47, 21.40, 14.50, 9.90, 6.70	1.47	NO	No point between 50 & 90%; R^2 < 0.8	recalc w/o outlier didn't improve fit, so outlier was not removed	A6 1b/NHK/CTR7/FAL
A10 1b/NHK/DF5/FAL/PD	DF	AA61PD	48400	779.388	1.203	10.37%	1	6	0.8164	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES		no outliers	A10 1b/NHK/CTR11/FAL
A11 1b/NHK/DF6/FAL/PD	DF	AA61PD	54700	880.837	1.706	4.22%	2	2	0.8960	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			A11 1b/NHK/CTR12/FAL
A12 1b/NHK/DF7/FAL/PD	DF	AA61PD	33200	534.622	0.372	17.37%	1	5	0.8678	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	NO	VC difference > 15%		A12 1b/NHK/CTR13/FAL/SLS
1b/NHK/DF3/FAL/PD	DF	AA61PD	46300	745.572	0.773	12.10%	1	5	0.9074	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			1b/NHK/CTR14/FAL/SLS

FENPROPATHRIN

IIVS															
A1	RF	AA61HY	1.38	0.004	0.552	4.86%	3	1	0.9698	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2 and 1X C1-C2	SLS-A1-N040317B
B1	DF	AA61HY	2.18	0.006	0.580	3.12%	5	3	0.9412	75.0, 34.1, 15.5, 7.04, 3.20, 1.46, 0.661, 0.301	2.2	YES		ppt in 2X C1-C3	SLS-B1-N040423A
B2	DF	AA61HY	1.67	0.005	0.600	4.40%	5	2	0.9440	75.0, 34.1, 15.5, 7.04, 3.20, 1.46, 0.661, 0.301	2.2	YES		ppt in 2X C1-C3	SLS-B2-N040424A
B3	DF	AA61HY	1.62	0.005	0.528	1.77%	5	2	0.9228	75.0, 34.1, 15.5, 7.04, 3.20, 1.46, 0.661, 0.301	2.2	YES		ppt in 2X C1-C3; ppt in 1X C1	SLS-B3-N040506A
ECBC															
AA61LJ-A1	RF	AA61LJ	4.46	0.013	0.569	6.52%	3	3	0.9479	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2 and 1X C1	SLS-P2
AA61LJ-B1	DF	AA61LJ	3.71	0.0106	1.025	3.17%	8	0	0.8224	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74	1.47	NO	no points between 50 - 100%	ppt in 2X C1-C5 and 1X C1	SLS-P8
AA61LJ-B2	DF	AA61LJ	2.94	0.008	1.265	0.48%	5	3	0.9897	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		ppt in 2X C1-C3	SLS-P10
AA61LJ-B3	DF	AA61LJ	3.38	0.010	0.779	5.84%	5	3	0.9503	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		ppt in 2X C1-C3 and 1X C1	SLS-P11
AA61LJ-B4	DF	AA61LJ	4.87	0.014	0.991	1.87%	5	3	0.9448	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		ppt in 2X C1-C3 and 1X C1	SLS-P23
FRAME															
FAL.NHK.A1.11/02/04	RF	AA61PT	5.51	0.016	1.226	1.06%	3	5	0.9610	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	C5 outliers removed by SD; ppt in 2X C1 and 1X C1-C2	FAL.NHK.SLS.11.02.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.PT.B1.26.02.04	DF	AA61PT	0.012	0.000	0.185	9.24%	8	0	0.4977	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	no points between 50-100%	ppt in 2X C1-C5 and 1X C1-C4	FAL.NHK.SLS/MO.26.02.03
FAL.NHK.PT.18.03.04 (B2 not in identifier)	DF	AA61PT	2.77	0.008	0.321	1.46%	4	1	0.7108	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.51, 0.24	2.15	YES		ppt in 2X C1 and 1X C1	FAL.NHK.SLS.18.03.03
FAL.NHK.PT.B3.19.03.04	DF	AA61PT	2.37	0.007	0.587	8.52%	5	2	0.9693	50.0, 23.3, 10.8, 5.03, 2.34, 1.09, 0.51, 0.24	2.15	YES		ppt in 2X C1	FAL.NHK.SLS.19.03.03
FAL.NHK.PT.B4.25.03.04	DF	AA61PT	1.56	0.004	0.693	8.69%	6	2	0.9644	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		ppt in 2X C1 and 1X C1-C4	FAL.NHK.SLS.25.03.03
GIBBERELLIC ACID															
IIVS															
A1	RF	AA61RE	NA	NA	0.542	1.18%	0	1	0.0000	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 - 50%	outlier in C7 removed by SD	SLS-A4-N040331N
B1	DF	AA61RE	2820	8.155	0.594	4.88%	1	4	0.9686	3750, 2344, 1465, 916, 572, 358, 224, 140	1.6	YES			SLS-B12-N041022B
B2	DF	AA61RE	2920	8.442	0.499	1.94%	1	2	0.9503	3750, 2679, 1913, 1367, 976, 697, 498, 356	1.4	YES			SLS-B113-N041029B
B3	DF	AA61RE	2680	7.735	0.646	1.50%	1	5	0.9492	3750, 2679, 1913, 1367, 976, 697, 498, 356	1.4	YES			SLS-B14-N041030A
ECBC															
AA61FR-A1	RF	AA61FR	NA	NA	0.958	1.55%	0	6	NA	2500, 250, 25, 2.5, 0.25, 0.025, 0.0025, 0.00025	10	RF	range finder		SLS-P22
AA61FR-B1	DF	AA61FR	2470	7.136	0.689	0.27%	4	4	0.9209	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES		ppt in 2X C1-C4 and 1X C1	SLS-P49
AA61FR-B2	DF	AA61FR	3270	9.429	1.151	0.64%	3	5	0.9334	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES		ppt in 2X C1-C5	SLS-P50
AA61FR-B3	DF	AA61FR	2810	8.118	0.643	1.28%	4	4	0.9736	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES		ppt in 2X C1-C3 and 1X C1	SLS-P53
FRAME															
FAL.NHK.GY.***A1.28.07.04 (should be 11.08.04)***	RF	AA61GY	NA	NA	0.596	2.46%	0	1	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.11.08.04
FAL.NHK.GY.B1.08.10.04	DF	AA61GY	3030	8.739	0.629	2.48%	1	7	0.8918	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	YES			FAL.NHK.SLS.08.10.03
FAL.NHK.GY.B2.20.10 .04	DF	AA61GY	3160	9.130	1.110	2.21%	1	2	0.9820	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	YES			FAL.NHK.SLS.20.10.04
FAL.NHK.GY.B3 .22.10.04	DF	AA61GY	2630	7.594	0.641	8.86%	1	1	0.8601	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	YES			FAL.NHK.SLS.22.10.04 (MO)
GLUTETHIMIDE															
IIVS															
A1	RF	AA61NN	119	0.546	0.579	1.28%	0	1	0.9782	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 - 50%	ppt in 2X C1	SLS-A5-N040401A
B1	DF	AA61NN	190	0.873	0.634	3.05%	4	3	0.9710	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 2X C1-C3	SLS-B4-N040513C
B2	DF	AA61NN	193	0.889	0.541	0.86%	4	2	0.9455	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 2X C1-C3	SLS-B5-N040514B
B3	DF	AA61NN	144	0.664	0.806	8.24%	4	4	0.9734	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES		ppt in 1X C1 and 2X C1	SLS-B6-N040716A
ECBC															
AA61FE-A1	RF	AA61FE	171	0.789	0.574	1.65%	1	6	0.9668	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P25
AA61FE-B1	DF	AA61FE	114	0.524	0.799	6.19%	3	5	0.9192	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P40
AA61FE-B2	DF	AA61FE	236	1.086	0.688	1.79%	2	1	0.9489	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P43

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61FE-B3	DF	AA61FE	210	0.966	1.015	6.51%	3	4	0.9724	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P45
FRAME															
FAL.NHK.KY.A1.24.09.04	RF	AA61KY	200	0.922	0.492	0.10%	1	1	0.0402	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.KY.B1.01.10.04	DF	AA61KY	222	1.021	1.023	10.48%	5	3	0.8909	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	NO	PC failed		FAL.NHK.SLS.01.10.04
FAL.NHK.KY.B2.07.10.04	DF	AA61KY	147	0.674	0.668	1.24%	6	2	0.9631	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			FAL.NHK.SLS.07.10.03
FAL.NHK.KY.B3.05.11.04	DF	AA61KY	195	0.899	0.502	0.78%	3	5	0.9246	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.05.11.04
FAL.NHK.KY.B4.10.11.04	DF	AA61KY	167	0.771	1.009	9.60%	3	3	0.9317	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.10.11.04
GLYCEROL															
IIVS															
A1	RF	AA61JF	NA	NA	0.446	6.43%	0	2	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0 - 50%		SLS-A1-N040317B
B1	DF	AA61JF	27500	298.392	0.509	14.14%	3	3	0.9818	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	YES			SLS-B12-N041022B
B2	DF	AA61JF	34200	371.354	0.519	9.50%	3	5	0.9761	101960, 72829, 52020, 37157, 26541, 18958, 13541, 9672	1.4	YES		130 ul of 2X doses were applied. Final conc. values adjusted in data sheets by SD; data from wells G3-G10 removed from EXCEL and PRISM analyses (by SD) since they were not dosed	SLS-B113-N041029B
B3	DF	AA61JF	25400	275.923	0.627	0.03%	3	4	0.9671	100000, 71429, 51020, 36443, 26031, 18593, 13281, 9486	1.4	YES			SLS-B14-N041030A
ECBC															
AA61HG-A1	RF	AA61HG	NA	NA	0.612	4.48%	0	7	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	no toxicity detected	SLS-P1
AA61HG-A2	RF	AA61HG	15600	168.961	0.497	3.56%	1	1	0.8792	100000, 10000, 1000, 100, 10, 1, 0.1, 0.01	10	RF	range finder		SLS-P3
AA61HG-B1	DF	AA61HG	51200	555.693	1.001	1.36%	1	3	0.9717	100000, 46512, 21633, 10062, 4680, 2177, 1012, 471	2.15	YES			SLS-P8
AA61HG-B2	DF	AA61HG	30500	330.969	0.880	0.09%	3	5	0.9505	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			SLS-P14
AA61HG-B3	DF	AA61HG	21100	229.503	0.481	14.05%	5	2	0.9533	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	YES			SLS-P16
FRAME															
FAL.NHK.RA.A1.11/02/04	RF	AA61RA	NA	NA	0.662	0.55%	0	0	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.11.02.04
FAL.NHK.RA.A2.18.02.04	DF	AA61RA	57300	621.996	0.180	11.45%	1	3	0.2547	100000, 68027, 46277, 31481, 21416, 14568, 9911, 6742	1.47	NO	PC failed	this is a definitive test since conc. series is different from A1 range finder	FAL.NHK.SLS.18.02.04
FAL.NHK.RA.B1.26.02.04	DF	AA61RA	21800	237.021	0.205	15.32%	2	1	0.9389	100000, 46512, 21633, 10062, 4680, 2177, 1012, 471	2.15	YES			FAL.NHK.SLS/NB.26.02.03
FAL.NHK.RA.B2.18.03.04	DF	AA61RA	8470	92.000	0.438	7.92%	4	4	0.9629	100000, 46512, 21633, 10062, 4680, 2177, 1012, 471	2.15	YES			FAL.NHK.SLS.18.03.03
FAL.NHK.RA.B3.19.03.04	DF	AA61RA	23800	258.100	0.407	10.70%	2	4	0.9425	100000, 46512, 21633, 10062, 4680, 2177, 1012, 471	2.15	YES			FAL.NHK.SLS.19.03.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
HALOPERIDOL															
IIVS															
A1	RF	AA61LW	2.86	0.008	0.589	2.46%	2	5	0.9764	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		SLS-A4-N040331N
B1	DF	AA61LW	4.51	0.012	0.585	0.93%	2	5	0.9715	50.0, 22.7, 10.3, 4.70, 2.13, 0.970, 0.441, 0.200	2.2	YES			SLS-B4-N040513C
B2	DF	AA61LW	3.11	0.008	0.576	4.43%	3	4	0.9736	50.0, 22.7, 10.3, 4.70, 2.13, 0.970, 0.441, 0.200	2.2	YES			SLS-B5-N040514B
B3	DF	AA61LW	2.24	0.006	0.764	4.42%	3	4	0.9571	50.0, 22.7, 10.3, 4.70, 2.13, 0.970, 0.441, 0.200	2.2	YES			SLS-B6-N040716A
ECBC															
AA61JC-A1	RF	AA61JC	4.88	0.013	0.947	6.60%	2	6	0.9383	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	ppt in 1X C1	SLS-P19
AA61JC-B1	DF	AA61JC	2.70	0.007	0.700	2.99%	4	3	0.9630	80.0, 37.2, 17.3, 8.05, 3.74, 1.74, 0.81, 0.38	2.15	YES			SLS-P41
AA61JC-B2	DF	AA61JC	3.66	0.010	0.687	7.99%	4	3	0.9516	40.0, 18.6, 8.65, 4.03, 1.87, 0.871, 0.405, 0.188	2.15	YES			SLS-P42
AA61JC-B3	DF	AA61JC	4.72	0.013	1.060	1.49%	4	4	0.9411	40.0, 18.6, 8.65, 4.03, 1.87, 0.871, 0.405, 0.188	2.15	YES			SLS-P44
FRAME															
FAL.NHK.PM.A1.11.08.04	RF	AA61PM	0.329	0.001	0.803	11.63%	3	3	0.8526	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	ppt in 1X C1-C2	FAL.NHK.SLS.11.08.04
FAL.NHK.PM.B1.08.10.04	DF	AA61PM	4.52	0.012	0.680	14.55%	2	4	0.9665	100, 31.8, 10.1, 3.2, 1.02, 0.322, 0.102, 0.0325	3.15	YES			FAL.NHK.SLS.08.10.03
FAL.NHK.PM.B2.22.10.04	DF	AA61PM	4.99	0.013	0.743	2.20%	2	5	0.9658	100, 31.8, 10.1, 3.2, 1.02, 0.322, 0.102, 0.0325	3.15	YES			FAL.NHK.SLS.22.10.04 (MO)
FAL.NHK.PM.B3.29.10.04	DF	AA61PM	1.64	0.004	0.629	7.30%	5	3	0.9621	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01,0.47	2.15	YES			FAL.NHK.SLS.29.10.04
HEXACHLOROPHENE															
IIVS															
A1	RF	AA61JN	0.025	0.00006	0.509	3.75%	2	3	0.9760	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	Due to high ppt in 2X C1-C2and 1X C1-C2; SD removed these two doses from Hill function analyses and set the bottom to 0	SLS-A1-N040317B
B1	DF	AA61JN	0.0223	0.00005	0.609	3.49%	3	3	0.9868	0.500, 0.227, 0.103, 0.047, 0.021, 0.010, 0.004, 0.002	2.2	YES			SLS-B1-N040423A
B2	DF	AA61JN	0.0186	0.00005	0.611	0.44%	4	1	0.9891	0.500, 0.227, 0.103, 0.047, 0.021, 0.010, 0.004, 0.002	2.2	YES			SLS-B2-N040424A
B3	DF	AA61JN	0.0227	0.00006	0.520	1.39%	3	2	0.9885	0.500, 0.227, 0.103, 0.047, 0.021, 0.010, 0.004, 0.002	2.2	YES			SLS-B3-N040506A
ECBC															
AA61ND-A1	RF	AA61ND	NA	NA	0.421	16.43%	3	0	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2 and 1X C1-C2	SLS-P4
AA61ND-B1	DF	AA61ND	0.0294	0.00007	0.684	6.18%	5	3	0.9590	0.200, 0.136, 0.093, 0.063, 0.043, 0.029, 0.020, 0.013	1.47	YES			SLS-P21

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61ND-B2	DF	AA61ND	0.0301	0.00007	0.891	1.12%	5	3	0.9862	0.200, 0.136, 0.093, 0.063, 0.043, 0.029, 0.020, 0.013	1.47	YES			SLS-P23
AA61ND-B3	DF	AA61ND	0.0221	0.00005	0.586	1.63%	2	6	0.9707	0.200, 0.136, 0.093, 0.063, 0.043, 0.029, 0.020, 0.013	1.47	YES			SLS-P25
FRAME															
FAL.NHK.HB.A2.26.02.03	RF	AA61HB	NA	NA	0.249	7.29%	NA	NA	0.0000	NA	NA	RF	range finder	SD says ppt binds or reacts with NR;gives "nonsense" data; tox. curve goes wrong direction; ppt in 1X C1-C3	FAL.NHK.SLS/MO.26.02.03
FAL.NHK.HB.B1.18.03.04	DF	AA61HB	NA	NA	0.654	5.98%	0	0	-1.2210	0.010, 0.003, 0.001, 0.00032, 0.00010, 0.0000322, 0.0000102, 0.0000032	3.15	NO	no points between 0-100%	SD notes incorrect range used; considers 100 ug/ml as start conc. w/ dil. factor 2.15	FAL.NHK.SLS.18.03.03
FAL.NHK.HB.B2.19.03.04	DF	AA61HB	NA	NA	0.523	6.30%	0	0	-1.2210	0.010, 0.003, 0.001, 0.00032, 0.00010, 0.0000322, 0.0000102, 0.0000032	3.15	NO	no points between 0-100%	SD notes incorrect range used; considers 100 ug/ml as start conc. w/ dil. factor 2.15	FAL.NHK.SLS.19.03.03
FAL.NHK.HB.B2.25.03.04 (should be B3)	DF	AA61HB	NA	NA	0.544	7.76%	0	0	0.1438	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	curve is going in the wrong direction	Data not analysed; chem. reacts w/ NR & gives false + results in columns C1-C4; cells in first 3-4 col. incorp. large amount of dye	FAL.NHK.SLS.25.03.03
FAL.NHK.HB.B3.26.03.04 (should be B4)	DF	AA61HB	NA	NA	0.652	15.30%	0	0	-1.2210	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	curve is going in the wrong direction	Data not analysed; chem. seems to react w/ NR & gives false + results in col. C1-C4; cells in first 3-4 col. Incorp. large amount of dye; ppt in 1X C1-C2	FAL.NHK.SLS.26.03.04
FAL.NHK.HB.B4.25.04.04 (should be B5)	DF	AA61HB	0.0521	0.00013	0.850	3.86%	4	2	0.9900	1.0, 0.465, 0.216, 0.101, 0.046, 0.022, 0.010, 0.005	2.15	YES			FAL.NHK.SLS.25.04.04
FAL.NHK.HB.B5.28.04.04 (should be B6)	DF	AA61HB	0.0619	0.00015	0.928	2.72%	4	1	0.9862	1.00, 0.465, 0.216, 0.101, 0.047, 0.022, 0.010, 0.005	2.15	YES			FAL.NHK.SLS.28.04.03
FAL.NHK.HB.13.05.04 (should be B7)	DF	AA61HB	NA	NA	0.603	2.36%	4	1	NA	1.00, 0.465, 0.216, 0.101, 0.047, 0.022, 0.010, 0.005	2.15	NO	no points between 50-100%; SD rejects test	odd plate; looks as if the dilutions ran left to right for top three wells & right to left for bottom three.	FAL.NHK.SLS.13.05.04
FAL.NHK.HB.B7.10.06.04 (should be B8)	DF	AA61HB	0.0233	0.00006	0.922	1.93%	5	3	0.9799	1.0, 0.47, 0.22, 0.10, 0.05, 0.022, 0.010, 0.0047	2.15	YES			FAL.NHK.SLS.10.06.04

LACTIC ACID

IIVS															
A1	RF	AA61FW	1360	15.114	0.573	1.92%	1	1	0.9351	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A2-N040320B
B1	DF	AA61FW	1260	13.976	0.552	3.33%	4	2	0.9915	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES			SLS-B1-N040423A
B2	DF	AA61FW	1210	13.377	0.561	10.36%	2	2	0.9868	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES			SLS-B2-N040424A
B3	DF	AA61FW	1470	16.344	0.458	4.02%	4	2	0.9836	5000, 3333, 2222, 1481, 988, 658, 439, 293	1.5	YES			SLS-B3-N040506A
ECBC															
AA61NL-A1	RF	AA61NL	1060	11.786	0.411	3.08%	1	1	0.8632	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P6

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61NL-B1	DF	AA61NL	1330	14.770	0.999	0.10%	3	4	0.9731	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P26
AA61NL-B2	DF	AA61NL	1310	14.418	0.909	0.66%	3	3	0.9901	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P28
AA61NL-B3	DF	AA61NL	1230	13.658	0.824	3.46%	3	5	0.9532	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P30
FRAME															
FAL.NHK.JT.A1.25.04.04	RF	AA61JT	1880	20.863	0.777	7.41%	1	1	0.7636	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.25.04.04
FAL.NHK.JT.B1.28.04.04	DF	AA61JT	1350	15.010	0.904	0.04%	3	5	0.9767	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			FAL.NHK.SLS.28.04.03
FAL.NHK.JT.B2.13.05.04	DF	AA61JT	1360	15.079	0.597	1.07%	3	4	0.9702	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			FAL.NHK.SLS.13.05.04
FAL.NHK.JT.B3.10.06.04	DF	AA61JT	1250	13.879	0.670	6.11%	3	1	0.9322	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			FAL.NHK.SLS.10.06.04

LINDANE

IIVS															
A1	RF	AA61PJ	46.8	0.161	0.634	0.78%	1	1	0.7927	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	ppt in 2x C1 and 1X C1	SLS-A3-N040331A
B1	DF	AA61PJ	15.7	0.054	0.547	10.52%	5	2	0.9540	200, 111, 61.7, 34.3, 19.1, 10.6, 5.88, 3.27	1.8	YES		ppt in 1X C1-C3 & 2X C1-C3; SD removed top 3 doses from Hill analyses; ppts and flattening of response curve were observed	SLS-B8-N040819A
B2	DF	AA61PJ	18.0	0.062	0.582	6.00%	4	2	0.9704	200, 111, 61.7, 34.3, 19.1, 10.6, 5.88, 3.27	1.8	YES		ppt in 1X C1-C3 & 2X C1-C2; SD removed top 3 doses from Hill analyses; ppts and flattening of response curve were observed	SLS-B9-N040820A
B3	DF	AA61PJ	13.2	0.045	0.532	6.43%	2	3	0.9626	200, 111, 61.7, 34.3, 19.1, 10.6, 5.88, 3.27	1.8	YES		ppt in 1X C1-C4 & 2X C1-C3; SD removed top 3 doses from Hill analyses; ppts and flattening of response curve were observed	SLS-B10-N040903A
ECBC															
AA61FK-A1	RF	AA61FK	40.6	0.140	0.821	9.29%	2	2	0.8809	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2; ppt in 1X C	SLS-P15
AA61FK-B1	DF	AA61FK	21.4	0.074	0.550	6.75%	5	2	0.9657	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES		ppt in 2X C1-C3; ppt in 1X C1-C4;	SLS-P49
AA61FK-B2	DF	AA61FK	15.5	0.053	0.558	2.09%	5	2	0.8770	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES		ppt in 2X C1-C3; ppt in 1X C1-C4	SLS-P53
AA61FK-B3	DF	AA61FK	20.3	0.070	0.619	6.30%	4	4	0.9653	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES		ppt in 2X C1-C3; ppt in 1X C1-C4	SLS-P55
FRAME															
FAL.NHK.KN.A1.14.05.04	RF	AA61KN	61.7	0.212	0.694	7.78%	2	1	0.8847	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1	FAL.NHK.SLS.14.05.03
FAL.NHK.KN.B1.20.08.04	DF	AA61KN	30.8	0.106	0.752	5.39%	6	2	0.9626	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES		ppt in 2X C1-C3; ppt in 1X C1-C6	FAL.NHK.SLS.20.08.04
FAL.NHK.KN.B2.29.10.04	DF	AA61KN	16.8	0.058	0.450	9.76%	7	1	0.9529	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES		ppt in 2X C1-C4; ppt in 1X C1-C5	FAL.NHK.SLS.29.10.04
FAL.NHK.KN.B3.05.11.04	DF	AA61KN	21.9	0.075	0.453	7.72%	6	2	0.9894	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES		ppt in 1X C1-C5	FAL.NHK.SLS.05.11.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
LITHIUM I CARBONATE															
IIVS															
A2	RF	AA61RN	839	11.355	0.736	1.65%	1	0	0.9100	1000, 100, 10, 1, 0.1,0.01, 0.001, 0.0001	10	RF	range finder		SLS-A2
B1	DF	AA61RN	524	7.092	0.364	1.54%	3	2	0.9453	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	YES			SLS-B1
B2	DF	AA61RN	519	7.024	0.26	7.33%	3	2	0.9436	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	YES			SLS-B2
B3	DF	AA61RN	571	7.728	0.315	8.55%	3	2	0.958	2000, 1333, 889, 593, 395, 263, 176, 117	1.5	YES			SLS-B3
ECBC															
AA61RR-A1	RF	AA61RR	767	10.380	0.750	3.35%	1	1	0.8957	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	range finder	SLS-P2
AA61RR-B1	DF	AA61RR	308	4.168	0.361	2.25%	6	2	0.9095	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES			SLS-P5
AA61RR-B2	DF	AA61RR	541	7.322	1.107	4.03%	4	4	0.9425	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES			SLS-P7
AA61RR-B3	DF	AA61RR	384	5.197	0.803	0.21%	5	3	0.9639	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES			SLS-P9
FRAME															
FAL.NHK.RM.A1.010803	RF	AA61RM	78.5	1.062	0.568	13.97%	2	5	0.7509	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.010803
FAL.NHK.RM.B1.080803	DF	AA61RM	378	5.116	0.794	1.03%	2	6	0.8188	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		high background	FAL.NHK.SLS.08.08.03
FAL.NHK.RM.B2.15.08.03	DF	AA61RM	518	7.010	0.433	6.00%	1	4	0.8092	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			FAL.NHK.SLS.15.08.03
FAL.NHK.RM.B3.23.08.03	DF	AA61RM	478	6.469	0.614	1.71%	2	4	0.8168	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	PC failed		FAL.NHK.SLS.230803
FAL.NHK.RM.B4.05.09.03	DF	AA61RM	303	4.101	0.095	9.10%	2	2	0.5447	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	low r2		FAL.NHK.SLS.050903
FAL.NHK.RM.B5.01.10.03	DF	AA61RM	887	12.004	1.302	0.06%	1	3	0.8807	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	PC failed		FAL.NHK.SLS.01.10.03
FAL.NHK.RM.B5.15.10.03 (should be B6?)	DF	AA61RM	471	6.374	0.529	0.71%	2	6	0.2797	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	NO	low r2		FAL.NHK.SLS.15.10.03
FAL.NHK.RM.28.11.03	DF	AA61RM	561	7.592	0.153	3.93%	1	5	0.7316	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES		challenging chemical; SMT accepts this test	FAL.NHK.SLS.28.11.03
MEPROBAMATE															
IIVS															
A1	RF	AA61LS	507	2.322	0.431	13.02%	1	2	0.8210	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A1-N040317B
B1	DF	AA61LS	631	2.890	0.650	3.10%	3	4	0.9748	2000, 1250, 781, 488, 305, 191, 119, 74.5	1.6	YES			SLS-B8-N040819A
B2	DF	AA61LS	705	3.228	0.691	2.97%	3	4	0.9666	2000, 1250, 781, 488, 305, 191, 119, 74.5	1.6	YES			SLS-B9-N040820A
B3	DF	AA61LS	537	2.460	0.649	2.00%	3	3	0.9670	2000, 1250, 781, 488, 305, 191, 119, 74.5	1.6	YES			SLS-B10-N040903A
ECBC															
AA61RJ-A1	RF	AA61RJ	324	1.49	0.677	2.99%	1	5	0.9463	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P2
AA61RJ-B1	DF	AA61RJ	746	3.419	1.112	0.28%	3	4	0.9663	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES			SLS-P8
AA61RJ-B2	DF	AA61RJ	883	4.045	1.180	2.65%	2	6	0.9767	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES			SLS-P10
AA61RJ-B3	DF	AA61RJ	653	2.992	0.784	1.54%	3	5	0.9321	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	YES			SLS-P11
FRAME															
FAL.NHK.HV.A1.11/02/04	RF	AA61HV	982	4.497	1.600	0.24%	1	4	0.8090	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	C8 outlier removed by SD	FAL.NHK.SLS.11.02.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.HV.A2.18/02/04	DF	AA61HV	4980	22.801	1.600	0.24%	1	4	0.4736	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	NO	PC failed	this is a definitive test since conc.series is different from A1 range finder	FAL.NHK.SLS.18.02.04
FAL.NHK.HV.B1.26/02/04	DF	AA61HV	30.8	0.141	0.254	10.02%	6	2	0.9661	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS/NB.26.02 .03
FAL.NHK.HV.B2.18/03/04	DF	AA61HV	77.8	0.356	0.378	0.13%	4	4	0.9274	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.18.03.03
FAL.NHK.HV.B3.25.03.04	DF	AA61HV	379	1.738	0.803	0.65%	2	5	0.7687	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.25.03.03
MERCURY II CHLORIDE															
IIVS															
A1	RF	AA61MX	3.25	0.012	0.485	7.23%	3	0	0.9831	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50 - 100%	ppt in 1X C1	SLS-A1-N040317B
B1	DF	AA61MX	4.54	0.017	0.632	0.90%	4	0	0.9852	20.0, 12.5, 7.81, 4.88, 3.05, 1.91, 1.19, 0.745	1.6	NO	no points between 50 - 100%		SLS-B1-N040423A
B2	DF	AA61MX	5.17	0.019	0.568	4.76%	0	2	0.9915	20.0, 12.5, 7.81, 4.88, 3.05, 1.91, 1.19, 0.745	1.6	NO	no points between 0 - 50%		SLS-B2-N040424A
B3	DF	AA61MX	5.10	0.019	0.495	6.71%	0	1	0.9819	20.0, 15.0, 11.3, 8.50, 6.39, 4.81, 3.61, 2.72	1.33	NO	no points between 0 - 50%		SLS-B3-N040506A
B4	DF	AA61MX	5.26	0.019	0.785	2.29%	2	3	0.9359	8.00, 7.27, 6.61, 6.01, 4.46, 4.97, 4.52, 4.11	1.1	YES			SLS-B6-N040716A
B5	DF	AA61MX	5.44	0.020	0.715	4.31%	1	3	0.9529	8.00, 7.27, 6.61, 6.01, 4.46, 4.97, 4.52, 4.11	1.1	YES			SLS-B7-N040717B
B6	DF	AA61MX	5.35	0.020	0.612	0.00%	2	2	0.9585	8.00, 7.27, 6.61, 6.01, 4.46, 4.97, 4.52, 4.11	1.1	YES			SLS-B8-N040819A
ECBC															
AA61KP-A1	RF	AA61KP	2.24	0.008	0.432	8.13%	3	1	0.9582	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1	SLS-P2
AA61KP-B1	DF	AA61KP	6.95	0.026	1.076	3.04%	1	1	0.9276	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			SLS-P8
AA61KP-B2	DF	AA61KP	7.87	0.029	1.169	3.40%	2	6	0.9666	10.0, 8.26, 6.83, 5.65, 4.67, 3.86, 3.19, 2.63	1.21	YES			SLS-P10
AA61KP-B3	DF	AA61KP	5.79	0.021	0.831	1.85%	2	5	0.9856	10.0, 8.26, 6.83, 5.65, 4.67, 3.86, 3.19, 2.63	1.21	YES			SLS-P11
FRAME															
FAL.NHK.HA.A1.11/02/04	RF	AA61HA	3.56	0.013	1.321	3.96%	3	0	0.9647	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1	FAL.NHK.SLS.11.02.04
FAL.NHK.HA.B1.18.03.04	DF	AA61HA	4.66	0.017	0.486	2.93%	2	3	0.9663	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			FAL.NHK.SLS.18.03.03
FAL.NHK.HA.B2.19.03.04	DF	AA61HA	4.98	0.018	0.533	9.73%	2	6	0.9174	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			FAL.NHK.SLS.19.03.03
FAL.NHK.HA.B2.25.03.04 (should be B3)	DF	AA61HA	6.56	0.024	0.533	4.35%	2	6	0.8230	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			FAL.NHK.SLS.25.03.03
METHANOL															
IIVS															
A1	RF	AA61FZ	601	18.763	0.567	1.73%	1	1	0.9073	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A5-N040401A
B1	DF	AA61FZ	2160	67.345	0.597	1.70%	1	7	0.8425	2000, 1250, 781, 488, 305, 191, 119, 74.5	1.6	YES			SLS-B4-N040513C
B2	DF	AA61FZ	1850	57.851	0.546	2.01%	1	4	0.9223	2000, 1250, 781, 488, 305, 191, 119, 74.5	1.6	YES			SLS-B5-N040514B
B3	DF	AA61FZ	2290	71.336	0.790	3.64%	1	3	0.9218	2500, 1563, 977, 610, 381, 238, 149, 93.1	1.6	YES			SLS-B6-N040716A
B4	DF	AA61FZ	NA	NA	0.707	6.86%	0	3	0.9030	2500, 1563, 977, 610, 381, 238, 149, 93.1	1.6	NO	no points between 0 - 50%		SLS-B7-N040717B
ECBC															

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61MJ-A1	RF	AA61MJ	NA	NA	0.909	0.96%	0	8	NA	2500, 250, 25, 2.5, 0.25, 0.025, 0.0025, 0.00025	10	RF	range finder; no points between 0 - 50%		SLS-P19
AA61MJ-B1	DF	AA61MJ	NA	NA	0.606	0.30%	0	4	NA	3500, 2381, 1620, 1102, 750, 510, 347, 236	1.47	NO	no points between 0 - 50%	0.02% DMSO in dosing solutions; highest stock conc. is 700,087 ug/ml	SLS-P48
AA61MJ-B2	DF	AA61MJ	NA	NA	0.759	0.65%	0	8	NA	3500, 2893, 2391, 1976, 1633, 1349, 1115, 922	1.21	NO	no points between 0 - 50%	no toxicity	SLS-P60
AA61MJ-B3	DF	AA61MJ	NA	NA	0.831	3.88%	0	8	NA	3500, 2893, 2391, 1976, 1633, 1349, 1115, 922	1.21	NO	no points between 0 - 50%	slight toxicity	SLS-P61
FRAME															
FAL.NHK.RG.A1.24.09.04	RF	AA61RG	635	19.829	0.632	0.55%	1	3	0.6562	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.RG.B1.01.10.04	DF	AA61RG	8610	268.725	1.078	6.69%	0	8	0.4209	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	PC failed; no points between 50-100%		FAL.NHK.SLS.01.10.04
FAL.NHK.RG.B2.07.10.04	DF	AA61RG	1360	42.297	0.649	3.62%	1	7	0.9324	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES			FAL.NHK.SLS.07.10.03
FAL.NHK.RG.B3.22.10.04	DF	AA61RG	2170	67.812	0.809	0.56%	0	8	0.9463	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	NO	no points between 0-50%		FAL.NHK.SLS.22.10.04 (NB)
FAL.NHK.RG.B4.28.10.04	DF	AA61RG	1100	34.301	0.625	8.71%	2	1	0.9422	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.28.10.04
FAL.NHK.RG.B5.05.11.04	DF	AA61RG	938	29.262	0.467	6.43%	2	6	0.5431	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.05.11.04
NICOTINE															
IIVS															
A1	RF	AA61HL	143	0.881	0.498	34.80%	1	1	0.9606	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; % VC difference > 15	volatility problem; VC1 OD values much lower than VC2; VC1 removed from subsequent analysis by SD	SLS-A4-N040331N
B1	DF	AA61HL	127	0.785	0.572	1.82%	4	4	0.9551	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES		outlier in C6 removed by SD: used plate sealer	SLS-B4-N040513C
B2	DF	AA61HL	128	0.791	0.552	4.42%	4	4	0.9558	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES			SLS-B5-N040514B
B3	DF	AA61HL	79.6	0.491	0.736	1.75%	5	3	0.9593	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES			SLS-B6-N040716A
ECBC															
AA61NA-A1	RF	AA61NA	225	1.390	0.541	27.12%	1	2	0.8258	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; % VC difference > 15	volatility problem	SLS-P38
AA61NA-B1(sealer)	DF	AA61NA	69.7	0.429	0.718	4.19%	5	2	0.8884	5000, 2326, 1082, 503, 234, 109, 51, 24	2.15	YES			SLS-P40
AA61NA-B2 (sealer)	DF	AA61NA	94.2	0.581	0.680	5.37%	5	3	0.9635	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES			SLS-P42
AA61NA-B3 (sealer)	DF	AA61NA	119	0.734	0.871	4.38%	5	3	0.9418	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES			SLS-P44
FRAME															
FAL.NHK.KL.A1.11.08.04	RF	AA61KL	277	1.706	0.455	16.01%	1	1	0.5525	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; % VC difference > 15		FAL.NHK.SLS.11.08.04
FAL.NHK.KL.B1.17.09.04	DF	AA61KL	553	3.412	0.487	26.34%	2	5	0.9450	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	NO	% VC difference > 15	outlier removed by SD; possible volatility problem	FAL.NHK.SLS.17.09.04
FAL.NHK.KL.B2.30.09.04	DF	AA61KL	80	0.493	0.478	10.61%	2	2	0.4411	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	NO	SD rejects curve	"roller coaster" curve; some low concentrations give high toxicity; SD rejects test	FAL.NHK.SLS.30.09.03
FAL.NHK.KL.B3.08.10.04	DF	AA61KL	193	1.191	0.552	19.76%	2	5	0.8957	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	NO	% VC difference > 15	volatility issue	FAL.NHK.SLS.08.10.03
FAL.NHK.KL.B4 .22.10.04	DF	AA61KL	91	0.561	0.730	2.67%	6	2	0.8631	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	2.15	YES			FAL.NHK.SLS.22.10.04 (NB)
FAL.NHK.KL.B5.29.10.04	DF	AA61KL	118	0.726	0.455	17.69%	5	3	0.9316	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	NO	% VC difference > 15		FAL.NHK.SLS.29.10.04
FAL.NHK.KL.B6.05.11.04	DF	AA61KL	224	1.380	0.376	14.23%	3	5	0.8894	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.05.11.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.KL.B7.12.11.04	DF	AA61KL	85.7	0.528	0.727	2.28%	5	3	0.9249	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.12.11.04
PARAQUAT															
IIVS															
A1	RF	AA61GD	84.5	0.329	0.578	2.76%	3	0	0.9874	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50 - 100%		SLS-A2-N040320B
B1	DF	AA61GD	50.4	0.196	0.564	3.71%	6	2	0.9776	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B1-N040423A
B2	DF	AA61GD	59.8	0.233	0.544	0.60%	5	3	0.9719	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B2-N040424A
B3	DF	AA61GD	50.1	0.194	0.496	3.71%	6	2	0.9679	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B3-N040506A
ECBC															
AA61MP-A1	RF	AA61MP	57.0	0.222	0.407	2.19%	2	2	0.9152	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P5
AA61MP-B1	DF	AA61MP	41.4	0.161	0.597	0.17%	5	3	0.9912	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			SLS-P20
AA61MP-B2	DF	AA61MP	50.7	0.197	1.009	3.67%	4	4	0.9822	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			SLS-P22
AA61MP-B3	DF	AA61MP	52.7	0.205	0.528	7.61%	5	3	0.9820	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			SLS-P24
FRAME															
FAL.NHK.HP.A1.26.03.04	RF	AA61HP	74.5	0.290	0.562	6.58%	2	1	0.9098	100000, 1000, 100,10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.26.03.04
FAL.NHK.HP.B1.25.04.04	DF	AA61HP	57.9	0.225	0.795	3.51%	4	4	0.9828	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.25.04.04
FAL.NHK.HP.B2.28.04.04	DF	AA61HP	60.1	0.234	0.815	1.88%	8	0	0.9066	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	NO	no points between 50 - 100%		FAL.NHK.SLS.28.04.03
FAL.NHK.HP.B3.11.06.04	DF	AA61HP	28.1	0.109	0.790	4.43%	4	4	0.8649	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	PC failed		FAL.NHK.SLS.11.06.04
FAL.NHK.HP.B4.23.06.04	DF	AA61HP	103	0.399	0.811	17.53%	3	3	0.9562	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	% VC difference > 15		FAL.NHK.SLS.23.06.04
FAL.NHK.HP.B5.25.06.04	DF	AA61HP	99.8	0.388	0.850	0.84%	3	2	0.9498	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.NHK.SLS.25.06.04
FAL.NHK.HP.B6.12.08.04	DF	AA61HP	55.7	0.217	0.880	2.31%	3	5	0.9207	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	NO	PC failed		FAL.NHK.SLS.12.08.04
FAL.NHK.HP-RB.B7.25.08.04	DF	AA61HP	132	0.515	0.635	4.72%	2	2	0.8927	500, 233, 108, 50.3, 23.4, 10.9, 5.1, 2.4	2.15	YES			FAL.NHK.SLS-RB.20.08.04
PARATHION															
IIVS															
A1	RF	AA61PS	95.7	0.329	0.684	5.51%	0	3	0.8685	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 50 - 100%	SD didn't use data from highest dose in Hill analyses due to the effects of ppts; ppt in 2X C1-C2 & 1X C1-C2	SLS-A3-N040331A
B1	DF	AA61PS	21.2	0.073	0.719	5.83%	6	2	0.9735	1000, 455, 207, 93.9, 42.7, 19.4, 8.82, 4.01	2.2	YES		ppt in 2X C1-C6; ppt in 1X C1-C4	SLS-B12-N041022B
B2	DF	AA61PS	37.8	0.130	0.656	1.73%	3	3	0.9754	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 2X C1-C3; ppt in 1X C1	SLS-B113-N041029B
B3	DF	AA61PS	28.1	0.097	0.752	0.68%	3	4	0.9677	100, 62.5, 39.1, 24.4, 15.3, 9.54, 5.96, 3.73	1.6	YES		ppt in 2X C1-C3; ppt in 1X C1-C2	SLS-B14-N041030A
ECBC															
AA61MD-A1	RF	AA61MD	16.0	0.055	0.846	0.38%	2	2	0.9789	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1-C2; ppt in 1X C	SLS-P39
AA61MD-B1	DF	AA61MD	25.8	0.088	0.995	5.19%	2	3	0.9372	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES		ppt in 2X C1-C2	SLS-P47
AA61MD-B2	DF	AA61MD	45.2	0.155	1.228	1.72%	2	6	0.9633	200, 93.0, 43.3, 20.1, 9.4, 4.4, 2.0, 0.9	2.15	YES		chunks in1X C1; ppt in 2X C1-C3	SLS-P51

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61MD-B3	DF	AA61MD	31.1	0.107	0.737	1.12%	3	5	0.9554	200, 93.0, 43.3, 20.1, 9.4, 4.4, 2.0, 0.9	2.15	YES		ppt in 2X C1-C3; ppt in 1X C1-C2	SLS-P53
FRAME															
FAL.NHK.KE.A1.20.10 .04	DF	AA61KE	87.1	0.299	1.237	0.40%	2	6	0.9819	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 1X C1	FAL.NHK.SLS.20.10.04
FAL.NHK.KE.B1.29.10.04	DF	AA61KE	33.3	0.114	0.455	24.83%	6	2	0.9604	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.06	2.15	NO	%VC difference >15	ppt in 2X C1-C3; ppt in C1-C5; volatility problem	FAL.NHK.SLS.29.10.04
FAL.NHK.KE.B2.03.11.04	DF	AA61KE	18.9	0.065	0.606	8.86%	6	2	0.9440	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.06	2.15	YES		ppt in 1X C1-C4	FAL.NHK.SLS.03.11.04
FAL.NHK.KE.B3.10.11.04	DF	AA61KE	NA	NA	1.144	4.04%	8	0	NA	1500, 1020, 694, 472, 321, 219, 149, 101	1.47	NO	no points between 50 - 100%	ppt in 2X C1-C5; ppt in 1X C1-C7	FAL.NHK.SLS.10.11.04
FAL.NHK.KE.B4.12.11.04	DF	AA61KE	32.1	0.110	0.809	3.24%	6	2	0.9806	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.06	2.15	YES		ppt in 2X C1-C4; ppt in 1X C1-C3	FAL.NHK.SLS.12.11.04
FAL.NHK.KE.B5.17.11.04	DF	AA61KE	42.7	0.146	0.855	10.63%	5	3	0.9385	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.06	2.15	YES		ppt in 2X C1-C4	FAL.NHK.SLS.17.11.04
PHENOBARBITAL															
IIVS															
A1	RF	AA61FG	378	1.630	0.575	0.41%	1	1	0.9186	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A5-N040401A
B1	DF	AA61FG	458	1.973	0.629	3.11%	3	4	0.9782	2000, 1111, 617, 343, 191, 106, 58.8, 32.7	1.8	YES		ppt in 1X C1 and 2X C1	SLS-B8-N040819A
B2	DF	AA61FG	362	1.560	0.655	0.89%	3	4	0.9861	2000, 1111, 617, 343, 191, 106, 58.8, 32.7	1.8	YES		ppt in 2X C1	SLS-B9-N040820A
B3	DF	AA61FG	322	1.387	0.623	0.79%	4	4	0.9867	2000, 1111, 617, 343, 191, 106, 58.8, 32.7	1.8	YES		ppt in 2X C1	SLS-B10-N040903A
ECBC															
AA61KV-A1	RF	AA61KV	436	1.875	0.953	0.85%	1	7	0.8831	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P56
AA61KV-B1	DF	AA61KV	569	2.450	0.593	0.65%	3	5	0.9763	3000, 1395, 649, 302, 140, 65, 30, 14	2.15	YES		ppt in 2X C1	SLS-P57
AA61KV-B2	DF	AA61KV	899	3.873	0.114	1.69%	2	4	0.8199	3000, 1395, 649, 302, 140, 65, 30, 14	2.15	YES		ppt in 2X C1	SLS-P58
AA61KV-B3	DF	AA61KV	611	2.631	0.831	1.41%	3	5	0.9887	3000, 1395, 649, 302, 140, 65, 30, 14	2.15	YES		ppt in 2X C1	SLS-P59
FRAME															
FAL.NHK.NJ.A1.24.09.04	RF	AA61NJ	253	1.089	0.619	11.58%	1	1	0.7751	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.NJ.B1.08.10.04	DF	AA61NJ	361	1.553	0.654	3.81%	2	6	0.9642	1500, 698, 3.25, 151, 70.2, 32.7, 15.2, 7.06	2.15	YES			FAL.NHK.SLS.08.10.03
FAL.NHK.NJ.B2.22.10.04	DF	AA61NJ	455	1.959	0.827	4.81%	3	4	0.9826	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.22.10.04 (NB)
FAL.NHK.NJ.B3.28.10.04	DF	AA61NJ	264	1.135	0.683	11.67%	3	5	0.9342	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.28.10.04
PHENOL															
IIVS															
A1	RF	AA61PG	34.4	0.366	0.617	98.64%	2	3	0.9801	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; % VC difference > 15	volatility problem; VC1 OD values much lower than VC2; VC1 removed from subsequent analysis by SD	SLS-A3-N040331A
B1	DF	AA61PG	79.3	0.842	0.522	2.09%	5	3	0.9749	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES			SLS-B1-N040423A
B2	DF	AA61PG	76.6	0.814	0.548	2.89%	3	3	0.9575	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES		ppt in 1X C1	SLS-B2-N040424A
B3	DF	AA61PG	86.5	0.919	0.473	0.39%	4	3	0.9620	2000, 909, 413, 188, 85.4, 38.8, 17.6, 8.02	2.2	YES		used plate sealer; ppt in 1X C1-C2	SLS-B3-N040506A
ECBC															
AA61FV-A1	RF	AA61FV	NA	NA	0.421	99.34%	1	1	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; % VC difference > 15	volatility problem	SLS-P12

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61FV-B1(sealer)	DF	AA61FV	62.8	0.667	0.622	8.17%	4	3	0.9585	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P32
AA61FV-B2 (sealer)	DF	AA61FV	78.5	0.834	0.668	7.31%	3	4	0.9576	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P34
AA61FV-B3 (sealer)	DF	AA61FV	36.1	0.383	0.318	2.99%	5	3	0.9402	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.7	2.15	YES			SLS-P36
FRAME															
FAL.NHK.MS.A1.14.05.04	RF	AA61MS	91.0	0.967	0.279	98.26%	3	0	0.2986	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; %VC difference > 15; no pts between 50-100%		FAL.NHK.SLS.14.05.03
FAL.NHK.MS.B1.12.08.04	DF	AA61MS	381	4.049	0.654	13.72%	1	2	0.8273	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	PC failed		FAL.NHK.SLS.12.08.04
FAL.NHK.MS.B2.19.08.04 (RB)	DF	AA61MS	170	1.805	0.168	46.79%	3	1	0.4991	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	PC failed; % VC difference > 15		FAL.NHK.SLS-RB.19.08.04
FAL.NHK.MS-NB.B3.25.08.04	DF	AA61MS	86.7	0.921	1.034	8.73%	4	3	0.9822	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.25.08.04
FAL.NHK.MS.B4.17.09.04	DF	AA61MS	94.6	1.005	0.760	15.15%	3	4	0.9736	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES		outlier removed by SD; potential volatility problem	FAL.NHK.SLS.17.09.04
FAL.NHK.MS.B5.30.09.04	DF	AA61MS	793	8.421	0.589	5.43%	1	0	0.8202	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	no points between 50 - 100%	SD removed data from C8 due to low OD; "roller coaster" curve	FAL.NHK.SLS.30.09.03
FAL.NHK.MS.B6.07.10.04	DF	AA61MS	98.4	1.046	0.650	8.37%	4	3	0.9794	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.07.10.03
PHENYLTHIOUREA															
IIVS															
A1	RF	AA61PV	467	3.066	0.775	1.12%	1	2	0.9466	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A3-N040331A
B1	DF	AA61PV	252	1.658	0.643	1.48%	5	3	0.9786	2500, 1389, 772, 429, 238, 132, 73.5, 40.8	1.8	YES		ppt in 2X C1	SLS-B8-N040819A
B2	DF	AA61PV	352	2.321	0.623	0.41%	4	4	0.9605	2500, 1389, 772, 429, 238, 132, 73.5, 40.8	1.8	YES		ppt in 2X C1	SLS-B9-N040820A
B3	DF	AA61PV	213	1.401	0.654	4.04%	5	3	0.9788	2500, 1389, 772, 429, 238, 132, 73.5, 40.8	1.8	YES		ppt in 2X C1-C2	SLS-B10-N040903A
ECBC															
AA61LN-A1	RF	AA61LN	294	1.930	0.995	4.15%	1	7	0.8497	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P39
AA61LN-B1	DF	AA61LN	362	2.380	0.577	2.20%	3	2	0.9609	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES			SLS-P41
AA61LN-B2	DF	AA61LN	306	2.012	0.705	1.12%	3	5	0.9632	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES			SLS-P43
AA61LN-B3	DF	AA61LN	422	2.771	0.972	5.43%	3	5	0.9477	2000, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	YES		ppt in 2X C1	SLS-P45
FRAME															
FAL.NHK.JB.A1.14.05.04	RF	AA61 JB	555	3.644	0.678	3.82%	1	7	0.9193	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.14.05.03
FAL.NHK.JB.B1.29.10.04	DF	AA61JB	335	2.201	0.575	8.89%	3	5	0.9804	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 2X C1	FAL.NHK.SLS.29.10.04
FAL.NHK.JB.B2.03.11.04	DF	AA61JB	373	2.452	0.526	0.65%	3	5	0.9615	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.03.11.04
FAL.NHK.JB.B3.05.11.04	DF	AA61JB	495	3.255	0.371	11.87%	3	1	0.8795	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES		ppt in 2X C1	FAL.NHK.SLS.05.11.04
PHYSOSTIGMINE															
IIVS															
A1	RF	AA61NF	136	0.494	0.555	4.16%	1	2	0.9514	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A5-N040401A
B1	DF	AA61NF	146	0.531	0.647	3.80%	4	4	0.9767	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B4-N040513C

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B2	DF	AA61NF	129	0.467	0.596	5.79%	3	3	0.9845	1000, 556, 309, 171, 95.3, 52.9, 29.4, 16.3	1.8	YES			SLS-B5-N040514B
B3	DF	AA61NF	141	0.511	0.834	1.84%	3	4	0.9527	500, 357, 255, 182, 130, 93.0, 66.4, 47.4	1.4	YES			SLS-B6-N040716A
ECBC															
AA61FT-A1	RF	AA61FT	123	0.447	0.863	2.71%	1	6	0.9452	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P38
AA61FT-B1	DF	AA61FT	158	0.575	0.691	3.04%	2	5	0.9669	700, 326, 151, 70.4, 32.8, 15.2, 7.09, 3.30	2.15	YES			SLS-P41
AA61FT-B2	DF	AA61FT	164	0.596	0.674	5.99%	2	3	0.9348	300, 204, 139, 94.4, 64.2, 43.7, 29.7, 20.2	1.47	YES			SLS-P43
AA61FT-B3	DF	AA61FT	169	0.612	1.001	2.86%	2	6	0.8953	300, 204, 139, 94.4, 64.2, 43.7, 29.7, 20.2	1.47	YES			SLS-P45
FRAME															
FAL.NHK.GT.A1.24.09.04	RF	AA61GT	153	0.555	0.662	6.01%	1	1	0.6638	1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.GT.B1.01.10.04	DF	AA61GT	225	0.819	1.035	7.61%	2	6	0.9354	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	PC failed		FAL.NHK.SLS.01.10.04
FAL.NHK.GT.B2.07.10.04	DF	AA61GT	107	0.387	0.508	1.13%	3	2	0.9741	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	wrong solvent used (medium); should be DMSO; SD will retest		FAL.NHK.SLS.07.10.03
FAL.NHK.GT.B3.08.10.04	DF	AA61GT	157	0.570	0.695	5.70%	3	5	0.9843	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	wrong solvent used (medium); should be DMSO; SD will retest		FAL.NHK.SLS.08.10.03
FAL.NHK.GT.B4.20.10 .04	DF	AA61GT	470	1.706	1.324	1.47%	1	5	0.9382	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.20.10.04
FAL.NHK.GT.B5.22.10.04	DF	AA61GT	0.366	0.001	0.767	7.78%	7	1	0.9929	1000, 317, 101, 32.0, 10.2, 3.22, 1.02, 0.32	3.15	YES		reach 100% cytotoxicityat C7	FAL.NHK.SLS.22.10.04 (NB)
FAL.NHK.GT.B6.28.10.04	DF	AA61GT	167	0.605	0.596	9.68%	3	4	0.9740	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.28.10.04

POTASSIUM I CHLORIDE

IIVS															
A2	RF	AA61FF	1490	19.987	0.680	4.54	0	1	0.9413	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF			SLS-A2
B1	DF	AA61FF	2040	27.364	0.355	1.41	4	4	0.9755	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	YES			SLS-B1
B2	DF	AA61FF	2120	28.437	0.274	8.41	2	4	0.9809	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	YES			SLS-B2
B3	DF	AA61FF	1810	24.279	0.295	8.80	4	3	0.984	10000, 6667, 4444, 2963, 1975, 1317, 878, 585	1.5	YES			SLS-B3
ECBC															
AA61KM-A1	RF	AA61KM	1460	19.584	0.687	3.96	1	6	0.8761	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF		range finder	SLS-P3
AA61KM-B1	DF	AA61KM	2650	35.547	0.949	0.35	3	5	0.9297	8000, 5442, 3702, 2518, 1714, 1166, 793, 539	1.47	YES			SLS-P7
AA61KM-B2	DF	AA61KM	2090	28.035	0.960	0.99	3	4	0.9645	8000, 5442, 3702, 2518, 1714, 1166, 793, 539	1.47	YES			SLS-P9
AA61KM-B3	DF	AA61KM	2250	30.181	0.797	5.97	3	4	0.9805	8000, 5442, 3702, 2518, 1714, 1166, 793, 539	1.47	NO	PC failed		SLS-P11
AA61KM-B4	DF	AA61KM	2940	39.437	0.666	2.17	3	3	0.9170	8000, 5442, 3702, 2518, 1714, 1166, 793, 539	1.47	YES			SLS-P19
FRAME															
FAL.NHK.MY.A1.010803	RF	AA61MY	1030	13.816	0.503	3.16	0	6	0.7001	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF			FAL.NHK.SLS.010803
FAL.NHK.MY.B1.080803	DF	AA61MY	1610	21.596	0.625	3.72	3	5	0.8175	5000, 3401, 2313, 1574, 1070, 728, 496, 337	1.47	YES		high background	FAL.NHK.SLS.08.08.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.MY.B2.15.08.03	DF	AA61MY	4760	63.850	0.250	36.21	1	2	0.2925	5000, 3401, 2313, 1574, 1070, 728, 496, 337	1.47	NO	% VC difference >15; low r2		FAL.NHK.SLS.15.08.03
FAL.NHK.MY.B3.23.08.03	DF	AA61MY	1880	25.218	0.554	7.67	2	6	0.7555	5000, 3401, 2313, 1574, 1070, 728, 496, 337	1.47	NO	PC failed		FAL.NHK.SLS.230803
FAL.NHK.MY.B4.28.08.04	DF	AA61MY	2860	38.364	0.385	5.19	2	6	0.8496	5000, 3401, 2313, 1574, 1070, 728, 496, 337	1.47	YES			FAL.NHK.SLS.280803
FAL.NHK.MY.B5.05.09.03	DF	AA61MY	NA	NA	0.113	NA	NA	NA	NA	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	NO		curve going in wrong direction; plate reversed 180 degrees when reading?	FAL.NHK.SLS.050903
FAL.NHK.MY.B5.15.10.03 (should be B6?)	DF	AA61MY	2390	32.059	0.482	3.11	1	6	0.8444	5000, 2326, 1082, 503, 234, 109, 50.6, 23.5	2.15	YES			FAL.NHK.SLS.15.10.03
POTASSIUM CYANIDE															
IIVS															
A1	RF	AA61KW	0.0006	0.00001	0.173	100.39%	3	0	0.7469	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 50 - 100%; % VC difference > 15	volatility problem; VC1 OD values much lower than VC2; VC1 removed from subsequent analysis bySD	SLS-A5-N040401A
B1	DF	AA61KW	NA	NA	0.656	2.12%	0	1	NA	0.100, 0.045, 0.021, 0.0094, 0.0043, 0.0019, 0.00088, 0.00040	2.2	NO	no points between 0 - 50%	used plate sealer; induced shift in response	SLS-B4-N040513C
B2	DF	AA61KW	NA	NA	0.541	1.12%	0	0	NA	0.100, 0.045, 0.021, 0.0094, 0.0043, 0.0019, 0.00088, 0.00040	2.2	NO	no points between 0 - 100%	no toxicity detected	SLS-B5-N040514B
B3	DF	AA61KW	19.2	0.295	0.670	0.68%	3	3	0.9761	100, 45.5, 20.7, 9.39, 4.27, 1.94, 0.882, 0.401	2.2	YES			SLS-B6-N040716A
B4	DF	AA61KW	16.6	0.255	0.613	5.27%	3	3	0.9799	100, 45.5, 20.7, 9.39, 4.27, 1.94, 0.882, 0.401	2.2	YES			SLS-B7-N040717B
B5	DF	AA61KW	14.8	0.227	0.584	5.68%	3	3	0.9770	100, 45.5, 20.7, 9.39, 4.27, 1.94, 0.882, 0.401	2.2	YES			SLS-B8-N040819A
ECBC															
AA61MN-A1	RF	AA61MN	NA	NA	0.017	103.07%	4	0	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no ponts between 50 - 100%; % VC difference > 15		SLS-P38
AA61MN-A2 (sealer)	RF	AA61MN	15.3	0.235	0.758	2.90%	2	3	0.9585	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P44
AA61MN-B1 (sealer)	DF	AA61MN	36.1	0.554	0.744	0.85%	3	4	0.9264	300, 140, 64.9, 30.2, 14.0, 6.53, 3.04, 1.41	2.15	YES			SLS-P46
AA61MN-B2 (sealer)	DF	AA61MN	29.4	0.452	0.939	0.10%	3	5	0.8814	300, 140, 64.9, 30.2, 14.0, 6.53, 3.04, 1.41	2.15	YES			SLS-P50
AA61MN-B3 (sealer)	DF	AA61MN	22.3	0.342	0.498	4.97%	3	2	0.9697	300, 140, 64.9, 30.2, 14.0, 6.53, 3.04, 1.41	2.15	YES			SLS-P52
FRAME															
FAL.NHK.GP.A1.24.09.04	RF	AA61GP	NA	NA	0.005	87.41%	0	0	-0.0679	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.GP.B1.01.10.04	DF	AA61GP	4.07	0.062	1.025	7.20%	0	6	0.2038	0.100, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	NO	PC failed; no points between 0-50%		FAL.NHK.SLS.01.10.04
FAL.NHK.B2.07.10.04	DF	AA61GP	16.4	0.251	0.331	40.76%	6	1	0.8792	5000, 1587, 504, 160, 50.8, 16.1, 5.12, 1.62	3.15	NO	%VC difference >15	volatility problems	FAL.NHK.SLS.07.10.03
FAL.NHK.GP.B3.20.10 .04	DF	AA61GP	NA	NA	1.150	0.46%	0	0	NA	500, 232, 108, 50.3, 23.9, 10.4, 5.06, 2.35	2.15	NO	no points between 0-100%		FAL.NHK.SLS.20.10.04
FAL.NHK.GP.B4.11.11.04	DF	AA61GP	NA	NA	0.679	9.53%	6	0	NA	2000, 1361, 926, 630, 428, 291,198,135	1.47	NO	no points between 50-100%	all concentrations were toxic	FAL.NHK.SLS.10.11.04
FAL.NHK.GP.B5.17.11.04	DF	AA61GP	71.9	1.105	0.622	22.40%	5	0	0.9016	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	NO	no points between 50-100%; %VC difference >15	outlier removed bySD	FAL.NHK.SLS.17.11.04
FAL.NHK.GP.B6.24.11.04	DF	AA61GP	53.2	0.817	0.906	10.92%	3	4	0.9588	500, 232, 108, 50.3, 23.9, 10.4, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.24.11.04
FAL.NHK.GP.B7.26.11.04	DF	AA61GP	11.9	0.182	0.460	1.72%	3	3	0.9363	500, 232, 108, 50.3, 23.9, 10.4, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.26.11.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.GP.B8.10.12.04	DF	AA61GP	202	3.107	0.993	1.92%	1	7	0.9318	500, 232, 108, 50.3, 23.9, 10.4, 5.06, 2.35	2.15	YES		SD has little confidence in values due to chem. volatility & interaction with plate sealer	FAL.NHK.SLS(MO).10.12.04
FAL.NHK.GP.B9.10.12.04	DF	AA61GP	31.6	0.484	0.903	1.34%	2	3	0.9469	500, 232, 108, 50.3, 23.9, 10.4, 5.06, 2.35	2.15	NO	PC failed	SD has little confidence in values due to chem. volatility & interaction with plate sealer	FAL.NHK.SLS.10.12.04
PROCAINAMIDE HCL															
IIVS															
A1	RF	AA61ML	3890	14.314	0.499	3.99%	0	0	0.9391	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0 - 100%		SLS-A4-N040331N
B1	DF	AA61ML	2210	8.143	0.558	0.88%	3	2	0.9836	10000, 7519, 5653, 4251, 3196, 2403, 1807, 1358	1.33	YES			SLS-B4-N040513C
B2	DF	AA61ML	1770	6.498	0.510	6.82%	4	1	0.8603	10000, 7519, 5653, 4251, 3196, 2403, 1807, 1358	1.33	YES			SLS-B5-N040514B
B3	DF	AA61ML	2100	7.740	0.694	1.43%	3	2	0.9920	10000, 7519, 5653, 4251, 3196, 2403, 1807, 1358	1.33	YES			SLS-B6-N040716A
ECBC															
AA61KC-A1	RF	AA61KC	5120	18.826	0.703	1.72%	0	4	0.9439	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0 - 50%		SLS-P18
AA61KC-B1	DF	AA61KC	1380	5.091	0.752	4.76%	5	2	0.9773	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P32
AA61KC-B2	DF	AA61KC	1350	4.963	0.410	2.83%	4	2	0.9664	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	YES			SLS-P37
AA61KC-B3	DF	AA61KC	1710	6.277	0.647	0.26%	2	4	0.9710	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	YES			SLS-P38
FRAME															
FAL.NHK.GV.A1.28.07.04	RF	AA61GV	1330	4.884	0.055	6.80%	1	1	0.6423	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.28.07.04
FAL.NHK.GV.B1.11.08.04	DF	AA61GV	1730	6.365	0.464	0.97%	1	1	0.9180	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS.11.08.04
FAL.NHK.GV.B2.17.09.04	DF	AA61GV	2030	7.478	0.775	4.46%	2	1	0.9417	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	YES			FAL.NHK.SLS.17.09.04
FAL.NHK.GV.B3.07.10.04	DF	AA61GV	1600	5.885	0.613	7.61%	3	3	0.9809	5000, 3401, 2314, 1574, 1071, 728, 496, 337	1.47	YES			FAL.NHK.SLS.07.10.03
2-PROPANOL															
IIVS															
A2	RF	AA61GC	28100	467.554	0.731	5.06	0	4	0.6596	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A2
A2 with plate cover	RF	AA61GC	9820	163.394	0.556	2.40	1	1	0.8691	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A3
B1	DF	AA61GC	15100	251.248	0.296	20.61	2	4	0.8006	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	NO	VC difference > 15%		SLS-B1
B1 with plate cover	DF	AA61GC	6610	109.983	0.316	4.51	3	3	0.9817	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES			SLS-B1
B2	DF	AA61GC	13600	226.290	0.233	23.35	2	4	0.8	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	NO	VC difference > 15%		SLS-B2
B2 with plate cover	DF	AA61GC	7570	125.957	0.243	9.58	2	3	0.9695	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES			SLS-B2

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B3	DF	AA61GC	19200	319.468	0.25	26.08	0	5	0.617	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	NO	VC difference > 15%; no points 50-100%; low R2		SLS-B3
B3 with plate cover	DF	AA61GC	7080	117.804	0.313	3.69	4	4	0.9821	20000, 14286, 10204, 7289, 5206, 3719, 2656, 1897	1.4	YES			SLS-B3
ECBC															
AA61JL-A1	RF	AA61JL	NA	NA	0.726	0.28	0	5	NA	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	no points between 0.1 - 50%; no r2 nor ICx values could be calculated	range finder	SLS-P2
AA61JL-B1	DF	AA61JL	NA	NA	0.457	63.96	6	1	NA	50000, 34014, 23139, 15740, 10707, 7284, 4955, 3370	1.47	NO	%VC difference > 15; no r2 nor ICx values could be calculated	Volatility of largest conc contaminated VC & others	SLS-P9
AA61JL-B2	DF	AA61JL	NA	NA	0.554	35.73	4	2	NA	50000, 34014, 23139, 15740, 10707, 7284, 4955, 3370	1.47	NO	PC failed; %VC difference > 15; no r2 nor ICx values could be calculated;	Volatility of largest conc contaminated VC & others	SLS-P11
AA61JL-B3 sealer	DF	AA61JL	4610	76.705	0.646	7.33	3	4	0.9280	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			SLS-P12
AA61JL-B4 sealer	DF	AA61JL	5450	90.682	0.480	2.76	2	5	0.8957	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			SLS-P18
AA61JL-B5 sealer	DF	AA61JL	5730	95.341	0.582	1.85	4	3	0.9429	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	YES			SLS-P19
FRAME															
FAL.NHK.NG.A1.30.07.03	RF	AA61NG	NA	NA	1.332	1.06	0	7	0.3849	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0.1 - 50%	Little toxicity	FAL.NHK.SLS.30.07.03
FAL.NHK.NG.B1.07.08.03	DF	AA61NG	1220	20.300	0.400	5.06	3	5	0.1851	10000, 6802, 4628, 3148, 2142, 1457, 991, 674.1	1.47	NO	low r2	SD wonders if chemical is a mitotic inhibitor	FAL.NHK.SLS.07.08.03
FAL.NHK.NG.B2.15.08.03	DF	AA61NG	2390	39.767	0.474	3.95	2	1	0.6756	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	NO	low r2		FAL.NHK.SLS.15.08.03
FAL.NHK.NG.B4.05.09.03 (plate sealer)	DF	AA61NG	21800	362.729	0.129	15.55	1	3	0.7750	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	NO	% VC difference >15	SD provided revised file to correct data entry error	FAL.NHK.SLS.050903
FAL.NHK.NG.B5.15.10.03 plate sealer and mineral oil	DF	AA61NG	7460	124.126	0.624	3.14	1	5	0.6032	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	NO	RF format; low r2		FAL.NHK.SLS.15.10.03
FAL.NHK.NG.B6.19.10.03 plate sealer	DF	AA61NG	5850	97.338	0.262	19.17	4	3	0.9245	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	NO	% VC difference >15		FAL.NHK.SLS.19.10.03
FAL.NHK.NG.B6.19.10.03 mineral oil	DF	AA61NG	5020	83.527	0.182	3.99	1	4	0.7943	20000, 13605, 9255, 6296, 4283, 2914, 1982, 1348	1.47	NO	Mineral oil	experimental	FAL.NHK.SLS.19.10.03
FAL.NHK.NG.B7.23.10.03 plate sealer	DF	AA61NG	2410	40.100	0.236	9.93	4	4	0.6362	20000, 9302, 4327, 2012, 936, 435, 202, 94	2.15	NO	low r2		FAL.NHK.SLS.23.10.03
FAL.NHK.NG.B7.23.10.03 mineral oil	DF	AA61NG	4710	78.369	0.251	8.11	3	3	0.5306	20000, 9302, 4327, 2012, 936, 435, 202, 94	2.15	NO	low r2		FAL.NHK.SLS.23.10.03
FAL.NHK.NG.B8.24.10.03 plate sealer	DF	AA61NG	5220	86.855	0.622	0.92	2	3	0.8150	20000, 9302, 4327, 2012, 936, 435, 202, 94	2.15	YES			FAL.NHK.SLS.24.10.03
FAL.NHK.NG.B8.24.10.03 mineral oil	DF	AA61NG	4730	78.702	0.709	2.74	2	4	0.7880	20000, 9302, 4327, 2012, 936, 435, 202, 94	2.15	NO	low r2; Mineral oil	experimental	FAL.NHK.SLS.24.10.03
FAL.NHK.NG.B9.05.11.03ps plate sealer	DF	AA61NG	4590	76.373	0.561	4.88	2	1	0.8354	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			FAL.NHK.SLS.05.11.03 (revised by study director)
FAL.NHK.NG.B9.05.11.03 min oil (mineral oil)	DF	AA61NG	4480	74.542	0.564	20.01	2	2	0.7822	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	NO	low r2; VC difference >15%; Mineral oil	experimental	FAL.NHK.SLS.05.11.03 (revised by study director)
FAL.NHK.NG.B10.07.11.03Ps plate sealer	DF	AA61NG	3010	50.083	0.243	1.37	3	1	0.7256	20000, 9302, 4327, 2012, 936, 435, 202, 94	2.15	YES		challenging chemical; SMT accepts this test	FAL.NHK.SLS.07.11.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.NG.B10.07.11.03.mo (mineral oil)	DF	AA61NG	2610	43.428	0.270	5.07	2	1	0.8214	20000, 9302, 4327, 2012, 936, 435, 202, 94	2.15	NO	Mineral oil	experimental	FAL.NHK.SLS.07.11.03
PROPRANOLOL															
IIVS															
Preliminary	RF	AA61GU	23.1	0.078	0.606	4.44%	0	0	0.9617	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001		RF	range finder		Preliminary
B1	DF	AA61GU	29.6	0.100	0.582	4.61%	2	1	0.9576	100, 56.3, 31.6, 17.8, 10.0, 5.6, 3.2, 1.8		YES			SLS-B1
B2	DF	AA61GU	26.9	0.091	0.764	0.61%	2	2	0.9790	100, 56.3, 31.6, 17.8, 10.0, 5.6, 3.2, 1.8		YES			SLS-B2
B3	DF	AA61GU	25.2	0.085	1.001	0.94%	2	4	0.9652	100, 56.3, 31.6, 17.8, 10.0, 5.6, 3.2, 1.8		YES			SLS-B3
B4	DF	AA61GU	32.7	0.111	0.907	4.02%	1	2	0.9864	100, 56.3, 31.6, 17.8, 10.0, 5.6, 3.2, 1.8		YES			SLS-B4
ECBC															
ECBC-NHK-Ib-01 AA61KH-A1	RF	AA61KH	15.8	0.053	1.006	0.13%	0	2	0.9629	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001		RF	range finder		SLS-P1
ECBC-NHK-Ib-02 AA61KH-B1	DF	AA61KH	33.1	0.112	1.153	0.37%	1	3	0.9724	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74		YES			SLS-P3
ECBC-NHK-Ib-03 AA61KH-B2	DF	AA61KH	40.1	0.136	1.216	7.40%	2	1	0.9856	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74		YES			SLS-P4
ECBC-NHK-Ib-04 AA61KH-B3	DF	AA61KH	41.6	0.141	1.153	5.14%	2	1	0.9683	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.91, 6.74		YES			SLS-P5
FRAME															
A1 1b/NHKRF1b/FAL/NM	RF	AA61NM	3.53	0.012	0.149	7.05%	0	3	0.8056	100, 20, 4, 0.8, 0.16, 0.032, 0.0064, 0.00128	5	RF	range finder		A1 1b/NHKCTR1/FAL/SLS
A2 1b/NHKRF2/FAL/NM	RF	AA61NM	8.66	0.029	0.475	9.32%	1	2	0.8193	100, 68.02, 46.27, 31.47, 21.40, 14.50, 9.90, 6.70	1.47	RF	range finder		A2 1b/NHKCTR2/FAL/SLS
A3 1b/NHK/DF2/FAL/NM	DF	AA61NM	24.4	0.082	0.042	11.04%	0	2	0.3257	30, 20.4, 13.8, 9.4, 6.42, 4.37, 2.97, 2.02	1.47	NO	No point between 10 & 50%; R^2 < 0.8; PC failed	NR crystal problems; used medium not normally used; removing outlier doesn't significantly improve R2	A3 1b/NHK/CTR4/FAL/
A4 1b/NHK/DF3/FAL/NM	DF	AA61NM	1.22	0.004	0.140	15.20%	0	4	0.0680	30, 20.4, 13.8, 9.4, 6.42, 4.37, 2.97, 2.02	1.47	NO	No point between 10 & 50% viability; R^2 < 0.8	NR crystal problems; used medium not normally used	A4 1b/NHK/CTR5/FAL
A5 1b/NHK/DF4/FAL/NM	DF	AA61NM	NA	NA	0.008	9.78%	0	0	NC	30, 20.4, 13.8, 9.4, 6.42, 4.37, 2.97, 2.02	1.47	NO	No points between 10 & 90%; no R^2 or ICx; PC failed	NR crystal problems; used medium not normally used; OD values of test wells no different than the background ODs; negative values for VC	A5 1b/NHK/CTR6/FAL
A6 1b/NHK/DF5/FAL/NM recalculated w/o outliers	DF	AA61NM	54.0	0.183	1.686	2.60%	0	8	0.7186	30, 20.4, 13.8, 9.4, 6.42, 4.37, 2.97, 2.02	1.47	NO	No point between 10 & 50%; R^2 < 0.8	removed two outliers; didn't reach IC50	A6 1b/NHK/CTR7/FAL
A8 1b/NHK/DF7/FAL/NM	DF	AA61NM	NA	NA	1.045	2.91%	0	5	NC	50, 34.01, 23.13, 15.74, 10.70, 7.28, 4.95,3.36	1.47	NO	No point between 10 & 50%; no R^2 or ICx	PRISM couldn't do calculations; didn't reach IC50; recalc w/o outliers didn't improve curve fit, so they have not been removed	A8 1b/NHK/CTR9/FAL
A9 1b/NHK/DF8/FAL/NM	DF	AA61NM	3.21	0.011	1.026	25.70%	0	4	0.1476	50, 34.01, 23.13, 15.74, 10.70, 7.28, 4.95,3.36	1.47	NO	VC difference > 15%; no point between 10 & 50%; R^2 < 0.8; PC failed	U-shaped dose-response	A9 1b/NHK/CTR10/FAL
A10 1b/NHK/DF9/FAL/NM	DF	AA61NM	42.8	0.145	0.954	2.32%	1	3	0.5573	350, 238.1, 162.0, 110.2, 75.0, 51.0, 34.7, 23.6	1.47	NO	R^2 < 0.8	no outliers; nonmonotonic response	A10 1b/NHK/CTR11/FAL
A11 1b/NHK/DF10/FAL/NM	DF	AA61NM	46.5	0.157	1.280	0.27%	1	2	0.8686	350, 238.1, 162.0, 110.2, 75.0, 51.0, 34.7, 23.6	1.47	YES		removed 3 outliers	A11 1b/NHK/CTR12/FAL
A12 1b/NHK/DG11/FAL/NM	DF	AA61NM	26.0	0.088	0.539	6.14%	3	0	0.8391	350, 238.1, 162.0, 110.2, 75.0, 51.0, 34.7, 23.6	1.47	NO	No point between 50 & 90%		A12 1b/NHK/CTR13/FAL/SLS

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
1b/NHK/DF12/FAL/NM	DF	AA61NM	43.4	0.147	0.650	5.04%	1	2	0.9265	350, 238.1, 162.0, 110.2, 75.0, 51.0, 34.7, 23.6	1.47	YES			1b/NHK/CTR14/FAL/SLS
1b/NHK/DF13/FAL/NM	DF	AA61NM	41.5	0.140	0.897	2.57%	2	2	0.9555	350, 238.1, 162.0, 110.2, 75.0, 51.0, 34.7, 23.6	1.47	YES			1b/NHK/CTR15/FAL/SLS
PROPYLPARABEN															
IIVS															
A1	RF	AA61PX	15.0	0.083	0.719	1.51%	2	2	0.9878	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A2-N040320B
B1	DF	AA61PX	13.4	0.075	0.631	1.14%	5	3	0.9849	200, 111, 61.7, 34.3, 19.1, 10.6, 5.88, 3.27	1.8	YES			SLS-B1-N040423A
B2	DF	AA61PX	15.2	0.085	0.664	3.40%	5	3	0.9935	200, 111, 61.7, 34.3, 19.1, 10.6, 5.88, 3.27	1.8	YES			SLS-B2-N040424A
B3	DF	AA61PX	12.9	0.072	0.512	1.92%	4	3	0.9841	200, 111, 61.7, 34.3, 19.1, 10.6, 5.88, 3.27	1.8	YES			SLS-B3-N040506A
ECBC															
AA61PK-A1	RF	AA61PK	14.8	0.082	0.534	9.07%	2	1	0.8856	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1 and 1X C1	SLS-P5
AA61PK-B1	DF	AA61PK	20.7	0.115	0.960	0.09%	4	4	0.9856	300, 140, 64.9, 30.2, 14.0, 6.53, 3.04, 1.41	2.15	YES			SLS-P27
AA61PK-B2	DF	AA61PK	15.9	0.088	1.059	0.57%	4	4	0.9647	300, 140, 64.9, 30.2, 14.0, 6.53, 3.04, 1.41	2.15	YES			SLS-P29
AA61PK-B3	DF	AA61PK	17.7	0.098	0.760	0.66%	4	4	0.9877	300, 140, 64.9, 30.2, 14.0, 6.53, 3.04, 1.41	2.15	YES			SLS-P30
FRAME															
FAL.NHK.HT.A1.26.03.04	RF	AA61HT	23.4	0.130	0.486	8.29%	2	2	0.7353	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		FAL.NHK.SLS.26.03.04
FAL.NHK.HT.A2.25.04.04	RF	AA61HT	NA	NA	0.729	50.05%	2	2	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; wrong desorb solution used in NRU; SD rejects test	same application date and PC as HT A1	FAL.NHK.SLS.26.03.04
FAL.NHK.HT.B1.28.04.04	DF	AA61HT	20.4	0.113	1.018	5.66%	2	3	0.9749	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.NHK.SLS.28.04.03
FAL.NHK.HT.B2.11.06.04	DF	AA61HT	10.7	0.060	0.892	2.02%	4	4	0.9211	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	PC failed		FAL.NHK.SLS.11.06.04
FAL.NHK.HT.B3.23.06.04	DF	AA61HT	NA	NA	0.521	99.17%	NA	NA	NA	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	NO	% VC difference > 15	no cells in VC2; no PRISM file	FAL.NHK.SLS.23.06.04
FAL.NHK.HT.B4.25.06.04	DF	AA61HT	15.3	0.085	1.063	4.00%	3	5	0.9548	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.NHK.SLS.25.06.04
FAL.NHK.HT.B5.20.08.04	DF	AA61HT	20.0	0.11072	0.906	0.85%	2	2	0.9443	100, 46.5, 21.6, 10.1, 4.68, 2.18, 1.01, 0.47	2.15	YES			FAL.NHK.SLS.20.08.04
SODIUM ARSENITE															
IIVS															
A1	RF	AA61MV	0.581	0.004	0.393	15.03%	2	1	0.9631	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	volatile effects in VC1 and VC2	SLS-A5-N040401A
B1	DF	AA61MV	0.440	0.003	0.590	11.98%	3	1	0.9426	30.0, 13.6, 6.20, 2.82, 1.28, 0.582, 0.265, 0.120	2.2	YES		used plate sealer	SLS-B4-N040513C
B2	DF	AA61MV	0.546	0.004	0.580	1.54%	4	1	0.9724	30.0, 13.6, 6.20, 2.82, 1.28, 0.582, 0.265, 0.120	2.2	YES		plate sealer used	SLS-B5-N040514B
B3	DF	AA61MV	0.424	0.003	0.666	3.98%	3	2	0.9931	30.0, 13.6, 6.20, 2.82, 1.28, 0.582, 0.265, 0.120	2.2	YES		plate sealer used	SLS-B6-N040716A
ECBC															
AA61KA-A1	RF	AA61KA	0.506	0.004	0.850	0.23%	3	2	0.9923	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P18

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61KA-B1	DF	AA61KA	1.05	0.008	0.822	1.69%	3	3	0.9450	8.00, 3.72, 1.73, 0.805, 0.374, 0.174, 0.081, 0.038	2.15	YES			SLS-P26
AA61KA-B2	DF	AA61KA	0.764	0.006	1.005	1.85%	4	4	0.9892	8.00, 3.72, 1.73, 0.805, 0.374, 0.174, 0.081, 0.038	2.15	YES			SLS-P28
AA61KA-B3	DF	AA61KA	0.555	0.004	0.801	0.43%	4	4	0.9804	8.00, 3.72, 1.73, 0.805, 0.374, 0.174, 0.081, 0.038	2.15	YES			SLS-P30
FRAME															
FAL.NHK.GS.A1.24.09.04	RF	AA61GS	0.056	0.0004	0.652	2.90%	1	3	0.9075	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.GS.B1.01.10.04	DF	AA61GS	1.07	0.008	0.961	3.18%	2	4	0.9814	10.0, 4.65, 2.16, 1.01, 0.47, 0.22, 0.10, 0.05	2.15	NO	PC failed		FAL.NHK.SLS.01.10.04
FAL.NHK.GS.B2.07.10.04	DF	AA61GS	0.275	0.002	0.516	3.33%	5	3	0.9843	10.0, 4.65, 2.16, 1.01, 0.47, 0.22, 0.10, 0.05	2.15	YES			FAL.NHK.SLS.07.10.03
FAL.NHK.GS.B3 .22.10.04	DF	AA61GS	0.545	0.004	0.712	5.53%	4	1	0.9815	10.0, 4.65, 2.16, 1.01, 0.47, 0.22, 0.10, 0.05	2.15	YES			FAL.NHK.SLS.22.10.04 (MO)
FAL.NHK.GS.B4 .28.10.04	DF	AA61GS	0.187	0.001	0.759	3.27%	6	2	0.9854	10.0, 4.65, 2.16, 1.01, 0.47, 0.22, 0.10, 0.05	2.15	YES			FAL.NHK.SLS.28.10.04

SODIUM CHLORIDE

IIVS															
A1	RF	AA61PE	2100	35.999	0.630	2.05%	1	1	0.9570	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A3-N040331A
B1	DF	AA61PE	NA	NA	0.549	1.11%	0	0	NA	1000, 625, 391, 244, 153, 95.4, 59.6, 37.3	1.6	NO	no points between 0 - 100%		SLS-B4-N040513C
B2	DF	AA61PE	NA	NA	0.518	0.68%	0	2	NA	1000, 625, 391, 244, 153, 95.4, 59.6, 37.3	1.6	NO	no points between 0 - 100%	toxicity not detected	SLS-B5-N040514B
B3	DF	AA61PE	3170	54.236	0.707	4.08%	3	4	0.9471	10000, 7143, 5102, 3644, 2603, 1859, 1328, 949	1.4	YES		outlier removed by SD	SLS-B6-N040716A
B4	DF	AA61PE	3470	59.332	0.599	10.23%	3	5	0.9518	10000, 7143, 5102, 3644, 2603, 1859, 1328, 949	1.4	YES			SLS-B7-N040717B
B5	DF	AA61PE	3770	64.460	0.550	2.04%	2	3	0.9280	10000, 7143, 5102, 3644, 2603, 1859, 1328, 949	1.4	YES			SLS-B8-N040819A
ECBC															
AA61JW-A1	RF	AA61JW	2250	38.485	0.817	2.63%	1	5	0.9346	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P12
AA61JW-B1	DF	AA61JW	3730	63.869	0.949	2.37%	3	5	0.9583	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P26
AA61JW-B2	DF	AA61JW	3740	64.016	0.999	4.56%	3	4	0.9559	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P28
AA61JW-B3	DF	AA61JW	3280	56.142	0.746	0.28%	3	5	0.9504	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES			SLS-P30
FRAME															
FAL.NHK.FM.A1.14.05.04	RF	AA61FM	2330	39.837	0.715	0.68%	1	4	0.9613	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.14.05.03
FAL.NHK.FM.B1.25.06.04	DF	AA61FM	366	6.256	0.954	1.08%	1	4	0.9769	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.25.06.04
FAL.NHK.FM.B2.12.08.04	DF	AA61FM	NA	NA	0.658	6.32%	0	0	NA	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	NO	PC failed; no points between 0 - 100%		FAL.NHK.SLS.12.08.04
FAL.NHK.FM.B3.19.08.04 nb	DF	AA61FM	NA	NA	0.397	0.95%	0	1	NA	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	NO	no points between 0 - 50%	no toxicity detected	FAL.NHK.SLS-NB.19.08.04
FAL.NHK.FM.B4.30.09.04	DF	AA61FM	NA	NA	0.558	4.48%	0	4	0.7866	2500, 930, 433, 201, 93.6, 43.5, 20.3, 9.42	2.15	NO	no points between 0 - 50%	toxicity curve begins to rise at high concentrations; maybe affecting NRU	FAL.NHK.SLS.30.09.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.FM.B5.05.11.04	DF	AA61FM	268	4.584	0.455	0.60%	1	6	0.8717	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	YES			FAL.NHK.SLS.05.11.04
FAL.NHK.FM.B3. 12.11.04 (should be B6)	DF	AA61FM	NA	NA	0.694	14.43%	0	3	NA	1000, 465, 216, 101, 46.8, 21.8, 10.1, 4.71	2.15	NO	no points between 0 - 50%	ppt in 1X C1-C4	FAL.NHK.SLS.12.11.04
FAL.NHK.FM.B7.17.11.04	DF	AA61FM	NA	NA	0.919	5.26%	0	8	NA	2000, 1527, 1165, 890, 679, 518, 396, 302	1.31	NO	no points between 0 - 50%		FAL.NHK.SLS.17.11.04
FAL.NHK.FM.B8.26.11.04	DF	AA61FM	2720	46.590	0.636	2.88%	2	6	0.9214	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			FAL.NHK.SLS.26.11.04

SODIUM DICHROMATE DIHYDRATE

IIVS															
A1	RF	AA61FP	0.390	0.001	0.545	2.40%	2	2	0.9955	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A4-N040331N
B1	DF	AA61FP	0.527	0.002	0.587	1.15%	3	4	0.9863	5.00, 2.78, 1.54, 0.857, 0.476, 0.265, 0.147, 0.082	1.8	YES			SLS-B4-N040513C
B2	DF	AA61FP	0.511	0.002	0.522	0.67%	4	4	0.9863	5.00, 2.78, 1.54, 0.857, 0.476, 0.265, 0.147, 0.082	1.8	YES			SLS-B5-N040514B
B3	DF	AA61FP	0.691	0.002	0.711	0.67%	4	4	0.9841	5.00, 2.78, 1.54, 0.857, 0.476, 0.265, 0.147, 0.082	1.8	YES			SLS-B6-N040716A
ECBC															
AA61NT-A1	RF	AA61NT	0.284	0.0010	0.542	1.94%	4	3	0.9819	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P16
AA61NT-B1	DF	AA61NT	0.781	0.003	0.837	1.68%	1	7	0.8935	1.00, 0.680, 0.463, 0.315, 0.214, 0.146, 0.099, 0.067	1.47	YES			SLS-P26
AA61NT-B2	DF	AA61NT	0.899	0.003	0.915	2.34%	2	6	0.9495	2.00, 1.361, 0.926, 0.630, 0.428, 0.291, 0.198, 0.135	1.47	YES			SLS-P28
AA61NT-B3	DF	AA61NT	0.673	0.002	0.762	1.72%	3	5	0.9680	2.00, 1.361, 0.926, 0.630, 0.428, 0.291, 0.198, 0.135	1.47	YES			SLS-P30
FRAME															
FAL.NHK.HK.A1.28.07.04	RF	AA61HK	0.112	0.000	0.059	15.81%	5	3	0.7460	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; % VC difference > 15		FAL.NHK.SLS.28.07.04
FAL.NHK.HK.***A1.28.07.04 (should be 11.08.04)***	RF	AA61HK	0.770	0.003	0.623	6.22%	1	1	0.9797	100, 31.6, 10.0, 3.17, 1.00, 0.317, 0.100, 0.0318	3.16	YES			FAL.NHK.SLS.11.08.04
FAL.NHK.HK-NB.B2.25.08.04	DF	AA61HK	48.8	0.164	0.877	4.03%	1	4	0.9276	100, 31.6, 10.0, 3.17, 1.00, 0.317, 0.100, 0.0318	3.16	NO	SD rejects		FAL.NHK.SLS.25.08.04
FAL.NHK.HK.B3.03.11.04	DF	AA61HK	0.512	0.002	0.518	1.50%	1	3	0.9921	100, 31.6, 10.0, 3.17, 1.00, 0.317, 0.100, 0.0318	3.16	YES		solvent listed as DMSO-- should be medium; SD confirmed medium was used	FAL.NHK.SLS.03.11.04
FAL.NHK.HK.B3.12.11.04 (should be B4)	DF	AA61HK	0.882	0.003	0.792	0.95%	5	3	0.9919	100, 31.6, 10.0, 3.17, 1.00, 0.317, 0.100, 0.0318	3.16	YES			FAL.NHK.SLS.12.11.04
FAL.NHK.HK.B4.24.11.04 (should be B5)	DF	AA61HK	1.24	0.004	1.060	0.46%	1	2	0.9962	100, 31.6, 10.0, 3.17, 1.00, 0.317, 0.100, 0.0318	3.16	YES			FAL.NHK.SLS.24.11.04

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
SODIUM I FLUORIDE															
IIVS															
A2	RF	AA61HF	50.2	1.196	0.624	2.61%	2	1	0.9754	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A2
B1	DF	AA61HF	50.1	1.193	0.355	6.81%	5	1	0.9643	300, 188, 117, 73.2, 45.8, 28.6, 17.9, 11.2	1.6	YES			SLS-B1
B2	DF	AA61HF	51.9	1.236	0.275	12.46%	5	2	0.9713	300, 188, 117, 73.2, 45.8, 28.6, 17.9, 11.2	1.6	YES			SLS-B2
B3	DF	AA61HF	49.1	1.169	0.321	2.29%	5	3	0.9679	300, 188, 117, 73.2, 45.8, 28.6, 17.9, 11.2	1.6	YES			SLS-B3
B6	DF	AA61HF	63.8	1.519	0.56	6.98%	4	4	0.9088	150, 115, 88.8, 68.3, 52.5, 40.4, 31.1, 23.9	1.46	YES			SLS-B7
ECBC															
AA61MG-A1	RF	AA61MG	35.2	0.838	0.673	0.47%	2	3	0.9552	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder	range finder	SLS-P2
AA61MG-B1	DF	AA61MG	55.0	1.310	0.359	0.67%	3	5	0.9146	1000, 300, 100, 30, 10, 3, 1, 0.3	3.33	YES			SLS-P5
AA61MG-B2	DF	AA61MG	41.3	0.984	0.855	2.57%	4	4	0.9376	150, 102.5, 69.4, 47.2, 32.1, 21.8, 14.9, 10.1	1.47	YES			SLS-P7
AA61MG-B3	DF	AA61MG	49.8	1.186	0.942	1.56%	4	4	0.9160	150, 102.5, 69.4, 47.2, 32.1, 21.8, 14.9, 10.1	1.47	YES			SLS-P9
FRAME															
FAL.NHK.RH.A1.010803	RF	AA61RH	3.94	0.094	1.113	4.56%	3	4	0.9474	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.010803
FAL.NHK.RH.B1.080803	DF	AA61RH	28.6	0.681	0.762	0.08	1	5	0.9046	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	YES	range finder format	high background	FAL.NHK.SLS.08.08.03
FAL.NHK.RH.B2.15.08.03	DF	AA61RH	45.2	1.076	0.549	0.03	4	3	0.9257	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.15.08.03
FAL.NHK.RH.B3.01.10.03	DF	AA61RH	51.2	1.219	1.140	0.01	4	4	0.9761	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	NO	PC failed	PC fails	FAL.NHK.SLS.01.10.03
FAL.NHK.RH.B3.15.10.03 (should be B4?)	DF	AA61RH	45.3	1.079	0.531	0.01	4	3	0.9771	500, 233, 108, 50.3, 23.4, 10.9, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.15.10.03
SODIUM HYPOCHLORITE															
IIVS															
A1	RF	AA61RD	1250	16.796	0.439	6.83%	0	2	0.9817	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0 - 50%		SLS-A1-N040317B
B1	DF	AA61RD	1620	21.787	0.530	4.61%	4	2	0.9847	10000, 5556, 3086, 1715,953, 529, 294, 163	1.8	YES			SLS-B1-N040423A
B2	DF	AA61RD	1460	19.642	0.571	5.89%	2	1	0.9828	10000, 5556, 3086, 1715,953, 529, 294, 163	1.8	YES			SLS-B2-N040424A
B3	DF	AA61RD	1820	24.389	0.515	7.20%	3	3	0.9820	4000, 2857, 2041, 1458, 1041, 744, 531, 379	1.4	YES			SLS-B3-N040506A
ECBC															
AA61HE-A1	RF	AA61HE	1030	13.874	0.465	7.39%	0	1	0.8508	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P3
AA61HE-B1	DF	AA61HE	1960	26.375	0.975	3.79%	2	3	0.9309	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	YES			SLS-P7
AA61HE-B2	DF	AA61HE	2390	32.151	1.161	1.44%	2	5	0.9791	5000, 3401, 2313, 1574, 1071, 728, 496, 337	1.47	YES			SLS-P9
AA61HE-B3	DF	AA61HE	1240	16.718	0.725	0.10%	4	3	0.9857	5000, 3401, 2313, 1574, 1071, 728, 496, 337	1.47	YES			SLS-P12
FRAME															
FAL.NHK.LU.A1.13.02.03	RF	AA61LU	955	12.829	0.077	1.41%	1	0	0.0662	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	rejected by SD due to bacterial contam. in some of the plates in this test series	FAL.NHK.SLS.13.02.03
FAL.NHK.LU.A2.20.02.03	DF	AA61LU	738	9.913	0.204	12.54%	6	1	0.9071	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES		this is a definitive test since conc. series is different from A1 RF	FAL.NHK.SLS.20.02.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.LU.B1.27.02.04	DF	AA61LU	NA	NA	0.492	9.65%	0	0	NA	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 0-100%; wrong solvent used	used wrong solvent; should be medium instead of DMSO	FAL.NHK.SLS.27.02.03
FAL.NHK.LU.B2.19.03.04	DF	AA61LU	1120	15.073	0.437	3.51%	2	6	0.9027	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.NHK.SLS.19.03.03
FAL.NHK.LU.B3.25.03.04	DF	AA61LU	1870	25.130	0.628	1.58%	1	2	0.7836	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.NHK.SLS.25.03.03
SODIUM OXALATE															
IIVS															
A1	RF	AA61GX	NA	NA	0.503	2.45%	0	2	NA	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; no points between 0 - 50%		SLS-A5-N040401A
B1	DF	AA61GX	252	1.879	0.631	2.24%	2	6	0.9647	500, 357, 255, 182, 130, 93.0, 66.4, 47.4	1.4	YES			SLS-B12-N041022B
B2	DF	AA61GX	428	3.191	0.565	1.71%	1	5	0.8879	510, 364, 260, 186, 133, 94.8, 67.7, 48.4	1.4	YES		130 ul of 2X doses were applied. Final conc. values adjusted in data sheets bySD	SLS-B113-N041029B
B3	DF	AA61GX	400	2.985	0.669	2.53%	1	7	0.8426	500, 357, 255, 182, 130, 93.0, 66.4, 47.4	1.4	YES			SLS-B14-N041030A
ECBC															
AA61LZ-A1	RF	AA61LZ	230	1.717	0.621	2.94%	2	6	0.9507	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P20
AA61LZ-B1	DF	AA61LZ	312	2.328	0.636	0.73%	3	5	0.8613	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		ppt in 1X C1-C3	SLS-P40
AA61LZ-B2	DF	AA61LZ	337	2.517	0.709	1.12%	2	6	0.9490	600, 408, 278, 189, 128, 87.4, 59.5, 40.5	1.47	YES		ppt in 1X C1-C2	SLS-P42
AA61LZ-B3	DF	AA61LZ	417	3.111	0.928	5.95%	1	5	0.9635	600, 408, 278, 189, 128, 87.4, 59.5, 40.5	1.47	YES		ppt in 1X C1	SLS-P44
FRAME															
FAL.NHK.RC.A1.24.09.04	RF	AA61RC	687	5.127	0.404	1.28%	2	0	0.6286	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1-C2	FAL.NHK.SLS.24.09.03
FAL.NHK.RC.B1.29.10.04	DF	AA61RC	134	1.002	0.598	5.63%	5	3	0.8555	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES		ppt In 1X C1-C5	FAL.NHK.SLS.29.10.04
FAL.NHK.RC.B2.03.11.04	DF	AA61RC	422	3.147	0.465	1.00%	1	7	0.7013	500, 340, 231, 157, 107, 72.8, 49.6, 33.7	1.47	YES			FAL.NHK.SLS.03.11.04
FAL.NHK.RC.B3.10.11.04	DF	AA61RC	384	2.863	1.082	0.92%	5	1	0.9714	2000, 1361, 926, 630, 428, 291,198,135	1.47	YES		ppt In 1X C1-C5	FAL.NHK.SLS.10.11.04
FAL.NHK.RC.B4.17.11.04	DF	AA61RC	460	3.435	1.002	2.39%	2	5	0.9280	1000, 680, 463, 315, 214, 146, 99.1, 67.4	1.47	YES			FAL.NHK.SLS.17.11.04
SODIUM SELENATE															
IIVS															
A2	RF	AA61FS	7.44	0.039	0.646	4.12	4	1	0.9744	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-A2
B1	DF	AA61FS	11.0	0.058	0.366	1.07	7	1	0.9841	556, 309, 172, 95.3, 53.0, 29.4, 16.3, 9.07	1.8	YES			SLS-B1
B2	DF	AA61FS	10.5	0.056	0.29	12.33	4	1	0.9854	556, 309, 172, 95.3, 53.0, 29.4, 16.3, 9.08	1.8	YES			SLS-B2
B3	DF	AA61FS	8.49	0.045	0.339	3.42	4	2	0.9763	100, 55.6, 30.9, 17.1, 9.5, 5.3, 2.94, 1.63	1.8	YES			SLS-B3
ECBC															
AA61LF-A1	RF	AA61LF	7.91	0.042	0.605	6.62	3	2	0.9431	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder	range finder	SLS-P1
AA61LF-B1	DF	AA61LF	7.99	0.042	0.361	5.82	7	1	0.9236	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES			SLS-P5
AA61LF-B3	DF	AA61LF	7.95	0.042	0.890	1.82	4	3	0.9492	100, 46.5, 21.6, 10.1, 4.7, 2.2, 1.0, 0.47	2.15	YES			SLS-P9
AA61LF-B4	DF	AA61LF	4.85	0.026	0.836	5.88	4	3	0.9845	100, 46.5, 21.6, 10.1, 4.7, 2.2, 1.0, 0.47	2.15	NO	PC failed		SLS-P11

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61LF-B5	DF	AA61LF	6.48	0.034	0.647	1.62	4	2	0.8997	100, 46.5, 21.6, 10.1, 4.7, 2.2, 1.0, 0.47	2.15	YES			SLS-P19
FRAME															
FAL.NHK.NS.A1.010803	RF	AA61NS	10.4	0.055	0.360	5.76	2	3	0.9256	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.010803
FAL.NHK.NS.A2.080803	RF	AA61NS	14.6	0.077	0.716	5.02	6	0	0.9642	250, 170, 116, 78.7, 53.6, 36.4, 24.8, 16.9	1.47	RF	range finder	high background	FAL.NHK.SLS.08.08.03
FAL.NHK.NS.B2.15.08.03 (should be B1)	DF	AA61NS	12.2	0.065	0.551	5.35	4	4	0.9509	50, 34.01, 23.14, 15.74, 10.71, 7.28, 4.96, 3.37	1.47	YES		this is the first definitive test	FAL.NHK.SLS.15.08.03
FAL.NHK.NS.B2.230803	DF	AA61NS	9.34	0.049	0.490	0.47	5	3	0.9542	50, 34.01, 23.14, 15.74, 10.71, 7.28, 4.96, 3.37	1.47	NO	PC failed		FAL.NHK.SLS.230803
FAL.NHK.NS.B3.28.08.06	DF	AA61NS	34.0	0.180	0.398	3.79	1	6	0.6981	50, 34.01, 23.14, 15.74, 10.71, 7.28, 4.96, 3.37	1.47	NO	low r2		FAL.NHK.SLS.280803
FAL.NHK.NS.B4.05.09.03	DF	AA61NS	9.14	0.048	0.207	7.21	6	2	0.9566	75, 51.02, 34.71, 23.61, 16.06, 10.93, 7.433, 5.06	1.47	YES			FAL.NHK.SLS.050903
FAL.NHK.NS.B5.01.10.03	DF	AA61NS	7.75	0.041	1.124	6.36	6	2	0.9147	75, 51.02, 34.71, 23.61, 16.06, 10.93, 7.433, 5.06	1.47	NO	PC failed		FAL.NHK.SLS.01.10.03
FAL.NHK.NS.B5.15.10.03 (should be B6?)	DF	AA61NS	27.0	0.143	0.565	1.67	2	4	0.9272	50, 34.01, 23.14, 15.74, 10.71, 7.28, 4.96, 3.37	1.47	YES			FAL.NHK.SLS.15.10.03
STRYCHNINE															
IIVS															
A1	RF	AA61JY	67.1	0.201	0.490	3.17%	1	1	0.8475	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder	ppt in 2X C1	SLS-A5-N040401A
B1	DF	AA61JY	59.0	0.176	0.606	1.54%	2	6	0.9699	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES		ppt in 2X C1	SLS-B12-N041022B
B2	DF	AA61JY	52.7	0.158	0.598	3.50%	2	6	0.9122	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES		ppt in 2X C1	SLS-B14-N041030A
B3	DF	AA61JY	53.5	0.160	0.616	2.26%	2	6	0.9020	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.4	YES		ppt in 2X C1	SLS-B15-N041110A
ECBC															
AA61NR-A1	RF	AA61NR	183	0.548	0.882	6.19%	1	6	0.8663	500, 50.0, 5.0, 0.50, 0.05, 0.005, 0.0005, 0.00005	10	RF	range finder		SLS-P39
AA61NR-B1	DF	AA61NR	66.5	0.199	0.878	3.32%	5	3	0.8150	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	YES		ppt in 2X C1-C8	SLS-P47
AA61NR-B2	DF	AA61NR	214	0.641	1.230	1.92%	2	6	0.9262	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	YES		ppt in 2X C1-C3	SLS-P50
AA61NR-B3	DF	AA61NR	72.3	0.216	0.593	3.86%	5	3	0.9316	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	YES		ppt in 2X C1-C5	SLS-P52
AA61NR-B4	DF	AA61NR	48.1	0.144	0.676	2.33%	6	2	0.9227	400, 272, 185, 126, 85.7, 58.3, 39.6, 27.0	1.47	YES		ppt in 2X C1	SLS-P54
FRAME															
FAL.NHK.FY.A1.24.09.04	RF	AA61FY	87.7	0.262	0.520	1.43%	1	0	-0.0136	100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.FY.B1.01.10.04	DF	AA61FY	60.3	0.180	0.965	14.61%	1	2	0.6474	125, 58.1, 27.0, 12.6, 5.85, 2.72, 1.27, 0.59	2.15	NO	PC failed		FAL.NHK.SLS.01.10.04
FAL.NHK.FY.B2.08.10.04	DF	AA61FY	83.9	0.251	0.595	2.95%	2	3	0.9088	250, 116, 54.1, 25.2, 11.7, 5.44, 2.53, 1.18	2.15	YES			FAL.NHK.SLS.08.10.03
FAL.NHK.FY.B3.29.10.04	DF	AA61FY	29.9	0.089	0.585	9.13%	4	3	0.9623	500, 232, 108, 50.3, 23.9, 10.4, 5.06, 2.35	2.15	YES			FAL.NHK.SLS.29.10.04
FAL.NHK.FY.B4.05.11.04	DF	AA61FY	43.8	0.131	0.475	5.37%	4	3	0.9636	500, 232, 108, 50.3, 23.9, 10.4, 5.06, 2.35	2.15	YES		outlier removed by SD	FAL.NHK.SLS.05.11.04
THALLIUM I SULFATE															
IIVS															
A1	RF	AA61KJ	0.0982	0.0002	0.448	10.68%	4	0	0.9741	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; no points between 50 - 100%		SLS-A1-N040317B

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B1	DF	AA61KJ	0.137	0.0003	0.574	0.51%	4	3	0.9864	1.00, 0.556, 0.309, 0.171, 0.095, 0.053, 0.029, 0.016	1.8	YES			SLS-B1-N040423A
B2	DF	AA61KJ	0.141	0.0003	0.553	1.22%	4	2	0.9838	1.00, 0.556, 0.309, 0.171, 0.095, 0.053, 0.029, 0.016	1.8	YES			SLS-B2-N040424A
B3	DF	AA61KJ	0.104	0.0002	0.471	0.27%	4	3	0.9906	1.00, 0.556, 0.309, 0.171, 0.095, 0.053, 0.029, 0.016	1.8	YES		Mimimal to no NRU in C1-C4 although visual observatios appeared as level 2.	SLS-B3-N040506A
ECBC															
AA61PB-A1	RF	AA61PB	NA	NA	0.610	3.77%	6	1	NA	500, 50.0, 5.00, 0.5, 0.05, 0.005, 0.0005, 0.00005	10	RF	range finder	ppt in 2X C1	SLS-P38
AA61PB-B1	DF	AA61PB	NA	NA	0.975	2.18%	0	8	NA	0.005, 0.00233, 0.00108, 0.0005, 0.00023, 0.00011, 0.00005, 0.00002	2.15	NO	no points between 0 - 50%		SLS-P46
AA61PB-B2	DF	AA61PB	0.313	0.0006	1.127	6.67%	2	6	0.8224	1.00, 0.465, 0.216, 0.101, 0.047, 0.022, 0.010, 0.005	2.15	YES			SLS-P50
AA61PB-B3	DF	AA61PB	0.132	0.0003	0.635	0.47%	4	4	0.9863	2.00, 0.930, 0.433, 0.201, 0.094, 0.044, 0.020, 0.009	2.15	YES		ppt in 2X C1	SLS-P52
AA61PB-B4	DF	AA61PB	0.149	0.0003	0.727	1.40%	4	4	0.9772	2.00, 0.930, 0.433, 0.201, 0.094, 0.044, 0.020, 0.009	2.15	YES			SLS-P54
FRAME															
FAL.NHK.GB.A1.13.02.03	RF	AA61GB	0.0708	0.0001	0.203	6.82%	3	3	0.6722	500, 50, 5, 0.5, 0.05, 0.005, 0.0005, 0.00005	10	RF	range finder	rejected by SD due to bacterial contam. in some of the plates in this test series	FAL.NHK.SLS.13.02.03
FAL.NHK.GB.B1.18.03.04	DF	AA61GB	0.167	0.0003	0.449	10.16%	3	2	0.9629	1.0, 0.47, 0.22, 0.10, 0.05, 0.022, 0.010, 0.0047	2.15	YES			FAL.NHK.SLS.18.03.03
FAL.NHK.GB.B2.19.03.04	DF	AA61GB	0.175	0.0003	0.448	0.84%	3	5	0.9714	1.0, 0.47, 0.22, 0.10, 0.05, 0.022, 0.010, 0.0047	2.15	YES			FAL.NHK.SLS.19.03.03
FAL.NHK.GB.B3.25.03.04	DF	AA61GB	0.118	0.0002	0.736	5.85%	4	3	0.9244	1.0, 0.47, 0.22, 0.10, 0.05, 0.022, 0.010, 0.0047	2.15	YES			FAL.NHK.SLS.25.03.03
TRICHLOROACETIC ACID															
IIVS															
A1	RF	AA61MR	661	4.043	0.513	1.38%	2	1	0.9403	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder	ppt in 1X C1	SLS-A4-N040331N
B1	DF	AA61MR	423	2.587	0.572	0.22%	5	2	0.9761	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES		ppt in 1X C1-C2	SLS-B4-N040513C
B2	DF	AA61MR	423	2.587	0.665	0.91%	4	2	0.9853	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES		ppt in 1X C1-C2	SLS-B5-N040514B
B3	DF	AA61MR	335	2.050	0.672	8.28%	3	2	0.9732	10000, 5556, 3086, 1715, 953, 529, 294, 163	1.8	YES		ppt in 1X C1-C2	SLS-B6-N040716A
ECBC															
AA61KT-A1	RF	AA61KT	348	2.132	0.561	3.44%	2	4	0.9560	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P17
AA61KT-B1	DF	AA61KT	400	2.448	0.789	0.01%	4	3	0.9754	7000, 3256, 1514, 704, 328, 152, 70.9, 33.0	2.15	YES			SLS-P33
AA61KT-B2	DF	AA61KT	366	2.243	0.666	4.87%	4	4	0.9886	7000, 3256, 1514, 704, 328, 152, 70.9, 33.0	2.15	YES			SLS-P35

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61KT-B3	DF	AA61KT	277	1.693	0.500	0.20%	4	4	0.9697	7000, 3256, 1514, 704, 328, 152, 70.9, 33.0	2.15	YES		ppt in 1X C1	SLS-P37
FRAME															
FAL.NHK.GH.A1.28.07.04	RF	AA61GH	627	3.835	0.053	4.54%	2	1	0.8134	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.28.07.04
FAL.NHK.GH.B1.11.08.04	DF	AA61GH	649	3.970	0.507	12.88%	4	4	0.8715	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS.11.08.04
FAL.NHK.GH.B2.27.08.04	DF	AA61GH	370	2.263	0.439	1.88%	4	4	0.8671	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES			FAL.NHK.SLS.27.08.04
FAL.NHK.GH.B3.17.09.04	DF	AA61GH	604	3.696	0.711	5.96%	4	4	0.9901	10000, 4651, 2163, 1006, 468, 218, 101, 47	2.15	YES		outlier removed by SD	FAL.NHK.SLS.17.09.04
1,1,1-TRICHLOROETHANE															
IIVS															
A1	RF	AA61KG	NA	NA	0.516	5.11%	0	1	NA	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder; no points between 0 - 50%		SLS-A5-N040401A
B1	DF	AA61KG	NA	NA	0.573	1.92%	0	5	-3.2450	10000, 7143, 5102, 3644, 2603, 1859, 1328, 949	1.4	NO	no points between 0 - 50%	ppt in 1X C1	SLS-B113-N041029B
B2	DF	AA61KG	NA	NA	0.677	2.29%	0	3	0.7130	12500, 8929, 6378, 4555, 3254, 2324, 1660, 1186	1.4	NO	no points between 0 - 50%	ppt in 1X C1-C3	SLS-B14-N041030A
B3	DF	AA61KG	9400	70.439	0.598	4.99%	0	2	0.8828	12500, 8929, 6378, 4555, 3254, 2324, 1660, 1186	1.4	NO	no points between 0 - 50%	ppt in 1X C1-C3; ppt in 2X C1-C4; test article was noted to form droplets and adhere to the dilution vesel; maximum plausible dose was tested.	SLS-B15-N041110A
ECBC															
AA61JV-A1(sealer)	RF	AA61JV	5300	39.702	0.614	8.77%	1	7	0.8101	10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001	10	RF	range finder		SLS-P20
AA61JV-B1(sealer)	DF	AA61JV	7530	56.469	0.920	1.02%	1	6	0.9418	10000, 6803, 4628, 3148, 2142, 1457, 991, 674	1.47	YES		ppt in 2X C1-C8	SLS-P46
AA61JV-B2 (sealer)	DF	AA61JV	8710	65.285	0.674	2.11%	1	6	0.9422	10000, 8264, 6830, 5645, 4665, 3855, 3186, 2633	1.21	YES		ppt in 2X C1; 1X C1 has large globules of chemical; outlier removed by SD	SLS-P48
AA61JV-B3 (sealer)	DF	AA61JV	8170	61.208	1.119	2.10%	1	7	0.8530	10000, 8264, 6830, 5645, 4665, 3855, 3186, 2633	1.21	YES		ppt in 2X C1-C4; 1X C1 has large globules of chemical;	SLS-P51
FRAME															
FAL.NHK.PN.A1.24.09.04	RF	AA61PN	NA	NA	0.472	8.81%	0	2	NA	10000, 1000, 100, 10, 1.0, 0.1, 0.01, 0.001	10	RF	range finder		FAL.NHK.SLS.24.09.03
FAL.NHK.PN.B1.29.10.04	DF	AA61PN	NA	NA	0.543	4.83%	0	0	0.9623	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 0-100%		FAL.NHK.SLS.29.10.04
FAL.NHK.PN.B2.19.11.04	DF	AA61PN	NA	NA	0.417	4.54%	0	1	NA	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	NO	no points between 0-50%		FAL.NHK.SLS.19.11.04
FAL.NHK.PN.B3.24.11.04	DF	AA61PN	NA	NA	1.211	2.37%	0	6	NA	10000, 4651, 2163, 1006, 468, 218, 101, 47.1	2.15	NO	no points between 0-50%	odd curve; two columns of data removed by SD (wells not seeded with cells?)	FAL.NHK.SLS.24.11.04
TRIETHYLENEMELAMINE															
IIVS															
A1	RF	AA61MT	1.64	0.008	0.690	3.71%	1	2	0.9531	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-A2-N040320B

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B1	DF	AA61MT	1.66	0.008	0.543	8.55%	3	5	0.9632	10.0, 5.56, 3.09, 1.71, 0.953, 0.529, 0.294, 0.163	1.8	YES			SLS-B1-N040423A
B2	DF	AA61MT	2.12	0.010	0.572	4.28%	3	3	0.9763	10.0, 5.56, 3.09, 1.71, 0.953, 0.529, 0.294, 0.163	1.8	YES			SLS-B2-N040424A
B3	DF	AA61MT	2.62	0.013	0.544	3.49%	2	4	0.9730	10.0, 5.56, 3.09, 1.71, 0.953, 0.529, 0.294, 0.163	1.8	YES			SLS-B3-N040506A
ECBC															
AA61GE-A1	RF	AA61GE	0.791	0.004	0.881	0.27%	0	7	0.9461	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder; no points between 0 - 50%	ppt in 2X C1	SLS-P13
AA61GE-B1	DF	AA61GE	1.33	0.007	0.642	6.27%	2	6	0.8577	5.00, 2.33, 1.08, 0.503, 0.234, 0.109, 0.051, 0.024	2.15	YES			SLS-P21
AA61GE-B2	DF	AA61GE	2.77	0.014	0.979	1.34%	1	6	0.9306	5.00, 2.33, 1.08, 0.503, 0.234, 0.109, 0.051, 0.024	2.15	YES			SLS-P23
AA61GE-B3	DF	AA61GE	0.964	0.005	0.561	1.05%	2	6	0.9283	5.00, 2.33, 1.08, 0.503, 0.234, 0.109, 0.051, 0.024	2.15	YES			SLS-P25
FRAME															
FAL.NHK.LB.A1.26.03.04	RF	AA61LB	1.13	0.006	0.805	2.56%	1	1	0.8822	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		FAL.NHK.SLS.26.03.04
FAL.NHK.LB.B1.25.04.04	DF	AA61LB	2.37	0.012	0.846	8.90%	1	3	0.9664	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			FAL.NHK.SLS.25.04.04
FAL.NHK.LB.B2.28.04.04	DF	AA61LB	2.22	0.011	0.851	4.98%	3	4	0.8151	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES			FAL.NHK.SLS.28.04.03
FAL.NHK.LB.B3.11.06.04	DF	AA61LB	2.18	0.011	0.975	1.63%	3	4	0.9221	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	PC failed		FAL.NHK.SLS.11.06.04
FAL.NHK.LB.B4.25.06.04	DF	AA61LB	1.49	0.007	1.155	0.33%	1	6	0.8420	10.0, 4.65, 2.16, 1.01, 0.468, 0.218, 0.101, 0.047	2.15	YES			FAL.NHK.SLS.25.06.04
TRIPHENYLTIN HYDROXIDE															
IIVS															
A1	RF	AA61JR	0.013	0.00004	0.729	1.45%	2	1	0.9887	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-A2-N040320B
B1	DF	AA61JR	0.015	0.00004	0.602	4.32%	2	0	0.9758	1.00, 0.556, 0.309, 0.171, 0.095, 0.053, 0.029, 0.016	1.8	NO	no points between 50 - 100%		SLS-B1-N040423A
B2	DF	AA61JR	0.015	0.00004	0.630	3.36%	2	0	0.9907	1.00, 0.556, 0.309, 0.171, 0.095, 0.053, 0.029, 0.016	1.8	NO	no points between 50 - 100%		SLS-B2-N040424A
B3	DF	AA61JR	0.012	0.00003	0.485	9.45%	3	2	0.9779	0.067, 0.045, 0.030, 0.020, 0.0132, 0.0088, 0.0059, 0.0039	1.5	YES			SLS-B3-N040506A
B4	DF	AA61JR	0.012	0.00003	0.658	0.37%	4	3	0.9917	0.067, 0.045, 0.030, 0.020, 0.013, 0.0088, 0.0059, 0.0039	1.5	YES			SLS-B8-N040819A
B5	DF	AA61JR	0.014	0.00004	0.610	0.07%	3	4	0.9907	0.067, 0.045, 0.030, 0.020, 0.013, 0.0088, 0.0059, 0.0039	1.5	YES			SLS-B9-N040820A
ECBC															
AA61LL-A1	RF	AA61LL	0.015	0.00004	0.542	3.67%	0	2	0.9880	10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder		SLS-P5

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
AA61LL-B1	DF	AA61LL	0.021	0.00006	1.065	0.78%	4	4	0.9633	0.080, 0.054, 0.037, 0.025, 0.017, 0.012, 0.008, 0.005	1.47	YES			SLS-P22
AA61LL-B2	DF	AA61LL	0.015	0.00004	0.599	0.01%	4	3	0.9832	0.080, 0.054, 0.037, 0.025, 0.017, 0.012, 0.008, 0.005	1.47	YES			SLS-P25
AA61LL-B3	DF	AA61LL	0.029	0.00008	0.987	5.68%	3	4	0.9754	0.080, 0.054, 0.037, 0.025, 0.017, 0.012, 0.008, 0.005	1.47	YES			SLS-P27
FRAME															
FAL.NHK.GG.A1.26.03.04	RF	AA61GG	0.010	0.00003	0.616	6.20%	2	0	0.8151	10.0, 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001	10	RF	range finder	ppt in 1X C1	FAL.NHK.SLS.26.03.04
FAL.NHK.GG.A2.25.04.04	DF	AA61GG	NA	NA	0.052	12.10%	2	6	NA	0.1, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	NO	wrong desorb solution used in NRU; SD rejects this test	ppt in 1X C1	FAL.NHK.SLS.25.04.04
FAL.NHK.GG.B1.28.04.04	DF	AA61GG	0.002	0.00001	0.877	1.40%	5	2	0.9884	0.100, 0.047, 0.022, 0.010, 0.005, 0.002, 0.001, 0.0005	2.15	YES			FAL.NHK.SLS.28.04.03
FAL.NHK.GG.B2.13.05.04	DF	AA61GG	0.003	0.00001	0.701	2.72%	2	3	0.9701	0.1, 0.0465, 0.0216, 0.0101, 0.0047, 0.0022, 0.0010, 0.0005	2.15	YES			FAL.NHK.SLS.13.05.04
FAL.NHK.GG.B3.10.06.04	DF	AA61GG	0.015	0.00004	0.894	5.53%	3	2	0.9727	0.100, 0. 68, 0.0463, 0.0315, 0.0214, 0.0146, 0.0099, 0.0067	1.47	YES			FAL.NHK.SLS.10.06.04
VALPROIC ACID															
IIVS															
A1	RF	AA61MZ	710	4.921	0.730	0.79%	1	2	0.9232	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A2-N040320B
B1	DF	AA61MZ	394	2.735	0.633	8.35%	4	4	0.9086	2500, 1563, 977, 610, 381, 238, 149, 93.1	1.6	YES			SLS-B8-N040819A
B2	DF	AA61MZ	512	3.548	0.676	4.33%	3	5	0.9566	2500, 1563, 977, 610, 381, 238, 149, 93.1	1.6	YES			SLS-B9-N040820A
B3	DF	AA61MZ	383	2.655	0.657	7.25%	3	4	0.9436	2500, 1563, 977, 610, 381, 238, 149, 93.1	1.6	YES			SLS-B10-N040903A
ECBC															
AA61JJ-A1	RF	AA61JJ	406	2.812	0.953	4.71%	1	1	0.9319	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-P15
AA61JJ-B1	DF	AA61JJ	575	3.991	0.920	0.13%	2	4	0.9458	1861, 865, 403, 187, 87.1, 40.5, 18.8, 8.8	2.15	YES			SLS-P27
AA61JJ-B2	DF	AA61JJ	484	3.358	0.963	0.38%	2	4	0.9533	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES		ppt in 2X C1-C2; oily	SLS-P29
AA61JJ-B3	DF	AA61JJ	344	2.383	0.717	0.17%	2	6	0.9570	2000, 930, 433, 201, 93.6, 43.5, 20.2, 9.4	2.15	YES		ppt in 2X C1; oily	SLS-P30
FRAME															
FAL.NHK.GK.A1.25.03.04	RF	AA61GK	NA	NA	0.666	0.25%	0	0	NA	2000, 200, 20, 2, 0.2, 0.02, 0.002, 0.0002	10	RF	range finder		FAL.NHK.SLS.25.03.03
FAL.NHK.GK.B1.25.04.04	DF	AA61GK	757	5.248	0.874	6.22%	3	5	0.8798	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	YES			FAL.NHK.SLS.25.04.04
FAL.NHK.GK.B2.28.04.04	DF	AA61GK	828	5.742	0.735	2.30%	3	5	0.8571	2500, 1701, 1157, 787,535, 364, 248, 169	1.47	YES		ppt in 2X C1	FAL.NHK.SLS.28.04.03
FAL.NHK.GK.B2.13.05.04 (should be B3)	DF	AA61GK	522	3.623	0.778	1.46%	2	3	0.9880	2500, 1163, 541, 252, 117, 54.4, 25.3, 11.8	2.15	YES			FAL.NHK.SLS.13.05.04
VERAPAMIL HCL															
IIVS															
A1	RF	AA61NH	78.3	0.160	0.566	5.81%	1	0	0.8763	100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001	10	RF	range finder; no points between 50 - 100%	SD chose to use bottom = 0 instead of bottom > 0;	SLS-A4-N040331N

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
B1	DF	AA61NH	67.5	0.137	0.656	5.17%	4	4	0.9864	200, 143, 102, 72.9, 52.1, 37.2, 26.6, 19.0	1.4	YES			SLS-B8-N040819A
B2	DF	AA61NH	71.0	0.144	0.669	0.10%	4	3	0.9788	200, 143, 102, 72.9, 52.1, 37.2, 26.6, 19.0	1.4	YES			SLS-B9-N040820A
B3	DF	AA61NH	60.1	0.122	0.577	7.59%	3	4	0.9794	200, 143, 102, 72.9, 52.1, 37.2, 26.6, 19.0	1.4	YES			SLS-B10-N040903A
ECBC															
AA61LY-A1	RF	AA61LY	64.6	0.131	0.423	5.73%	2	3	0.9492	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder	ppt in 2X C1	SLS-P17
AA61LY-B1	DF	AA61LY	65.3	0.133	0.821	0.23%	4	4	0.9735	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES			SLS-P33
AA61LY-B2	DF	AA61LY	71.0	0.144	0.861	1.55%	4	4	0.9820	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES		ppt in 2X C1	SLS-P35
AA61LY-B3	DF	AA61LY	45.2	0.092	0.455	1.81%	3	4	0.9523	800, 372, 173, 80.5, 37.4, 17.4, 8.1, 3.8	2.15	YES		ppt in 2X C1	SLS-P37
FRAME															
FAL.NHK.MC.A1.28.07.04	RF	AA61MC	81.1	0.165	0.070	23.68%	2	1	0.6033	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; % VC difference > 15		FAL.NHK.SLS.28.07.04
FAL.NHK.MC.B1.20.08.04	DF	AA61MC	73.3	0.149	0.892	3.87%	1	4	0.9216	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.06	2.15	YES		ppt in 2X C1-C3; outliers removed by SD	FAL.NHK.SLS.20.08.04
FAL.NHK.MC.B2.08.10.04	DF	AA61MC	50.0	0.102	0.728	0.31%	3	3	0.9778	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.06	2.15	YES		ppt in 2X C1	FAL.NHK.SLS.08.10.03
FAL.NHK.MC.B3.20.10 .04	DF	AA61MC	115	0.233	1.206	5.67%	1	2	0.9892	1500, 698, 325, 151, 70.2, 32.7, 15.2, 7.06	2.15	YES			FAL.NHK.SLS.20.10.04
XYLENE															
IIVS															
A1	RF	AA61MA	871	8.203	0.746	0.09%	1	0	0.8848	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder		SLS-A3-N040331A
B1	DF	AA61MA	374	3.524	0.700	5.04%	3	2	0.7194	2000, 1429, 1020, 729, 521, 372, 266, 190	1.4	YES		well-to-well variability in 3 lowest doses observed	SLS-B8-N040819A
B2	DF	AA61MA	700	6.592	0.660	6.57%	2	3	0.7739	2000, 1429, 1020, 729, 521, 372, 266, 190	1.4	YES		ppt in 2X C1-C3; variability in 4 highest doses observed; top 2 doses not included in the Hill analysis	SLS-B9-N040820A
B3	DF	AA61MA	385	3.631	0.629	2.40%	2	2	0.8182	2000, 1429, 1020, 729, 521, 372, 266, 190	1.4	YES		ppt in 2X C1-C4; variability in 7 highest doses observed;Top dose not included in Hill analysis (SD decision)	SLS-B10-N040903A
ECBC															
AA61GM-A1	RF	AA61GM	164	1.545	1.075	3.37%	0	5	0.9337	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 - 50%	ppt in 2X C1	SLS-P13
AA61GM-B1	DF	AA61GM	NA	NA	1.106	0.20%	0	8	NA	800, 544, 370, 252, 171, 117, 79.3, 53.9	1.47	NO	no points between 0 - 50%		SLS-P47
AA61GM-B2	DF	AA61GM	NA	NA	0.675	0.96%	0	5	NA	2000, 1361, 926, 630, 428, 291, 198, 135	1.47	NO	no points between 0 - 50%	ppt in 2X C1-C5	SLS-P49
AA61GM-B3	DF	AA61GM	NA	NA	0.699	4.39%	0	4	NA	4000, 3306, 2732, 2258, 1866, 1542, 1275, 1053	1.21	NO	no points between 0 - 50%	ppt in 2X C1-C8; no toxicity detected	SLS-P53
FRAME															
FAL.NHK.JG.A1.14.05.04	RF	AA61JG	NA	NA	0.725	2.43%	0	0	NA	1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001	10	RF	range finder; no points between 0 - 100%		FAL.NHK.SLS.14.05.03
FAL.NHK.JG.B1.08.10.04	DF	AA61JG	NA	NA	0.834	13.03%	0	7	0.3835	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 0 - 50%		FAL.NHK.SLS.08.10.03

NHK NRU Reference Substance Data

Experiment ID NHK Cells	Assay Type[1]	Substance ID	IC_{50} (ug/mL)	IC_{50} (mM)	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability	Notes	PC ID
FAL.NHK.JG.B2.22.10.04	DF	AA61JG	3130	29.444	0.798	7.28%	0	6	0.6066	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 0 - 50%		FAL.NHK.SLS.22.10.04 (NB)
FAL.NHK.JG.B3.28.10.04	DF	AA61JG	NA	NA	0.559	1.04%	0	0	NA	2500, 1701, 1157, 787, 535, 364, 248, 169	1.47	NO	no points between 0 - 100%		FAL.NHK.SLS.28.10.04

Abbreviations: ppt=Precipitate; SD=Study Director; RF=Range Finder; DF=Definitive Test; PC=Positive Control; C1 - C8=Concentration series applied to the the cells. C1 is the highest concentration and C8 is lowest; NA=Not Available; RC=Registry of Cytotoxicity; 2X=Two times the concentration applied to the cells; VC=Vehicle Control; R2=Coefficient of Determination; OD=Optical Density; ID=Identification. Substance ID was the code assigned by the chemical distributor (BioReliance Corp.). Experiment ID and PC ID are test identification numbers assigned by the cytotoxicity testing laboratory.

[1] Range finder or definitive test
[2] Mean OD value for all VC wells in test plate
[3] Difference of right and left VC column of wells in the test plate
[4] % Viability values between 0 and 50% viability; test acceptance criterion. Phase Ib used the range of 10 -50%.
[5] % Viability values between 50 and 100% viability; test acceptance criterion. Phase Ib used the range of 50 - 90%.
[6] Calculated value from the Prism® software
[7] Reference substance concentrations applied to the cells
[8] Step-wise dilution factor used to determine reference substance exposure concentrations
[9] Determination for whether test meets or doesn't meet test acceptance criteria; not applied to RF tests
Shaded boxes identify values that do not meet the specific test acceptance criteria

Appendix I3

3T3 NRU Positive Control (SLS) Data

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3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
ECBC												
Phase Ia												
SLS-B1	45.2	0.157	13-Aug-02	0.187	17.06%	1	1	0.8361	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	VC difference > 15%.
SLS-B2	40.4	0.140	27-Aug-02	0.385	3.88%	3	4	0.7841	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	Inadequate curve fit.
SLS-B3	38.6	0.134	27-Aug-02	0.410	0.04%	1	5	0.8376	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B4	33.3	0.116	28-Aug-02	0.288	15.91%	1	2	0.9378	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	VC difference > 15%.
SLS-B5	26.6	0.092	28-Aug-02	0.233	4.43%	2	4	0.8086	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	Inadequate curve fit.
SLS-B6 (25 ug/ml NR 1 hr)	39.5	0.137	4-Sep-02	0.255	7.59%	1	2	0.9621	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	R&D: 3 replicate ODs/concentration
SLS-B7 (50 ug/ml NR 1 hr)	39.1	0.136	4-Sep-02	0.330	3.18%	1	2	0.9749	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	R&D: 3 replicate ODs/concentration
SLS-B8 (25 ug/ml NR 3 hr)	36.5	0.126	4-Sep-02	0.508	3.64%	1	3	0.9639	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	R&D: 3 replicate ODs/concentration
SLS-B9 (50 ug/ml NR 3 hr)	33.1	0.115	4-Sep-02	0.457	1.39%	1	4	0.9678	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	R&D: 3 replicate ODs/concentration
SLS-B11	42.9	0.149	9-Sep-02	0.349	6.33%	1	2	0.9332	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B12	35.3	0.123	10-Sep-02	0.326	5.41%	1	3	0.9211	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B13	33.0	0.114	10-Sep-02	0.414	6.50%	1	4	0.8802	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B14 (33 ug/ml NR)	37.6	0.130	11-Sep-02	0.347	1.97%	1	3	0.9241	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B15 (33 ug/ml NR)	42.8	0.148	11-Sep-02	0.303	3.16%	1	1	0.8408	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	Inadequate curve fit.
SLS-B16 (33 ug/ml NR)	34.8	0.121	11-Sep-02	0.345	3.43%	1	2	0.9770	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B17 (33 ug/ml NR)	34.3	0.119	11-Sep-02	0.389	17.94%	0	4	0.8377	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	VC difference > 15%. No points between 10 & 50%.
SLS-B18	39.2	0.136	17-Sep-02	0.430	7.88%	1	2	0.9472	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B19	44.7	0.155	17-Sep-02	0.422	13.89%	1	1	0.9389	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B20	34.8	0.121	17-Sep-02	0.445	4.12%	1	3	0.9364	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B21	38.6	0.134	17-Sep-02	0.402	1.66%	1	3	0.8969	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B22	43.5	0.151	18-Sep-02	0.394	2.94%	1	1	0.9271	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-B23	39.7	0.138	18-Sep-02	0.423	1.71%	1	2	0.9253	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
SLS-B24	45.6	0.158	18-Sep-02	0.283	10.48%	0	2	0.8502	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	No points between 10 & 50%.
SLS-B25	44.6	0.155	18-Sep-02	0.311	13.03%	1	0	0.8784	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	No points between 50 & 90%.
Phase Ib												
ECBC-3T3-Ib-01 SLS-P1	34.0	0.118	22-Jan-03	0.300	2.23%	1	3	0.9245	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
ECBC-3T3-Ib-01 SLS-P2	31.3	0.109	22-Jan-03	0.214	2.18%	1	4	0.8744	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
ECBC-3T3-Ib-02 SLS-P3	13.2	0.046	29-Jan-03	0.270	23.27%	2	3	0.8703	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	VC difference > 15%; IC50 out of range
ECBC-3T3-Ib-03 SLS-P4	56.1	0.195	4-Feb-03	0.438	7.34%	1	2	0.8206	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	IC50 out of range
ECBC-3T3-Ib-04 SLS-P5	43.0	0.149	25-Feb-03	0.750	3.31%	1	1	0.9827	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
ECBC-3T3-Ib-05 SLS-P7	40.8	0.141	26-Feb-03	0.443	6.47%	1	1	0.9702	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
ECBC-3T3-Ib-06 SLS-P9	44.9	0.156	4-Mar-03	0.450	3.57%	1	1	0.9403	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
ECBC-3T3-Ib-07 SLS-P12	37.3	0.129	11-Mar-03	0.568	10.54%	1	4	0.9314	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
ECBC-3T3-Ib-08 SLS-P13	47.2	0.164	18-Mar-03	0.517	6.58%	1	1	0.9566	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
Phase II												
SLS-P1	41.4	0.144	17-Jun-03	0.409	4.01%	3	3	0.9561	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P2	36.1	0.125	17-Jun-03	0.452	16.14%	3	4	0.9411	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	% VC difference > 15
SLS-P3	44.5	0.154	24-Jun-03	0.427	8.32%	3	3	0.9434	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P4	39.5	0.137	24-Jun-03	0.460	0.14%	3	4	0.9202	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P5	44.2	0.153	1-Jul-03	0.619	2.60%	3	4	0.9365	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P6	37.8	0.131	1-Jul-03	0.563	3.20%	2	4	0.9361	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P7	42.1	0.146	8-Jul-03	0.485	5.48%	1	5	0.9162	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P8	41.5	0.144	8-Jul-03	0.630	4.97%	2	4	0.9461	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P9	40.3	0.140	15-Jul-03	0.450	6.36%	1	5	0.9250	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P10	35.2	0.122	15-Jul-03	0.629	4.12%	3	3	0.9751	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P11	38.7	0.134	22-Jul-03	0.488	3.70%	2	4	0.9769	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P12	39.1	0.136	22-Jul-03	0.554	1.92%	3	4	0.9760	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P13	41.6	0.144	29-Jul-03	0.700	0.18%	3	4	0.9440	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P14	40.7	0.141	29-Jul-03	0.730	3.11%	3	4	0.9663	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P15	43.2	0.150	5-Aug-03	0.649	0.59%	2	4	0.9591	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P16	44.1	0.153	6-Aug-03	0.276	3.23%	4	4	0.9790	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P17	37.3	0.129	31-Aug-03	0.710	5.38%	2	4	0.9482	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P18 sealer	32.4	0.112	31-Aug-03	0.545	4.39%	3	3	0.8897	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	R&D
SLS-P19	41.4	0.144	1-Sep-03	0.613	2.00%	3	3	0.9625	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P20	38.4	0.133	9-Sep-03	0.350	0.88%	3	4	0.9350	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P21	43.0	0.149	23-Sep-03	0.650	3.04%	2	4	0.9637	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P22	41.2	0.143	29-Oct-03	0.406	1.21%	3	4	0.9289	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P23	41.8	0.145	4-Nov-03	0.378	8.20%	4	4	0.9577	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P24	44.7	0.155	5-Nov-03	0.333	3.43%	4	3	0.9518	80, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
Phase III												
SLS-P1	37.5	0.130	13-Jan-04	0.355	3.82%	3	3	0.8860	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P2	34.9	0.121	13-Jan-04	0.442	8.96%	3	3	0.9641	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P3	40.8	0.142	21-Jan-04	0.461	4.62%	2	3	0.9751	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P4	29.4	0.102	21-Jan-04	0.511	3.62%	2	3	0.9672	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range
SLS-P5	43.7	0.151	27-Jan-04	0.299	2.09%	3	4	0.9766	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P6	42.8	0.148	27-Jan-04	0.384	1.89%	2	3	0.9558	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P7	43.1	0.149	3-Feb-04	0.378	6.60%	4	4	0.9779	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P8	38.5	0.134	3-Feb-04	0.379	7.38%	2	4	0.9662	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P9	38.5	0.134	10-Feb-04	0.375	8.36%	3	4	0.9315	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P10	35.9	0.124	10-Feb-04	0.374	3.25%	3	4	0.9640	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P11	40.5	0.140	24-Feb-04	0.297	2.83%	3	4	0.9554	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P12	37.3	0.129	24-Feb-04	0.334	0.02%	2	3	0.9665	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P13	39.3	0.136	25-Feb-04	0.385	0.30%	3	4	0.9624	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P14	37.9	0.132	25-Feb-04	0.422	5.43%	4	4	0.9561	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P15	44.7	0.155	2-Mar-04	0.526	3.85%	2	5	0.9840	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P16	41.9	0.145	2-Mar-04	0.605	0.29%	2	4	0.9739	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P17	38.9	0.135	3-Mar-04	0.453	7.56%	3	4	0.9496	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P18	35.5	0.123	3-Mar-04	0.522	0.59%	3	3	0.9404	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P19	41.3	0.143	9-Mar-04	0.539	7.29%	3	4	0.9586	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P20	37.7	0.131	9-Mar-04	0.535	0.73%	2	4	0.9731	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P21	42.7	0.148	16-Mar-04	0.563	0.59%	2	3	0.9849	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P22	38.9	0.135	16-Mar-04	0.548	0.03%	3	4	0.9759	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P23	43.4	0.150	23-Mar-04	0.632	3.43%	3	4	0.9714	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P24	42.1	0.146	23-Mar-04	0.707	2.19%	2	4	0.9858	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P25	52.7	0.183	30-Mar-04	0.667	2.75%	2	5	0.9661	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range
SLS-P26	43.0	0.149	30-Mar-04	0.623	0.88%	3	3	0.9556	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P27	45.9	0.159	6-Apr-04	0.521	2.17%	2	4	0.9766	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P28	43.9	0.152	6-Apr-04	0.614	1.41%	3	4	0.9785	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P29	46.3	0.161	13-Apr-04	0.477	4.37%	3	5	0.9579	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P30	43.1	0.149	13-Apr-04	0.609	1.67%	1	5	0.9420	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P31	44.1	0.153	20-Apr-04	0.473	5.99%	1	5	0.9456	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P32	39.4	0.136	20-Apr-04	0.481	2.79%	3	4	0.9762	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P33	44.8	0.155	27-Apr-04	0.434	8.49%	2	4	0.9548	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P34	42.1	0.146	27-Apr-04	0.448	8.96%	3	4	0.9624	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P35	49.3	0.171	4-May-04	0.611	1.23%	3	4	0.9828	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P36	42.4	0.147	4-May-04	0.680	4.09%	2	4	0.9626	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P37	44.8	0.155	11-May-04	0.588	2.31%	2	5	0.9713	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P38	43.2	0.150	11-May-04	0.682	3.69%	3	4	0.9645	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P39	37.8	0.131	18-May-04	0.418	7.64%	3	4	0.9578	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P40	37.0	0.128	18-May-04	0.408	1.70%	2	4	0.9541	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P41	45.0	0.156	25-May-04	0.506	2.77%	2	5	0.9772	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P42	42.1	0.146	25-May-04	0.575	1.65%	2	4	0.9733	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P43	42.8	0.148	15-Jun-04	0.698	6.20%	3	4	0.9689	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P44	42.2	0.146	15-Jun-04	0.695	8.92%	4	4	0.9648	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P45	45.9	0.159	22-Jun-04	0.561	1.81%	3	5	0.9718	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P46	46.1	0.160	22-Jun-04	0.650	1.33%	2	5	0.9772	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P47	40.2	0.139	29-Jun-04	0.421	8.18%	4	4	0.9603	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P48	37.6	0.130	29-Jun-04	0.468	10.36%	3	4	0.9512	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P49	40.2	0.139	13-Jul-04	0.325	12.65%	4	4	0.9524	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P50	NA	NA	20-Jul-04	0.414	4.06%	1	1	NA	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range
SLS-P51	NA	NA	20-Jul-04	0.414	16.20%	1	5	NA	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range; % VC difference > 15;
SLS-P52	NA	NA	27-Jul-04	0.471	14.02%	3	1	NA	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range
SLS-P53	NA	NA	27-Jul-04	0.555	8.43%	5	1	NA	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P54	44.1	0.153	10-Aug-04	0.797	1.55%	3	5	0.9653	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P55	45.1	0.156	10-Aug-04	0.658	5.46%	3	4	0.9570	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P56	NA	NA	17-Aug-04	0.372	34.25%	2	5	NA	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	PC failed; % VC difference > 15
SLS-P57	40.4	0.140	17-Aug-04	0.523	6.59%	4	4	0.9579	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P58	47.1	0.163	24-Aug-04	0.477	4.19%	2	5	0.9215	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P59	40.6	0.141	24-Aug-04	0.462	7.30%	4	4	0.9589	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P60	53.7	0.186	31-Aug-04	0.754	3.56%	2	6	0.8457	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range
SLS-P61	60.1	0.208	31-Aug-04	0.726	3.36%	2	6	0.9203	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	NO	IC50 out of range
SLS-P62	43.4	0.150	14-Sep-04	0.635	5.64%	2	5	0.9006	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P63	41.4	0.144	14-Sep-04	0.625	6.52%	2	5	0.9614	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P64	37.4	0.130	28-Sep-04	0.473	6.10%	3	4	0.9400	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P65	38.8	0.135	28-Sep-04	0.394	4.91%	3	4	0.9681	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P66	37.0	0.128	5-Oct-04	0.520	3.86%	2	4	0.9495	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P67	33.4	0.116	5-Oct-04	0.554	4.23%	3	3	0.9603	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P68	42.7	0.148	19-Oct-04	0.472	0.62%	2	5	0.9632	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P69	43.6	0.151	19-Oct-04	0.349	0.38%	1	5	0.9659	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P70	39.7	0.138	26-Oct-04	0.468	3.33%	3	4	0.9687	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P71	44.9	0.156	27-Oct-04	0.504	3.38%	2	3	0.9416	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P72	45.8	0.159	2-Nov-04	0.517	1.76%	3	5	0.9405	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P73	45.7	0.158	2-Nov-04	0.517	0.08%	2	5	0.9685	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
SLS-P74	46.6	0.161	16-Nov-04	0.510	0.42%	2	5	0.9461	80.0, 66.1, 54.6, 45.2, 37.3, 30.8, 25.5, 21.1	1.21	YES	
FAL												
Phase Ia												
B1(1a/3T3/DF1/FAL/SLS)	53.9	0.187	3-Sep-02	0.402	11.18%	0	1	0.9577	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 10 & 50% viability
B2(1a/3T3/DF2/FAL/SLS)	NA	NA	3-Sep-02	0.419	15.17%	1	1	0.7691	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	Bad values for 6.3 ug/mL wells. VC difference > 15%.
B3(1a/3T3/DF3/FAL/SLS)	50.8	0.176	3-Sep-02	0.420	3.73%	0	1	0.9583	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 10 & 50% viability
B4(1a/3T3/DF4/FAL/SLS)	44.4	0.154	3-Sep-02	0.490	2.60%	1	1	0.9800	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	
B5(1a/3T3/DF5/FAL/SLS)	51.0	0.177	3-Sep-02	0.503	8.01%	0	1	0.9812	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 10 & 50% viability
B6(1a/3T3/DF6/FAL/SLS)	49.8	0.173	3-Sep-02	0.441	6.29%	1	0	0.9517	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 50 & 90% viability.
B7(1a/3T3/DF7/FAL/SLS)	54.2	0.188	4-Sep-02	0.408	5.64%	0	1	0.8134	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 10 & 50% viability
B8(1a/3T3/DF8/FAL/SLS)	50.2	0.174	4-Sep-02	0.337	34.90%	0	1	0.8010	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	VC difference > 15%. No point between 10 & 50% viability
B9(1a/3T3/DF9/FAL/SLS)	52.1	0.181	4-Sep-02	0.484	0.79%	0	1	0.9657	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 10 & 50% viability
B10(1a/3T3/DF10/FAL/SLS)	52.5	0.182	4-Sep-02	0.459	7.20%	0	1	0.9389	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 10 & 50% viability
B11(1a/3T3/DF11/FAL/SLS)	46.4	0.161	4-Sep-02	0.509	6.94%	0	3	0.9422	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	No point between 10 & 50% viability
1a/3T3/DF14/FAL/SLS	23.0	0.080	18-Sep-02	0.900	3.51%	1	3	0.8277	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	NO	Inadequate curve fit.
1a/3T3/DF15/FAL/SLS	46.7	0.162	18-Sep-02	0.547	7.61%	1	0	0.9736	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	NO	No point between 50 & 90% viability

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
1a/3T3/DF16/FAL/SLS	42.4	0.147	18-Sep-02	0.590	21.70%	1	0	0.9833	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	NO	VC difference > 15%. No point between 50 & 90% viability.
1a/3T3/DF17/FAL/SLS	46.6	0.161	18-Sep-02	0.442	4.00%	1	0	0.8646	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	NO	No point between 50 & 90% viability
1a/3T3/DF18/FAL/SLS	22.6	0.078	18-Sep-02	0.920	4.36%	2	3	0.8319	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	NO	Inadequate curve fit.
1a/3T3/DF19/FAL/SLS	23.1	0.080	18-Sep-02	0.936	4.30%	1	3	0.8350	150, 102, 69.4, 47.2, 32.1, 21.9, 14.9, 10.1	1.47	NO	Inadequate curve fit.
1a/3T3/DF28/FAL/SLS	48.0	0.166	22-Oct-02	0.488	9.05%	0	1	0.9570	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	No point between 10 & 50% viability
1a/3T3/DF29/FAL/SLS	50.7	0.176	22-Oct-02	0.579	10.46%	0	3	0.8773	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	No point between 10 & 50% viability
1a/3T3/DF30/FAL/SLS	42.0	0.146	23-Oct-02	0.768	6.31%	1	3	0.9433	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	YES	
1a/3T3/DF31/FAL/SLS	46.8	0.162	23-Oct-02	0.795	2.60%	0	4	0.9321	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	No point between 10 & 50% viability
1a/3T3/DF32/FAL/SLS	49.0	0.170	23-Oct-02	0.784	0.24%	0	1	0.9725	100, 68.0, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	No point between 10 & 50% viability
1a3T3DF33FALSLS	48.9	0.169	30-Oct-02	0.676	2.03%	1	2	0.9532	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF34FALSLS	48.0	0.166	30-Oct-02	0.636	4.77%	1	2	0.9788	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF35FALSLS	48.7	0.169	30-Oct-02	0.684	2.23%	1	2	0.9811	100, 76.9, 59.2, 45.5, 35, 26.9, 20.7, 15.9	1.30	YES	
1a3T3DF36FALSLS	53.0	0.184	30-Oct-02	0.545	4.83%	1	1	0.8486	100, 76.9, 59.2, 45.5, 35, 26.9, 20.7, 15.9	1.30	NO	Inadequate curve fit.
1a3T3DF37FALSLS	50.8	0.176	31-Oct-02	0.660	1.09%	1	3	0.9261	100, 76.9, 59.2, 45.5, 35, 26.9, 20.7, 15.9	1.30	YES	
1a3T3DF38FALSLS+	51.4	0.178	31-Oct-02	0.612	9.54%	1	4	0.9057	100, 76.9, 59.2, 45.5, 35, 26.9, 20.7, 15.9	1.30	YES	
1a3T3DF39FALSLS	51.3	0.178	31-Oct-02	0.630	0.19%	1	2	0.9749	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF40FALSLS	52.5	0.182	31-Oct-02	0.669	6.97%	1	1	0.9879	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF41FALSLS+	47.1	0.163	5-Nov-02	0.581	3.57%	1	3	0.9757	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF42FALSLS	46.8	0.162	5-Nov-02	0.564	11.34%	1	3	0.9468	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF43FALSLS	36.6	0.127	6-Nov-02	0.649	6.40%	1	3	0.8929	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF44FALSLS+	44.8	0.155	6-Nov-02	0.605	1.06%	2	3	0.9258	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF45FALSLS	40.7	0.141	12-Nov-02	0.618	0.88%	1	3	0.9756	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF46FALSLS	42.3	0.147	12-Nov-02	0.665	0.86%	1	3	0.9599	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
1a3T3DF47FALSLS	42.1	0.146	12-Nov-02	0.674	3.71%	1	2	0.9811	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF48FALSLS	37.9	0.131	13-Nov-02	0.531	15.94%	2	3	0.8139	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	NO	VC difference > 15%.
1a3T3DF49FALSLS	38.7	0.134	13-Nov-02	0.561	14.96%	1	3	0.8648	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF50FALSLS	40.6	0.141	13-Nov-02	0.533	11.42%	2	3	0.9179	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF51FALSLS	40.3	0.140	20-Nov-02	0.689	0.29%	1	3	0.9478	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF52FALSLS	42.5	0.147	20-Nov-02	0.780	1.37%	1	3	0.9682	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1a3T3DF53FALSLS	39.9	0.138	20-Nov-02	0.692	7.30%	2	3	0.9403	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
Phase Ib												
1b3T3CRT1FALSLS	34.4	0.119	4-Dec-02	0.618	16.76%	3	2	0.8479	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	NO	VC difference > 15%
1b3T3CTR2FALSLS	48.8	0.169	10-Dec-02	0.545	6.73%	1	2	0.9409	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1b3T3CTRFALSLS	24.5	0.085	17-Dec-02	0.453	1.97%	1	0	0.8653	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	NO	IC50 out of range; no points between 50 & 90% viability
1b3T3CTRFALSLS	43.5	0.151	7-Jan-03	0.597	2.23%	1	2	0.9631	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1b3T3CTRFALSLS	50.9	0.176	8-Jan-03	0.271	14.37%	1	1	0.9136	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	NR crystals in plate; stopped after 1 h
1b3T3CRTFALSLS	43.2	0.150	14-Jan-03	0.625	3.68%	1	3	0.9163	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1b3T3CRT2FALSLS	32.4	0.112	14-Jan-03	0.417	5.55%	1	2	0.9377	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
1b3T3CRTFALSLS	70.1	0.243	15-Jan-03	0.432	2.31%	1	2	0.9000	82.6, 67.7, 56.0, 42.29, 38.25, 31.61, 26.13,21.59	1.21	YES	IC50 out of range
1b3T3CRTFALSLS	35.3	0.122	21-Jan-03	0.651	1.86%	1	2	0.9727	100.00, 82.64, 68.30, 56.45, 46.65, 38.55, 31.86, 26.33	1.21	YES	
1b3T3CRTFALSLS	38.1	0.132	28-Jan-03	0.181	17.95%	1	0	0.9716	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	NO	NR crystals in plate; stopped after 1 h; VC difference > 15%; no point between 50 & 90% viability
1b3T3CRTFALSLS	58.7	0.204	29-Jan-03	0.646	8.07%	0	2	0.9573	100, 68.02, 46.28, 31.48, 21.42, 14.57, 9.91, 6.74		NO	No point between 10 & 50% viability
1b3T3CRTFALSLS	44.3	0.154	4-Feb-03	0.662	0.79%	1	1	0.9848	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
1b3T3CRTFALSLS	36.8	0.128	5-Feb-03	0.566	1.65%	1	1	0.9867	100, 82.645, 68.301, 56.447, 46.651, 38.554, 31.863, 26.333	1.21	YES	
1b3T3CRTFALSLS	48.0	0.166	26-Feb-03	0.310	15.17%	1	2	0.9457	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
Phase II												
A1SLS190603	49.1	0.170	17-Jun-03	1.031	2.49%	2	5	0.7802	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	NO	r2 too low
FAL.3T3.SLS2.A1.200603	54.6	0.189	18-Jun-03	0.684	6.26%	4	3	0.9851	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.A2.26.06.03	50.8	0.176	24-Jun-03	0.483	3.45%	3	4	0.9788	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.A2.27.06.03	50.7	0.176	25-Jun-03	0.564	0.19%	2	2	0.9878	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.B1.03.07.03	57.5	0.199	1-Jul-03	0.516	7.13%	1	4	0.9913	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	IC50 out of range
FAL.3T3.SLS.04.07.03	55.8	0.193	2-Jul-03	0.562	4.86%	4	3	0.9788	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	NO	IC50 out of range
FAL.3T3.SLS.10.07.03	52.5	0.182	8-Jul-03	0.640	0.86%	2	3	0.9794	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.11.07.03	50.6	0.175	9-Jul-03	0.533	2.92%	2	3	0.9869	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.17.07.03	50.2	0.174	15-Jul-03	0.708	0.81%	2	3	0.9905	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.18.07.03	43.2	0.150	16-Jul-03	0.502	5.68%	2	3	0.9763	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.25.07.03	47.6	0.165	23-Jul-03	0.435	5.81%	1	2	0.9633	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.070803	30.5	0.106	5-Aug-03	0.725	0.11%	7	1	0.9204	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	NO	IC50 out of range
FAL.3T3.SLS.080803	36.2	0.126	6-Aug-03	0.463	1.17%	5	3	0.7811	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	NO	low r2
FAL.3T3.SLS.120903	39.4	0.137	10-Sep-03	0.768	4.53%	3	4	0.8322	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.180903	45.2	0.157	16-Sep-03	0.401	0.69%	4	3	0.9582	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.190903	45.0	0.156	17-Sep-03	0.377	0.62%	1	2	0.9790	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.250903	35.7	0.124	23-Sep-03	0.379	4.55%	3	2	0.9738	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.031003	51.2	0.178	1-Oct-03	0.596	5.23%	2	4	0.9344	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.171003	37.5	0.130	15-Oct-03	0.398	9.90%	3	2	0.9763	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.301003	49.8	0.173	28-Oct-03	0.310	12.63%	4	1	0.9702	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
FAL.3T3.SLS.301003 (should be 311003)	39.6	0.137	29-Oct-03	0.313	8.62%	3	3	0.9886	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
Phase III												
FAL.3T3.SLS.080104	55.0	0.191	6-Jan-04	0.615	0.20%	4	4	0.9771	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.09/01/04	53.3	0.185	7-Jan-04	0.592	7.04%	4	4	0.9727	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.15/01/04	67.0	0.232	13-Jan-04	0.841	1.98%	2	6	0.8901	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	NO	IC50 out of range
FAL.3T3.SLS.16/01/04	30.4	0.105	14-Jan-04	1.161	0.39%	6	2	0.8932	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.22/01/04	35.7	0.124	20-Jan-04	0.382	7.11%	3	2	0.9685	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL3T3.23-01-04	30.8	0.107	21-Jan-04	0.792	2.31%	2	2	0.9194	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL3T3.SLS.29-01-04	41.4	0.144	27-Jan-04	0.467	0.43%	5	3	0.9671	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.29/01/04	44.3	0.153	28-Jan-04	0.453	1.44%	4	4	0.9721	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	YES	
FAL.3T3.SLS.5/02/04	26.9	0.093	3-Feb-04	0.417	2.14%	4	0	0.9317	100, 82.6, 68.5, 56.5, 46.7, 38.6, 31.9, 26.4	1.21	NO	recalculated values: IC50 out of range; no points between 50-100
FAL.3T3.SLS.06/02/04	38.8	0.135	4-Feb-04	0.427	4.23%	5	3	0.9136	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL3T3.SLS.25.02.04	47.9	0.166	23-Feb-04	0.637	2.29%	3	4	0.9829	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.17/03/04	49.8	0.173	15-Mar-04	0.356	5.91%	4	3	0.9831	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.01/04/04	44.0	0.152	30-Mar-04	0.404	1.46%	2	2	0.9593	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.29/04/04	42.3	0.147	27-Apr-04	0.310	2.34%	3	5	0.9881	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.30/04/04	31.3	0.108	28-Apr-04	0.249	4.22%	6	1	0.9874	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.06/05/04	40.7	0.141	4-May-04	0.320	9.70%	2	3	0.9897	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.07/05/04	40.2	0.139	5-May-04	0.313	0.03%	3	3	0.9865	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.20/05/04	45.2	0.157	18-May-04	0.422	3.24%	2	3	0.9797	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.21.05.04	32.7	0.114	19-May-04	0.337	0.94%	2	2	0.9720	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.27/05/04	44.2	0.153	25-May-04	0.406	5.89%	3	3	0.9466	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.24.06.04	40.6	0.141	22-Jun-04	0.434	3.69%	4	3	0.9826	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
FAL.3T3.SLS.08.07.04	39.7	0.138	6-Jul-04	0.324	7.16%	2	3	0.9659	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.09.07.04	40.3	0.140	7-Jul-04	0.408	2.92%	2	3	0.9765	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.16.07.04	35.6	0.124	14-Jul-04	0.402	5.43%	2	2	0.9676	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.17.09.04	40.3	0.140	15-Sep-04	0.411	1.89%	3	3	0.9796	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.23.09.04	40.7	0.14126	21-Sep-04	0.333	2.60%	2	3	0.9718	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.14.10.04	42.9	0.14860	12-Oct-04	0.320	5.42%	3	2	0.9901	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	
FAL.3T3.SLS.04.11.04	39.9	0.13836	2-Nov-04	0.259	2.51%	4	3	0.9816	100, 82.6, 68.3, 56.4, 46.7, 38.6, 31.9, 26.3	1.21	YES	

IIVS

Phase Ia

B1	NA	NA	24-Aug-02	0.306	17.18%	1	0	0.5129	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	VC difference > 15%. No points between 50 & 90% viability.
B2	53.7	0.186	24-Aug-02	0.280	38.89%	1	0	0.3966	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	VC difference > 15%. No points between 50 & 90% viability.
B3	34.7	0.120	25-Aug-02	0.452	1.92%	0	1	0.9877	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	No points between 10 & 50% viability.
B4	34.2	0.119	25-Aug-02	0.428	4.07%	0	3	0.9664	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	No points between 10 & 50% viability.
B5	35.9	0.125	26-Aug-02	0.409	3.71%	0	1	0.9872	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	No points between 10 & 50% viability.
B6	39.0	0.135	26-Aug-02	0.382	0.09%	0	0	0.9649	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	No points between 10 & 90% viability.
B7	35.7	0.124	27-Aug-02	0.302	2.98%	0	2	0.9773	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	No points between 10 & 50% viability.
B8	36.1	0.125	27-Aug-02	0.299	6.86%	0	1	0.9792	100, 56.2, 31.6, 17.8, 10, 5.63, 3.17, 1.78	1.78	NO	No points between 10 & 50% viability.
B9	41.5	0.144	29-Aug-02	0.342	6.02%	1	1	0.9831	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B10	45.1	0.156	29-Aug-02	0.358	1.51%	1	1	0.9664	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B11	43.8	0.152	30-Aug-02	0.366	4.26%	1	0	0.9936	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	NO	No points between 50 & 90% viability.
B12	44.6	0.155	30-Aug-02	0.359	0.95%	1	1	0.9864	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B13	44.5	0.154	4-Sep-02	0.538	0.37%	1	1	0.9799	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
B14	43.9	0.152	4-Sep-02	0.491	6.43%	1	1	0.9869	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B15	37.8	0.131	5-Sep-02	0.357	9.90%	1	1	0.9906	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B16	40.4	0.140	5-Sep-02	0.336	10.55%	1	1	0.9832	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B17	39.7	0.138	6-Sep-02	0.464	2.31%	1	2	0.9780	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B18	38.1	0.132	6-Sep-02	0.426	11.25%	1	1	0.9910	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B19	36.7	0.127	7-Sep-02	0.378	4.90%	1	1	0.9928	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B20	36.5	0.127	7-Sep-02	0.354	12.49%	1	1	0.9954	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
B21	46.7	0.162	8-Sep-02	0.453	0.44%	0	2	0.9800	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	NO	No points between 10 & 50% viability.
B22	41.8	0.145	8-Sep-02	0.439	0.63%	1	1	0.9802	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10, 6.8	1.47	YES	
Phase Ib												
A1 Preliminary	41.1	0.143	15-Jan-03	0.389	8.42%	1	1	0.9890	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10.0, 6.81	1.47	YES	
B1	43.5	0.151	22-Jan-03	0.569	6.41%	1	1	0.9822	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10.0, 6.81	1.47	YES	
B2	44.8	0.155	29-Jan-03	0.514	2.88%	1	1	0.9830	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10.0, 6.81	1.47	YES	
B3	38.5	0.133	5-Feb-03	0.519	1.00%	1	1	0.9854	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10.0, 6.81	1.47	YES	
B4	49.4	0.171	12-Feb-03	0.548	10.23%	0	2	0.9770	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10.0, 6.81	1.47	NO	No points between 10 and 50%; IC50 out of range
B5	41.9	0.145	26-Feb-03	0.507	5.41%	1	1	0.9747	100, 68.1, 46.4, 31.6, 21.5, 14.7, 10.0, 6.81	1.47	YES	
Phase II												
A1	41.3	0.143	23-Jul-03	0.546	3.97%	1	3	0.9902	100, 66.7, 44.4, 29.6, 19.8, 13.2, 8.78, 5.85	1.50	YES	
B1	39.6	0.137	28-Jul-03	0.375	1.11%	1	5	0.9559	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	YES	
B2	38.8	0.135	29-Jul-03	0.529	5.36%	2	5	0.9711	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	YES	
B3	30.0	0.104	30-Jul-03	0.527	1.74%	1	4	0.9854	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	NO	IC50 out of range
B4	42.6	0.148	13-Aug-03	0.483	7.35%	1	5	0.9891	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	YES	
B5	39.1	0.136	16-Sep-03	0.510	6.44%	3	5	0.9568	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	YES	
B6	38.2	0.132	23-Sep-03	0.433	2.75%	1	5	0.9668	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
B7	38.9	0.135	24-Sep-03	0.479	2.49%	1	5	0.9710	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	YES	
B8	45.2	0.157	1-Oct-03	0.547	3.52%	1	5	0.9798	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.5	1.40	YES	
Phase III												
A1	42.1	0.146	3-Feb-04	0.429	3.86%	2	5	0.9691	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	
A2	42.4	0.147	10-Feb-04	0.494	0.10%	2	4	0.9874	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	
A3	41.0	0.142	17-Feb-04	0.458	1.06%	1	4	0.9858	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
A4	37.2	0.129	9-Mar-04	0.417	7.26%	1	4	0.9893	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
A5	33.0	0.114	23-Mar-04	0.346	1.01%	2	3	0.9758	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B1	45.9	0.159	26-Jul-04	0.399	0.81%	1	5	0.9709	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	
B2	44.5	0.154	27-Jul-04	0.379	5.70%	3	4	0.9828	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	
B3	40.1	0.139	28-Jul-04	0.344	14.50%	2	5	0.9364	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	
B4	42.2	0.146	23-Aug-04	0.493	3.37%	1	3	0.9874	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	
B5	47.2	0.164	24-Aug-04	0.485	7.64%	2	2	0.9864	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B6	46.1	0.160	28-Sep-04	0.462	1.12%	1	4	0.9824	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B7	40.7	0.141	1-Oct-04	0.372	10.21%	1	5	0.9808	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B8	41.2	0.143	4-Oct-04	0.427	0.90%	1	4	0.9826	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B9	43.4	0.150	12-Oct-04	0.413	4.72%	1	5	0.9758	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B10	43.7	0.151	13-Oct-04	0.465	2.54%	2	5	0.9833	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	
B11	42.3	0.147	2-Nov-04	0.398	4.84%	1	3	0.9920	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B12	32.5	0.113	9-Nov-04	0.355	1.15%	1	3	0.9888	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B13	41.6	0.144	10-Nov-04	0.362	5.53%	1	4	0.9831	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	

3T3 NRU Positive Control (SLS) Data

Experiment ID[1] 3T3 Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
B14	21.4	0.074	16-Nov-04	0.445	4.98%	3	3	0.9568	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	NO	IC50 out of range
B15	43.5	0.151	8-Dec-04	0.442	2.26%	1	3	0.9932	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B16	37.2	0.129	14-Dec-04	0.436	5.18%	1	5	0.9757	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B17	43.2	0.150	15-Dec-04	0.373	3.10%	1	3	0.9869	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49	1.40	YES	
B18	41.0	0.142	19-Jan-05	0.385	1.43%	1	3	0.9739	100, 71.4, 51.0, 36.4, 26.0, 18.6, 13.3, 9.49,	1.40	YES	

Abbreviations: NR=Neutral red; R&D=Research and development; PC=Positive control; C1 - C8=Concentration series applied to the the cells. C1 is the highest concentration and C8 is lowest; NA=Not Available; RC=Registry of Cytotoxicity;
[1] PC test ID
[2] Mean OD value for all VC wells in test plate
[3] Difference of right and left VC column of wells in the test plate
[4] % Viability values between 0 and 50% viability; test acceptance criterion; Phases Ia and Ib = number of points between 10 - 50%
[5] % Viability values between 50 and 100% viability; test acceptance criterion; Phases Ia and Ib = number of points between 50 - 90%
[6] Calculated value from the Prism® software
[7] Reference substance concentrations applied to the cells
[8] Step-wise dilution factor
[9] Determination whether test meets or doesn't meet test acceptance criteria
Shaded boxes identify values that do not meet the specific test acceptance criteria

Acceptance Limits for PC IC_{50}

Phase	ECBC (ug/mL)	FAL (ug/mL)	IIVS (ug/mL)
Ib (3T3)	28.8 – 47.7	25.2 – 59.5	34.5 – 47.3
II (3T3)	26.4 – 56.3	31.5 – 54.9	33.6 – 50.6
III (3T3)	30.8 – 51.6	27.2 – 64.7	31.8 – 49.3

Appendix I4

NHK NRU Positive Control (SLS) Data

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NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
ECBC												
Phase Ia												
SLS-B1	5.47	0.019	12-Aug-02	0.559	13.30%	1	0	0.9772	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	26% highest viability. No points between 50 & 90% viability.
SLS-B2	5.92	0.021	12-Aug-02	0.782	3.07%	1	0	0.9717	100, 68, 46.3, 31.5, 21.4, 14.6, 9.9, 6.7	1.47	NO	32% highest viability. No points between 50 & 90% viability.
SLS-B3	3.40	0.012	12-Sep-02	0.285	21.73%	3	0	0.8182	50, 34, 23.2, 15.8, 10.7, 7.3, 5, 3.4	1.47	NO	VC difference > 15%. No points between 50 & 90% viability.
SLS-B4	3.91	0.014	12-Sep-02	0.369	3.41%	3	0	0.8615	50, 34, 23.2, 15.8, 10.7, 7.3, 5, 3.4	1.47	NO	No points between 50 & 90% viability.
SLS-B5	7.02	0.024	9-Sep-02	2.277	5.94%	1	4	0.9229	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B6	4.77	0.017	9-Sep-02	1.898	5.47%	2	4	0.8750	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B7)	4.90	0.017	9-Sep-02	2.301	2.51%	2	3	0.9331	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B8	5.61	0.019	9-Sep-02	2.312	4.42%	2	4	0.9273	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	NO	< 8 of 12 vehicle control replicates.
SLS-B9	6.65	0.023	10-Sep-02	1.181	6.10%	1	5	0.8680	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B10	3.71	0.013	10-Sep-02	1.007	7.50%	4	2	0.9338	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B11	3.84	0.013	9-Sep-02	1.531	11.76%	3	3	0.9413	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B12 (no re-feed)	4.10	0.014	16-Sep-02	0.763	7.92%	2	3	0.9683	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B13 (re-feed)	2.78	0.010	16-Sep-02	0.404	10.90%	3	2	0.9131	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B14 (no re-feed)	2.82	0.010	16-Sep-02	0.924	0.12%	3	2	0.9583	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B15 (re-feed)+	3.42	0.012	16-Sep-02	0.271	2.12%	3	2	0.8829	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B16 (no re-feed)	2.71	0.009	23-Sep-02	0.313	9.38%	2	2	0.9026	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B17 (re-feed)+	3.13	0.011	23-Sep-02	0.078	14.92%	2	2	0.7987	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	NO	Inadequate curve fit.
SLS-B18 (no re-feed)	3.19	0.011	23-Sep-02	0.258	19.12%	3	2	0.8196	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	NO	VC difference > 15%.
SLS-B19 (re-feed)	3.19	0.011	23-Sep-02	0.079	4.56%	2	3	0.6930	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	NO	Inadequate curve fit.
SLS-B20	3.48	0.012	9-Oct-02	0.892	1.31%	2	3	0.9455	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	
SLS-B21	3.17	0.011	9-Oct-02	0.863	0.47%	3	2	0.9539	23.2, 15.8, 10.7, 7.3, 5, 3.4, 2.3, 1.6	1.47	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
Phase Ib												
ECBC-NHK-Ib-01 SLS-P2	3.98	0.014	23-Jan-03	0.861	0.42%	1	4	0.9559	20, 13.6, 9.25, 6.28, 4.27, 2.90, 1.97, 1.34	1.47	YES	
ECBC-NHK-Ib-01 SLS-P1	4.57	0.016	23-Jan-03	0.788	2.50%	2	4	0.9326	20, 13.6, 9.24, 6.28, 4.26, 2.90, 1.97, 1.34	1.47	YES	
ECBC-NHK-Ib-02 SLS-P3	2.20	0.008	28-Jan-03	1.023	6.41%	2	2	0.9391	20, 13.6, 9.24, 6.28, 4.26, 2.90, 1.97, 1.34	1.47	YES	
ECBC-NHK-Ib-03 SLS-P4	3.16	0.011	3-Feb-03	1.135	1.67%	2	3	0.9623	20, 13.6, 9.24, 6.28, 4.26, 2.90, 1.97, 1.34	1.47	YES	
ECBC-NHK-Ib-04 SLS-P5	3.76	0.013	10-Feb-03	1.267	0.53%	2	2	0.9559	20, 13.6, 9.24, 6.28, 4.26, 2.90, 1.97, 1.34	1.47	YES	
ECBC-NHK-Ib-05 SLS-P7	3.75	0.013	24-Feb-03	1.154	1.28%	2	3	0.9757	20, 13.6, 9.24, 6.28, 4.26, 2.90, 1.97, 1.34	1.47	YES	
ECBC-NHK-Ib-05 SLS-P6	3.92	0.014	24-Feb-03	1.135	4.94%	1	4	0.9316	20, 13.6, 9.24, 6.28, 4.26, 2.90, 1.97, 1.34	1.47	YES	
ECBC-NHK-Ib-06 SLS-P8	3.05	0.011	17-Mar-03	0.964	7.32%	2	3	0.9603	20, 13.6, 9.24, 6.28, 4.26, 2.90, 1.97, 1.34	1.47	YES	
Phase II												
SLS-P1	2.78	0.010	16-Jun-03	0.610	5.82%	4	2	0.9491	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P2	2.76	0.010	16-Jun-03	0.671	11.64%	6	2	0.9346	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P3	2.38	0.008	23-Jun-03	0.583	2.99%	6	2	0.9074	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P4	2.46	0.009	23-Jun-03	0.607	0.81%	3	2	0.9167	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P5	1.96	0.007	30-Jun-03	0.380	4.50%	7	1	0.8647	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P7	2.38	0.008	7-Jul-03	1.023	4.31%	6	2	0.8829	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P8	2.34	0.008	7-Jul-03	0.967	1.28%	6	2	0.9475	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P9	2.76	0.010	14-Jul-03	1.054	5.19%	6	2	0.8590	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P10	2.53	0.009	14-Jul-03	0.950	3.83%	6	2	0.9316	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P11	6.64	0.023	21-Jul-03	0.823	4.52%	3	4	0.9677	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	NO	IC50 out of range
SLS-P12	5.75	0.020	21-Jul-03	0.748	1.27%	3	5	0.9376	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P13	7.88	0.027	28-Jul-03	0.088	4.75%	3	1	0.7990	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	NO	IC50 out of range
SLS-P15	3.00	0.010	25-Aug-03	0.139	7.92%	4	3	0.8397	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P16	3.55	0.012	31-Aug-03	0.660	0.75%	4	4	0.8686	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P17 sealer	3.64	0.013	31-Aug-03	0.642	4.51%	4	4	0.9055	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	NO	R&D experiment
SLS-P18	3.50	0.012	1-Sep-03	0.471	7.27%	4	3	0.9184	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P19	2.68	0.009	2-Sep-03	0.761	0.66%	6	2	0.9106	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
SLS-P20	3.14	0.011	2-Sep-03	0.761	6.29%	4	4	0.8461	20.0, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.3	1.47	YES	
Phase III												
SLS-P1	2.71	0.009	14-Jan-04	0.602	1.54%	6	2	0.9562	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P2	2.41	0.008	14-Jan-04	0.593	2.01%	5	2	0.9500	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P3	2.75	0.010	4-Feb-04	0.514	2.25%	5	3	0.9521	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P4	3.48	0.012	4-Feb-04	0.545	2.19%	5	3	0.9372	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P5	2.87	0.010	9-Feb-04	0.400	20.23%	6	2	0.9787	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	NO	% VC difference >15
SLS-P6	2.95	0.010	9-Feb-04	0.582	1.37%	5	3	0.9743	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P7	4.26	0.015	22-Mar-04	1.064	1.54%	4	4	0.9309	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P8	4.65	0.016	22-Mar-04	1.026	2.48%	4	4	0.9055	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P9	5.62	0.019	29-Mar-04	1.172	6.87%	3	5	0.9149	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P10	5.19	0.018	29-Mar-04	1.211	2.79%	3	5	0.8495	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P11	3.27	0.011	5-Apr-04	0.760	3.46%	5	3	0.9345	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P12	3.07	0.011	12-Apr-04	0.781	2.78%	5	3	0.9583	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P13	2.64	0.009	12-Apr-04	0.847	1.72%	6	2	0.9227	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P14	3.09	0.011	19-Apr-04	0.911	3.10%	5	3	0.9541	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P15	2.39	0.008	19-Apr-04	0.840	2.00%	5	2	0.9495	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P16	2.57	0.009	26-Apr-04	0.594	0.48%	6	2	0.9722	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P17	2.59	0.009	26-Apr-04	0.507	1.33%	6	2	0.9605	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P18	2.36	0.008	3-May-04	0.667	2.30%	4	3	0.9382	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P19	3.28	0.011	3-May-04	0.786	0.06%	5	3	0.9557	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P20	2.10	0.007	10-May-04	0.684	2.79%	6	2	0.9517	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P21	2.71	0.009	10-May-04	0.591	0.47%	5	2	0.9609	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P22	3.62	0.013	24-May-04	0.967	0.75%	4	4	0.9317	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P23	3.57	0.012	24-May-04	0.944	1.32%	4	4	0.9164	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P24	1.78	0.006	14-Jun-04	0.623	4.06%	6	1	0.9431	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P25	2.37	0.008	14-Jun-04	0.523	5.18%	6	2	0.9303	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P26	3.46	0.012	21-Jun-04	0.901	0.40%	4	4	0.8960	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P27	3.41	0.012	21-Jun-04	1.021	0.50%	4	4	0.9365	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P28	2.45	0.008	28-Jun-04	0.946	1.45%	6	2	0.9476	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P29	2.34	0.008	28-Jun-04	0.918	3.97%	6	2	0.9517	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P30	2.65	0.009	6-Jul-04	0.784	0.62%	5	3	0.9483	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P31	2.85	0.010	6-Jul-04	0.673	0.82%	4	3	0.9655	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P32	2.53	0.009	12-Jul-04	0.626	2.25%	6	2	0.9348	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P33	2.28	0.008	12-Jul-04	0.756	2.45%	6	2	0.9521	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P34	2.58	0.009	19-Jul-04	0.759	0.59%	5	2	0.9536	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P35	2.71	0.009	19-Jul-04	0.781	1.21%	5	3	0.9599	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P36	2.72	0.009	26-Jul-04	0.373	0.31%	4	3	0.9411	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P37	2.50	0.009	26-Jul-04	0.427	1.21%	6	2	0.9482	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P38	3.26	0.011	2-Aug-04	0.628	12.01%	3	4	0.8904	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P39	2.59	0.009	2-Aug-04	0.839	3.43%	5	3	0.9302	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P40	2.74	0.010	9-Aug-04	0.632	3.96%	5	3	0.9279	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P41	2.90	0.010	9-Aug-04	0.663	2.35%	5	3	0.9480	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P42	2.94	0.010	16-Aug-04	0.697	0.23%	5	2	0.9599	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P43	3.04	0.011	16-Aug-04	0.751	0.50%	5	3	0.9240	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P44	2.46	0.009	23-Aug-04	0.908	2.01%	6	2	0.9487	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P45	3.38	0.012	23-Aug-04	0.926	1.47%	5	3	0.9464	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P46	4.04	0.014	30-Aug-04	0.936	2.46%	4	4	0.9318	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P47	4.58	0.016	30-Aug-04	0.943	1.02%	4	4	0.8656	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P48	2.64	0.009	7-Sep-04	0.721	6.39%	5	3	0.9543	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P49	1.99	0.007	7-Sep-04	0.641	0.69%	4	2	0.9585	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
SLS-P50	2.99	0.010	13-Sep-04	1.123	3.25%	5	3	0.8908	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P51	3.72	0.013	13-Sep-04	1.042	0.19%	4	4	0.9217	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P52	2.70	0.009	27-Sep-04	0.529	1.54%	6	2	0.9508	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P53	2.76	0.010	27-Sep-04	0.604	1.75%	4	2	0.9270	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P54	3.45	0.012	4-Oct-04	0.745	0.79%	4	4	0.9265	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P55	3.12	0.011	4-Oct-04	0.639	5.10%	3	3	0.9318	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P56	3.77	0.013	18-Oct-04	0.826	1.61%	5	3	0.9471	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P57	3.02	0.010	25-Oct-04	0.612	1.55%	4	3	0.9690	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
SLS-P58	2.83	0.010	26-Oct-04	0.155	8.34%	3	3	0.9318	20.0, 13.61, 9.26, 6.30, 4.28, 2.91, 1.98, 1.35	1.47	YES	
FAL												
Phase Ia												
B1(1a/NHK/DF4/FAL/SLS)	8.13	0.028	9-Sep-02	1.333	6.67%	1	2	0.9823	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B2(1a/NHK/DF5/FAL/SLS)	7.63	0.026	9-Sep-02	1.294	6.43%	1	2	0.9889	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B3(1a/NHK/DF6/FAL/SLS)+	8.06	0.028	9-Sep-02	1.289	6.39%	1	2	0.9839	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B4(1a/NHK/DF7/FAL/SLS)	4.62	0.016	9-Sep-02	1.169	13.44%	1	1	0.9683	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B5(1a/NHK/DF8/FAL/SLS)	5.23	0.018	9-Sep-02	1.089	9.96%	1	1	0.9645	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B6(1a/NHK/DF12/FAL/SLS)	5.19	0.018	9-Sep-02	1.184	9.32%	1	1	0.9253	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B7(1a/NHK/DF14/FAL/SLS)	6.72	0.023	11-Sep-02	0.333	0.73%	2	2	0.8307	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	NO	Inadequate curve fit.
B8(1a/NHK/DF15/FAL/SLS)	7.79	0.027	11-Sep-02	1.000	11.26%	1	1	0.9666	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B9(1a/NHK/DF16/FAL/SLS)	7.63	0.026	11-Sep-02	1.076	8.62%	1	2	0.9339	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
B10(1a/NHK/DF17/FAL/SLS)+	5.30	0.018	11-Sep-02	1.698	7.44%	1	1	0.9810	400, 200, 100, 50, 25, 12.5, 6.3, 3.1	2.00	YES	
1 (no re-feed)	7.70	0.027	23-Sep-02	1.534	4.79%	1	5	0.9328	20, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.4	1.47	NO	405 nm OD subtracted from 540 nm OD
3 (no re-feed)	8.66	0.030	23-Sep-02	1.559	0.38%	1	5	0.9202	20, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.4	1.47	NO	405 nm OD subtracted from 540 nm OD
2 (re-feed)	6.84	0.024	23-Sep-02	1.485	1.38%	1	3	0.9695	20, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.4	1.47	NO	405 nm OD subtracted from 540 nm OD

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
4 (re-feed)	5.60	0.019	23-Sep-02	1.301	14.78%	1	4	0.8851	20, 13.6, 9.3, 6.3, 4.3, 2.9, 2.0, 1.4	1.47	NO	405 nm OD subtracted from 540 nm OD
5 (no re-feed)	8.26	0.029	25-Sep-02	1.122	9.11%	2	2	0.8930	25, 17, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	NO	405 nm OD subtracted from 540 nm OD
6 (no re-feed)	11.75	0.041	25-Sep-02	0.633	16.43%	2	4	0.6280	25, 17, 11.6, 7.87, 5.35, 3.64, 2.48, 1.69	1.47	NO	405 nm OD subtracted from 540 nm OD. VC difference > 15%.
1a/NHK/DF23/FAL/SLS	3.33	0.012	22-Oct-02	0.246	8.25%	2	0	0.9216	50, 34, 23, 15.7, 10.7, 7.3, 4.9, 3.4	1.47	NO	No point between 50 & 90% viability
1a/NHK/DF24/FAL/SLS	4.63	0.016	23-Oct-02	0.493	3.46%	2	1	0.9721	50, 34, 23, 15.7, 10.7, 7.3, 4.9, 3.4	1.47	YES	
1a/NHK/DF25/FAL/SLS	3.22	0.011	23-Oct-02	0.393	41.08%	3	0	0.8731	50, 34, 23, 15.7, 10.7, 7.3, 4.9, 3.4	1.47	NO	VC difference > 15%. No point between 50 & 90% viability.
1a/NHK/DF26/FAL/SLS	4.45	0.015	23-Oct-02	0.505	20.88%	2	1	0.9385	50, 34, 23, 15.7, 10.7, 7.3, 4.9, 3.4	1.47	NO	VC difference > 15%.
1a/NHK/DF27/FAL/SLS	4.41	0.015	23-Oct-02	0.484	7.93%	2	1	0.9076	50, 34, 23, 15.7, 10.7, 7.3, 4.9, 3.4	1.47	YES	
1a/NHK/DF28/FAL/SLS	6.66	0.023	24-Oct-02	0.693	1.54%	1	2	0.8672	50, 34, 23, 15.7, 10.7, 7.3, 4.9, 3.4	1.47	YES	
1a/NHK/DF29/FAL/SLS	5.57	0.019	24-Oct-02	0.545	9.79%	1	1	0.9244	50, 34, 23, 15.7, 10.7, 7.3, 4.9, 3.4	1.47	YES	
1a/NHK/DF30/FAL/SLS	14.43	0.050	19-Nov-02	1.094	2.67%	1	6	0.6304	30.0, 23.08, 17.75, 13.65, 10.50, 8.08, 6.22, 4.78	1.30	NO	Inadequate curve fit.
1a/NHK/DF31/FAL/SLS+	13.38	0.046	19-Nov-02	1.354	3.71%	2	6	0.6670	30.0, 23.08, 17.75, 13.65, 10.50, 8.08, 6.22, 4.78	1.30	NO	Inadequate curve fit.
1a/NHK/DF32/FAL/SLS	13.37	0.046	19-Nov-02	0.890	3.18%	2	5	0.6136	30.0, 23.08, 17.75, 13.65, 10.50, 8.08, 6.22, 4.78	1.30	NO	Inadequate curve fit.
1a/NHK/DF33/FAL/SLS+	11.89	0.041	19-Nov-02	0.766	7.34%	3	3	0.8476	30.0, 23.08, 17.75, 13.65, 10.50, 8.08, 6.22, 4.78	1.30	YES	
Phase Ib												
A1 1b/NHKCTR1/FAL/SLS	3.74	0.013	11-Dec-02	0.164	7.05%	1	1	0.9725	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
A2 1b/NHKCTR2/FAL/SLS	6.46	0.022	13-Dec-02	0.743	9.94%	1	5	0.8017	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
A3 1b/NHK/CTR4/FAL / recalculated w/o outlier	4.88	0.017	14-Jan-03	0.086	3.20%	2	4	0.7526	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	NO	$R^2 < 0.8$

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
A4 1b/NHK/CTR5/FAL	3.12	0.011	15-Jan-03	0.146	3.42%	2	1	0.8444	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
A5 1b/NHK/CTR6/FAL	NC	#VALUE!	17-Jan-03	0.003	286.96%	1	0	NC	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	NO	VC difference > 15%; no point between 50 & 90%; no R^2 or ICx
A6 1b/NHK/CTR7/FAL	7.80	0.027	27-Jan-03	1.210	2.15%	2	2	0.9626	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
A8 1b/NHK/CTR9/FAL	5.48	0.019	3-Feb-03	0.935	12.58%	1	4	0.9362	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
A9 1b/NHK/CTR10/FAL	4.12	0.014	4-Feb-03	0.648	23.68%	2	4	0.7160	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	NO	VC difference > 15%; R^2 < 0.8
A10 1b/NHK/CTR11/FAL	3.92	0.014	19-Mar-03	1.068	6.94%	2	3	0.8868	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
A11 1b/NHK/CTR12/FAL	5.08	0.018	20-Mar-03	1.542	0.79%	3	3	0.8792	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
A12 1b/NHK/CTR13/FAL/SLS	3.14	0.011	23-Mar-03	0.403	13.53%	3	1	0.8720	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
1b/NHK/CTR14/FAL/SLS	3.32	0.012	24-Mar-03	0.831	3.67%	1	2	0.9652	15, 11.54, 8.88, 6.83, 5.25, 4.04, 3.11, 2.39	1.30	YES	
1b/NHK/CTR15/FAL/SLS	2.91	0.010	2-May-03	0.973	0.92%	2	2	0.9586	15, 10.2, 6.94, 4.72, 3.21, 2.19, 1.49, 1.01	1.47	YES	
1b/NHK/DF1/FAL/SLS	4.52	0.016	2-May-03	0.843	5.43%	2	2	0.9229	10, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
Phase II												
FAL.NHK.SLS.30.07.03	3.10	0.011	7-Jul-03	1.114	4.61%	3	4	0.9350	12.0, 8.2, 5.6, 3.2, 2.6, 1.8, 1.2, 0.8	1.47	YES	
FAL.NHK.SLS.010803	1.34	0.005	30-Jul-03	0.609	2.17%	3	2	0.9358	12.0, 8.2, 5.6, 3.2, 2.6, 1.8, 1.2, 0.8	1.47	YES	
FAL.NHK.SLS.07.08.03	1.40	0.005	5-Aug-03	0.526	4.20%	4	2	0.9077	12.0, 8.2, 5.6, 3.2, 2.6, 1.8, 1.2, 0.8	1.47	YES	
FAL.NHK.SLS.08.08.03	1.74	0.006	6-Aug-03	0.810	2.34%	4	3	0.9517	12.0, 8.2, 5.6, 3.2, 2.6, 1.8, 1.2, 0.8	1.47	YES	
FAL.NHK.SLS.13.08.03	2.75	0.010	11-Aug-03	0.639	0.03%	4	4	0.3154	10, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	low r2
FAL.NHK.SLS.15.08.03	3.56	0.012	13-Aug-03	0.462	6.70%	3	5	0.8954	10, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.230803	3.03	0.011	21-Aug-03	0.401	0.35%	4	2	0.7230	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	NO	low r2
FAL.NHK.SLS.280803	3.45	0.012	26-Aug-03	0.454	2.31%	2	3	0.9372	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
FAL.NHK.SLS.050903	3.20	0.011	3-Sep-03	0.110	8.54%	2	3	0.9158	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.01.10.03	4.59	0.016	29-Sep-03	1.292	1.62%	2	6	0.9168	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.01.10.03	5.50	0.019	29-Sep-03	0.895	20.89%	2	5	0.9276	10, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	% VC difference >15
FAL.NHK.SLS.15.10.03	2.90	0.010	13-Oct-03	0.547	4.65%	3	5	0.8927	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.19.10.03	3.85	0.013	17-Oct-03	0.340	2.89%	3	5	0.9637	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.23.10.03	4.90	0.017	21-Oct-03	0.279	8.61%	3	2	0.7996	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.24.10.03	2.96	0.010	22-Oct-03	0.932	1.31%	3	5	0.9119	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.05.11.03	3.69	0.013	3-Nov-03	0.515	1.10%	3	5	0.8516	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.07.11.03	3.95	0.014	5-Nov-03	0.351	4.18%	3	3	0.9316	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
FAL.NHK.SLS.28.11.03	3.46	0.012	26-Nov-03	0.174	6.01%	3	5	0.9543	10, 6.8, 4.6, 3.14, 2.14, 1.5, 0.9, 0.68	1.47	YES	
Phase III												
FAL.NHK.SLS.11.02.04	5.28	0.018	9-Feb-04	1.131	1.33%	2	6	0.9062	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	bottom not set to 0
FAL.NHK.SLS.11.02.04	4.83	0.017	9-Feb-04	1.131	1.33%	2	6	0.8318	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	r2 < 0.85
FAL.NHK.SLS.13.02.03	3.63	0.013	11-Feb-04	0.106	6.36%	4	4	0.7409	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	r2 < 0.85
FAL.NHK.SLS.18.02.04	6.22	0.022	16-Feb-04	0.155	6.02%	2	2	0.4330	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	r2 < 0.85; IC50 out of range
FAL.NHK.SLS.20.02.03	2.24	0.008	18-Feb-04	0.254	1.35%	4	4	0.9233	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS/NB.26.02.03	3.25	0.011	24-Feb-04	0.292	4.37%	4	4	0.9347	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS/MO.26.02.03	4.04	0.014	24-Feb-04	0.280	4.67%	3	3	0.9265	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.27.02.03	2.78	0.010	25-Feb-04	0.472	3.50%	3	5	0.9173	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.18.03.03	4.48	0.016	16-Mar-04	0.424	2.34%	3	5	0.8934	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.19.03.03	2.76	0.010	17-Mar-04	0.555	1.67%	3	5	0.8882	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.25.03.03	2.93	0.010	23-Mar-04	0.584	8.67%	4	4	0.9493	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.26.03.04	3.96	0.014	24-Mar-04	0.593	3.86%	3	5	0.9244	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.28.04.03	3.06	0.011	26-Apr-04	0.762	0.95%	3	5	0.9561	10.0, 6.8, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
FAL.NHK.SLS.13.05.04	2.79	0.010	11-May-04	0.612	0.80%	4	4	0.9782	10, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.14.05.03	3.80	0.013	12-May-04	0.594	7.47%	3	3	0.9301	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.25.0	2.62	0.009	23-Jun-04	1.347	0.43%	4	4	0.8730	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.28.07.04	NA	NA	26-Jul-04	0.073	22.93%	2	5	0.7622	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	% VC differnece > 15; r2 too low
FAL.NHK.SLS.11.08.04	3.77	0.013	9-Aug-04	0.512	4.88%	3	5	0.8470	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.12.08.04	5.86	0.020	10-Aug-04	0.701	8.17%	2	1	0.9776	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	IC50 out of range
FAL.NHK.SLS-RB.19.08.04	4.49	0.016	17-Aug-04	0.337	0.10%	3	1	0.7397	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	r2 too low
FAL.NHK.SLS-NB.19.08.04	1.85	0.006	17-Aug-04	0.537	10.04%	3	4	0.8589	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.20.08.04	3.70	0.013	18-Aug-04	0.738	8.90%	3	5	0.9750	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.25.08.04	3.56	0.012	23-Aug-04	0.991	2.23%	2	6	0.8697	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS-RB.20.08.04 (should be 25.08.04)	5.20	0.018	23-Aug-04	0.645	2.80%	2	1	0.8472	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.27.08.04	3.00	0.010	23-Aug-04	0.546	7.84%	3	5	0.8783	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.17.09.04	3.30	0.011	15-Sep-04	0.803	1.34%	3	5	0.9408	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.30.09.***03***	2.78	0.010	28-Sep-04	0.562	3.86%	3	4	0.9559	5000, 2326, 1082, 503, 234, 109, 50.6, 23.6	1.47	YES	
FAL.NHK.SLS.01.10.04	8.25	0.029	29-Sep-04	1.103	3.49%	1	7	0.9669	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	IC50 out of range
FAL.NHK.SLS.07.10.***03***	2.23	0.008	5-Oct-04	0.602	6.09%	4	4	0.9488	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
FAL.NHK.SLS.08.10.***03***	2.91	0.010	6-Oct-04	0.827	4.33%	3	5	0.9222	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.20.10.04	4.95	0.017	18-Oct-04	1.231	5.58%	2	6	0.9099	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.22.10.04 (NB)	3.62	0.013	20-Oct-04	0.675	0.86%	3	5	0.9405	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.28.10.04	3.39	0.012	26-Oct-04	0.641	7.85%	3	5	0.9366	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.29.10.04	2.33	0.008	27-Oct-04	0.502	1.46%	4	4	0.9531	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.03.11.04	3.19	0.011	1-Nov-04	0.447	8.60%	3	5	0.9331	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.05.11.04	2.16	0.007	3-Nov-04	0.538	0.62%	4	4	0.9467	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.10.11.04	4.07	0.014	8-Nov-04	1.011	0.89%	2	6	0.9210	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.12.11.04	3.76	0.013	10-Nov-04	0.742	3.04%	2	6	0.9085	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.17.11.04	4.04	0.014	15-Nov-04	1.050	1.74%	2	6	0.8732	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.19.11.04	3.91	0.014	17-Nov-04	0.509	4.62%	3	3	0.9793	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.24.11.04	4.09	0.014	22-Nov-04	1.124	2.91%	2	6	0.8654	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.26.11.04	3.00	0.010	24-Nov-04	0.620	1.45%	3	5	0.9524	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS(MO).10.12.04	6.02	0.021	8-Dec-04	1.017	1.35%	2	6	0.8137	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	YES	
FAL.NHK.SLS.10.12.04	4.18	0.014	8-Dec-04	0.928	0.25%	3	5	0.9170	10.0, 6.80, 4.63, 3.15, 2.14, 1.46, 0.99, 0.67	1.47	NO	IC50 out of range; low r2

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
IIVS												
Phase Ia												
B1	3.70	0.013	19-Aug-02	0.785	11.83%	1	5	0.8579	10, 5.6, 3.2, 1.8, 1.0, 0.6, 0.3, 0.2	1.79	YES	
B2	2.93	0.010	19-Aug-02	0.778	5.60%	1	6	0.8406	10, 5.6, 3.2, 1.8, 1.0, 0.6, 0.3, 0.2	1.79	YES	
B3	59.28	0.206	24-Aug-02	1.883	3.30%	1	6	0.0862	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	NO	Major precipitation problems
B4	10.06	0.035	24-Aug-02	1.680	8.59%	0	2	0.6253	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	NO	Major precipitation problems. No points between 10 & 50%.
B5	3.72	0.013	25-Aug-02	1.129	7.89%	1	5	0.9213	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B6	3.88	0.013	25-Aug-02	1.130	5.10%	1	5	0.8956	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B7	3.57	0.012	26-Aug-02	1.083	7.51%	1	6	0.8251	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B8	3.30	0.011	26-Aug-02	0.867	11.48%	3	5	0.8592	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B9	3.85	0.013	27-Aug-02	0.985	10.80%	2	5	0.8840	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B10	3.72	0.013	27-Aug-02	1.026	2.70%	1	6	0.8212	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B11	4.92	0.017	4-Sep-02	1.240	0.59%	1	5	0.8987	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B12	4.13	0.014	4-Sep-02	1.218	4.81%	1	6	0.8888	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B13	4.02	0.014	5-Sep-02	1.082	0.78%	1	6	0.8669	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B14	4.18	0.014	5-Sep-02	1.111	3.22%	1	6	0.8742	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B15	4.36	0.015	6-Sep-02	0.693	12.53%	1	6	0.8170	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B16	5.07	0.018	6-Sep-02	0.747	12.82%	2	6	0.7516	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	NO	Inadequate curve fit.
B17	3.70	0.013	7-Sep-02	0.550	3.51%	1	5	0.8953	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B18	3.50	0.012	7-Sep-02	0.558	9.32%	1	6	0.8518	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B19	3.45	0.012	8-Sep-02	0.658	10.32%	1	6	0.8785	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B20	3.03	0.011	8-Sep-02	0.682	5.43%	2	5	0.9061	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B23 (no re-feed)	3.54	0.012	21-Sep-02	1.084	4.29%	2	4	0.9573	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B23 (re-feed)	3.46	0.012	21-Sep-02	0.824	4.80%	2	3	0.9531	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	

NHK NRU Positive Control (SLS) Data

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B24 (no re-feed)	3.89	0.013	21-Sep-02	1.120	0.13%	1	5	0.9361	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B24 (re-feed)	3.72	0.013	21-Sep-02	0.784	2.36%	2	4	0.9265	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B25 (no re-feed)	3.92	0.014	22-Sep-02	1.078	1.34%	1	5	0.9426	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B25 (re-feed)	4.19	0.015	22-Sep-02	0.938	2.24%	2	5	0.9540	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B26 (no re-feed)	3.44	0.012	22-Sep-02	1.037	7.19%	2	3	0.9495	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B26 (re-feed)	3.64	0.013	22-Sep-02	0.775	4.29%	2	4	0.9491	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B27 (no re-feed)	2.87	0.010	23-Sep-02	1.050	1.79%	2	5	0.8907	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B27 (re-feed)	2.68	0.009	23-Sep-02	0.841	2.77%	2	5	0.9212	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B28 (no re-feed)	3.30	0.011	23-Sep-02	1.029	0.04%	2	5	0.9088	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
B28 (re-feed)	2.78	0.010	23-Sep-02	0.819	3.87%	3	4	0.9476	10, 6.8, 4.6, 3.2, 2.2, 1.5, 1.0, 0.7	1.47	YES	
Phase Ib												
Preliminary	2.78	0.010	4-Jan-03	0.631	3.03%	3	3	0.9588	10, 6.8, 4.6, 3.2, 2.2, 1.47, 1.0, 0.68	1.47	YES	
B1	2.98	0.010	17-Jan-03	0.518	0.50%	2	5	0.9403	10, 6.8, 4.6, 3.2, 2.2, 1.47, 1.0, 0.68	1.47	YES	
B2	3.31	0.011	18-Jan-03	0.726	9.52%	2	3	0.9621	10, 6.8, 4.6, 3.2, 2.2, 1.47, 1.0, 0.68	1.47	YES	
B3	3.00	0.010	31-Jan-03	0.845	3.64%	2	4	0.9420	10, 6.8, 4.6, 3.2, 2.2, 1.47, 1.0, 0.68	1.47	YES	
B4	3.64	0.013	1-Feb-03	0.781	1.49%	2	4	0.9550	10, 6.8, 4.6, 3.2, 2.2, 1.47, 1.0, 0.68	1.47	YES	
Phase II												
A2	3.11	0.011	9-Aug-03	0.682	5.04%	3	4	0.9538	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B1	3.24	0.011	16-Aug-03	0.351	7.73%	3	3	0.9661	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B2	4.42	0.015	17-Aug-03	0.26	3.34%	2	4	0.9394	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B3	4.10	0.014	18-Aug-03	0.284	4.05%	3	2	0.9569	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B4	4.52	0.016	25-Aug-03	0.201	2.12%	2	4	0.9434	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B7	3.98	0.014	29-Aug-03	0.605	7.45%	2	4	0.945	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B8	6.56	0.023	13-Sep-03	0.512	9.47%	1	4	0.8297	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	NO	IC50 out of range

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
B9	5.85	0.020	14-Sep-03	0.551	4.08%	2	3	0.9042	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	NO	IC50 out of range
B10	5.25	0.018	15-Sep-03	0.475	1.75%	2	3	0.8811	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	NO	IC50 out of range
B11	6.15	0.021	16-Sep-03	0.38	1.21%	1	3	0.7715	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	NO	IC50 out of range; low r2
B12	4.27	0.015	29-Sep-03	0.642	4.75%	2	5	0.924	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B13	4.27	0.015	29-Sep-03	0.242	1.41%	2	4	0.928	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B14	3.98	0.014	30-Sep-03	0.317	1.85%	2	5	0.9696	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	YES	
B15	6.36	0.022	1-Oct-03	0.294	0.97%	2	2	0.8797	10, 6.7, 4.4, 3.0, 2.0, 1.3, 0.88, 0.59	1.50	NO	IC50 out of range
Phase III												
A1	2.88	0.010	15-Mar-04	0.474	1.95%	3	5	0.9576	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
A2	3.42	0.012	18-Mar-04	0.581	5.05%	2	6	0.9176	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
A3	3.90	0.014	29-Mar-04	0.610	0.07%	3	5	0.8815	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
A4	2.67	0.009	29-Mar-04	0.509	3.50%	3	5	0.9629	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
A5	2.65	0.009	30-Mar-04	0.533	5.08%	3	5	0.9534	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B1	2.84	0.010	21-Apr-04	0.621	3.08%	4	4	0.9377	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B2	3.38	0.012	22-Apr-04	0.526	2.69%	3	5	0.9568	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B3	2.79	0.010	4-May-04	0.531	6.18%	3	5	0.9469	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B4	4.20	0.015	11-May-04	0.528	11.31%	2	6	0.8904	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B5	3.51	0.012	12-May-04	0.537	7.15%	2	6	0.9149	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	

NHK NRU Positive Control (SLS) Data

Experiment ID[1] NHK Cells	IC_{50} (ug/mL)	IC_{50} (mM)	Reference Substance Application Date	Mean VC OD[2]	Difference of right/left VC from mean VC[3]	Number of Points 0 - 50 %[4]	Number of Points 50 - 100 %[5]	R^2 [6]	Exposure Concentrations (ug/mL)[7]	Dilution Factor[8]	Acceptable Tests[9]	Rationale for Unacceptability
B6	2.72	0.009	14-Jul-04	0.629	6.79%	3	5	0.9380	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B7	2.58	0.009	15-Jul-04	0.611	0.67%	3	5	0.9646	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B8	2.95	0.010	17-Aug-04	0.587	10.35%	3	4	0.9304	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B9	3.08	0.011	18-Aug-04	0.554	1.95%	3	4	0.9609	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B10	4.14	0.014	1-Sep-04	0.597	6.80%	2	6	0.9448	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B11	3.55	0.012	2-Sep-04	0.669	1.77%	2	6	0.9438	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B12	2.93	0.010	20-Oct-04	0.599	3.40%	3	5	0.9561	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B13	2.50	0.009	27-Oct-04	0.629	3.01%	3	5	0.9645	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B14	3.10	0.011	28-Oct-04	0.702	3.78%	3	5	0.9615	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	
B15	2.51	0.009	8-Nov-04	0.623	2.50%	4	4	0.9151	10.0, 6.67, 4.44, 2.96, 1.98, 1.32, 0.88, 0.59	1.50	YES	

Abbreviations: NR=Neutral red; R&D=Research and development; PC=Positive control; C1 - C8=Concentration series applied to the the cells. C1 is the highest concentration and C8 is lowest; NA=Not Available; RC=Registry of Cytotoxicity;
[1] PC test ID
[2] Mean OD value for all VC wells in test plate
[3] Difference of right and left VC column of wells in the test plate
[4] % Viability values between 0 and 50% viability; test acceptance criterion; Phases Ia and Ib = number of points between 10 - 50%
[5] % Viability values between 50 and 100% viability; test acceptance criterion; Phases Ia and Ib = number of points between 50 - 90%
[6] Calculated value from the Prism® software
[7] Reference substance concentrations applied to the cells
[8] Step-wise dilution factor
[9] Determination whether test meets or doesn't meet test acceptance criteria
Shaded boxes identify values that do not meet the specific test acceptance criteria

Acceptance Limits for PC IC_{50}

Phase	ECBC (ug/mL)	FAL (ug/mL)	IIVS (ug/mL)
Ib (NHK)	1.40 – 6.67	1.34 – 13.6	2.57 – 4.79
II (NHK)	1.22 – 6.10	0 – 11.1	2.10 – 5.04
III (NHK)	0.07 – 7.11	0.57 – 5.82	1.94 – 5.61

Appendix J

LD_{50} and Toxicity Category Predictions

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Appendix J

The data presented in this appendix support the analyses in **Section 6**. For the analysis in **Appendices J1** through **J6,** the IC_{50} values for each reference substance are the geometric mean of the geometric mean IC_{50} values obtained for each laboratory. IC_{50} data for the same reference substances were used with each regression/test method evaluated. Sixty-seven chemicals were evaluated for the 3T3 NRU test method and 68 chemicals were evaluated for the NHK NRU test method. Of the original 72 chemicals tested, epinephrine bitartrate, colchicine, and propylparaben were excluded due to the lack of rat oral reference LD_{50} data. Carbon tetrachloride and methanol were excluded from the 3T3 NRU evaluations because no laboratory attained sufficient toxicity in any experiment for the calculation of an IC_{50}. Carbon tetrachloride was also excluded from the NHK NRU evaluations because no laboratory attained sufficient toxicity in any test for the calculation of an IC_{50}.

RC Millimole Regression: Appendices J1 (3T3 NRU) and J2 (NHK NRU)

Log LD_{50} (mmol/kg) = 0.435 log IC_{50} (mM) + 0.625

Appendices J1 and **J2** support the analysis of outlier substances presented in **Section 6.2**. Predicted LD_{50} values in mmol/kg and mg/kg (conversion from the mmol/kg values) for each reference substance were determined for each test method using the respective IC_{50} values in the RC millimole regression. Epinephrine bitartrate, colchicine, and propylparaben were included in this analysis for a more complete comparison with the results of the RC. The predicted log LD_{50} value was subtracted from the observed log LD_{50} value (initial values in **Table 3-2** from the RC, HSDB, or RTECS® were converted to mmol/kg) and the difference (positive or negative) was compared to the RC criterion for outliers (0.699). Reference substances with absolute values greater than 0.699 were identified as positive or negative outliers to the RC millimole regression. The observed LD_{50} value (mg/kg) was used to assign each reference chemical to an observed toxicity category (GHS acute oral classification [UN 2005]). The predicted LD_{50} value (mg/kg) was used to determine the reference substance's predicted toxicity category.

RC Rat-Only Millimole Regression: Appendices J3 (3T3 NRU) and J4 (NHK NRU)

Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621

Appendices J3 and **J4** support the accuracy analyses for GHS acute oral toxicity category predictions presented in **Section 6.4.2**. As described in **Section 6.3.1**, the RC rat-only millimole regression was calculated using the RC IC_{50} and LD_{50} values for the 282 chemicals that had rat oral LD_{50} values. The observed LD_{50} values, which were the reference LD_{50} values (mg/kg) from **Table 4-2**, were used to assign each reference substance to an observed toxicity category (GHS acute oral classification [UN 2005]). The predicted LD_{50} value (mg/kg) was used to determine the reference substance's predicted toxicity category.

RC Rat-Only Weight Regression: Appendices J5 (3T3 NRU) and J6 (NHK NRU)

Log LD_{50} (mg/kg) = 0.372 log IC_{50} (μg/mL) + 2.024

Appendices J5 and **J6** support the accuracy analyses for GHS acute oral toxicity category predictions presented in **Section 6.4.2**. As described in **Section 6.3.2**, the RC rat-only weight regression was calculated using the RC IC_{50} and LD_{50} data for the 282 chemicals that had rat oral LD_{50} values. The regression data were converted into weight units (i.e., LD_{50} values as mg/kg and IC_{50} values as μg/mL). The observed LD_{50} values, which were the reference LD_{50} values (mg/kg) from **Table 4-2**, were used to assign each reference substance to an observed toxicity category (GHS acute oral classification [UN 2005]). Predicted LD_{50} values in mg/kg for each reference substance were determined for each NRU test method using the respective NRU IC_{50} values in the RC rat-only weight regression. The predicted LD_{50} value (mg/kg) was used to determine the reference substance's predicted toxicity category.

Comparison of RC Rat-Only Millimole Regression and the RC Rat-Only Weight Regression for the Prediction of LD_{50} for Low or High Molecular Weight Substances

Appendix J7 supports **Section 6.6.2**, which compares the under- and over-prediction of acute oral toxicity (i.e., using LD_{50} values) for low and high molecular weight substances for the RC rat-only millimole regression and the RC rat-only weight regression. The analysis uses the RC IC_{50} and LD_{50} values for the 282 RC substances with rat oral LD_{50} data, which are provided in **Appendix K-3**.

Appendix J1

3T3 NRU Predictions: RC Millimole Regression

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3T3 NRU Predictions: RC Millimole Regression

RC Millimole Regression: Log LD_{50} (mmol/kg) = 0.435 log IC_{50} (mM) + 0.625

Reference Substance[1]	Log Observed LD_{50} (mmol/kg)	Observed LD_{50} (mg/kg)	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[4]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	3T3 Log IC_{50} (mM)[5]	3T3 IC_{50} (ug/mL)[5]	Log Observed LD_{50} - Log Predicted LD_{50} (mmol/kg)[6]	Outlier[7]
1,1,1-Trichloroethane	1.888	10298	> 5000	1.576	5022	> 5000	2.186	20453	0.312	
2-Propanol	1.988	5843	> 5000	1.392	1483	300-2000	1.764	3489	0.595	
5-Aminosalicylic Acid	1.704	7749	> 5000	1.076	1824	300-2000	1.037	1667	0.628	
Acetaminophen	1.201	2404	2000-5000	0.407	385.9	300-2000	-0.501	47.7	0.795	Positive
Acetonitrile	1.966	3798	2000-5000	1.620	1711	300-2000	2.287	7951	0.346	
Acetylsalicylic Acid	0.744	1000	300-2000	0.875	1351	300-2000	0.574	676	-0.131	
Aminopterin	-2.167	3	< 5	-1.480	15	5-50	-4.839	0.006	-0.687	
Amitriptyline HCl	0.061	361	300-2000	-0.092	254	50-300	-1.648	7.05	0.153	
Arsenictrioxide	-1.000	20	5-50	-0.236	115	50-300	-1.980	2.07	-0.764	Negative
Atropine Sulfate	-0.036	639	300-2000	0.207	1119	300-2000	-0.961	76.0	-0.243	
Boric Acid	1.634	2660	2000-5000	1.267	1143.6	300-2000	1.476	1850	0.367	
Busulfan	-2.090	2	< 5	0.407	629	300-2000	-0.501	77.7	-2.497	Negative
Cadmium chloride	-0.319	88	50-300	-0.484	60	50-300	-2.549	0.518	0.165	
Caffeine	-0.005	192	50-300	0.579	737	300-2000	-0.105	153	-0.584	
Carbamazepine	0.918	1957	300-2000	0.468	695	300-2000	-0.360	103	0.450	
Chloral Hydrate	0.462	479	300-2000	0.644	729	300-2000	0.044	183	-0.182	
Chloramphenicol	1.021	3393	2000-5000	0.453	918	300-2000	-0.395	130	0.568	
Citric Acid	1.194	3000	2000-5000	0.886	1477.5	300-2000	0.600	765	0.308	
Colchicine	-1.82	6	5-50	-1.144	28.7	5-50	-4.066	0.034	-0.680	
Cupric Sulfate Pentahydrate	0.080	300	50-300	0.268	462	300-2000	-0.822	37.6	-0.188	
Cycloheximide	-2.148	2	< 5	-0.757	49.3	5-50	-3.177	0.187	-1.391	Negative
Dibutyl Phthalate	1.635	11998	> 5000	0.274	523	300-2000	-0.807	43.4	1.361	Positive
Dichlorvos (DDVP)	-1.114	17	5-50	0.149	311	300-2000	-1.095	17.7	-1.262	Negative
Diethyl Phthalate	1.588	8602	> 5000	0.487	683	300-2000	-0.316	107	1.100	Positive
Digoxin	-1.637	18	5-50	0.519	2580	2000-5000	-0.244	445	-2.156	Negative
Dimethylformamide	1.583	2800	2000-5000	1.432	1974	300-2000	1.854	5224	0.152	
Diquat Dibromide Monohydra	-0.173	243	50-300	-0.094	291	50-300	-1.654	8.04	-0.079	
Disulfoton	-2.137	2	< 5	0.696	1363	300-2000	0.163	400	-2.833	Negative
Endosulfan	-1.354	18	5-50	-0.175	272	50-300	-1.840	5.88	-1.179	Negative

3T3 NRU Predictions: RC Millimole Regression

Reference Substance[1]	Log Observed LD_{50} (mmol/kg)	Observed LD_{50} (mg/kg)	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[4]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	3T3 Log IC_{50} (mM)[5]	3T3 IC_{50} (ug/mL)[5]	Log Observed LD_{50} - Log Predicted LD_{50} (mmol/kg)[6]	Outlier[7]
Epinephrine bitartrate	-1.92	4	< 5	0.298	662	300-2000	-0.752	59.0	-2.219	Negative
Ethanol	2.483	14008	> 5000	1.561	1675	300-2000	2.151	6523	0.922	Positive
Ethyleneglycol	2.140	8567	> 5000	1.754	3522	2000-5000	2.595	24436	0.386	
Fenpropathrin	-1.288	18	5-50	0.114	454	300-2000	-1.175	23.3	-1.402	Negative
Gibberellic Acid	1.260	6305	> 5000	1.214	5664	> 5000	1.353	7810	0.047	
Glutethimide	0.441	600	300-2000	0.590	846	300-2000	-0.079	181	-0.149	
Glycerol	2.139	12691	> 5000	1.679	4394	2000-5000	2.422	24345	0.461	
Haloperidol	-0.468	128	50-300	-0.153	264	50-300	-1.788	6.13	-0.315	
Hexachlorophene	-0.824	61	50-300	-0.239	235	50-300	-1.987	4.19	-0.585	
Lactic Acid	1.617	3730	2000-5000	1.290	1757	300-2000	1.529	3044	0.327	
Lindane	-0.585	76	50-300	0.444	808	300-2000	-0.416	112	-1.029	Negative
Lithium carbonate	1.206	1187	300-2000	1.008	753	300-2000	0.881	562	0.198	
Meprobamate	0.561	794	300-2000	0.778	1309	300-2000	0.351	490	-0.217	
Mercury Chloride	-2.434	1	< 5	-0.166	185	50-300	-1.819	4.12	-2.268	Negative
Nicotine	-0.511	50	5-50	0.776	969	300-2000	0.347	361	-1.287	Negative
Paraquat	-0.509	80	50-300	0.144	358.14	300-2000	-1.106	20.1	-0.652	
Parathion	-2.161	2	< 5	0.237	503	300-2000	-0.891	37.4	-2.398	Negative
Phenobarbital	-0.154	163	50-300	0.800	1465	300-2000	0.402	586	-0.954	Negative
Phenol	0.643	414	300-2000	0.559	341	300-2000	-0.152	66.3	0.085	
Phenylthiourea	-1.705	3	< 5	0.501	482	300-2000	-0.285	79.0	-2.206	Negative
Physostigmine	-1.787	5	< 5	0.183	420	300-2000	-1.015	26.6	-1.970	Negative
Potassium cyanide	-0.824	10	5-50	0.506	209	50-300	-0.274	34.6	-1.330	Negative
Potassium chloride	1.543	2602	2000-5000	1.355	1689	300-2000	1.678	3555	0.188	
Procainamide HCl	0.856	1950	300-2000	0.716	1414	300-2000	0.210	441	0.140	
Propranolol	0.201	470	300-2000	0.050	332	300-2000	-1.321	14.1	0.151	
Propylparaben	1.550	6326	> 5000	0.260	328	300-2000	-0.840	26.1	1.290	Positive
Sodium Arsenite	-0.501	41	5-50	-0.347	58	50-300	-2.234	0.759	-0.154	
Sodium Chloride	1.710	2998	2000-5000	1.456	1669	300-2000	1.910	4746	0.254	
Sodium Dichromate Dihydrat	-0.719	57	50-300	-0.552	84	50-300	-2.706	0.587	-0.167	
Sodium Hypochlorite	2.078	8910	> 5000	1.123	989	300-2000	1.145	1040	0.955	Positive

3T3 NRU Predictions: RC Millimole Regression

Reference Substance[1]	Log Observed LD_{50} (mmol/kg)	Observed LD_{50} (mg/kg)	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[4]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	3T3 Log IC_{50} (mM)[5]	3T3 IC_{50} (ug/mL)[5]	Log Observed LD_{50} - Log Predicted LD_{50} (mmol/kg)[6]	Outlier[7]
Sodium Oxalate	0.063	155	50-300	0.383	323.4	300-2000	-0.557	37.142	-0.319	
Sodium fluoride	0.632	180	50-300	0.742	232	50-300	0.269	78.0	-0.110	
Sodium selenate	-2.072	2	< 5	0.271	352.7	300-2000	-0.814	29.023	-2.343	Negative
Strychnine	-2.144	2	< 5	0.483	1017	300-2000	-0.326	158	-2.627	Negative
Thallium Sulfate	-1.241	29	5-50	-0.231	296	50-300	-1.968	5.43	-1.009	Negative
Trichloroacetic Acid	1.486	4999	2000-5000	0.948	1449	300-2000	0.742	902	0.538	
Triethylenemelamine	-2.310	1	< 5	-0.626	48	5-50	-2.875	0.272	-1.684	Negative
Triphenyltin Hydroxide	-0.921	44	5-50	-1.258	20	5-50	-4.329	0.017	0.337	
Valproic Acid	1.009	1471	300-2000	0.955	1299	300-2000	0.758	826	0.054	
Verapamil HCl	-0.658	108	50-300	0.126	656	300-2000	-1.148	34.9	-0.783	Negative
Xylene	1.607	4300	2000-5000	0.987	1030	300-2000	0.832	721	0.621	

Abbreviations: 3T3=Neutral red uptake with mouse fibroblast 3T3 cell line

[1]Carbon tetrachloride and methanol were excluded because IC_{50} values could not be determined. Initial LD_{50} from **Table 3-2** converted to mmol/kg. Initial LD_{50} values came largely from the RC (1983/84 RTECS®) for RC susbtances and from the current Hazardous Substances Data Bank (HSDB) or RTECS® and electronic database searches for non-RC substances.

[2]Globally Harmonized System (GHS) hazard classification (UN 2005):

Abbreviation	Category	Oral LD_{50} Limits
<5	1	$LD_{50} \leq 5$ mg/kg
5-50	2	$5 < LD_{50} \leq 50$ mg/kg
50-300	3	$50 < LD_{50} \leq 300$ mg/kg
300-2000	4	$300 < LD_{50} \leq 2000$ mg/kg
2000-5000	5	$2000 < LD_{50} \leq 5000$ mg/kg
>5000	Unclassified	$LD_{50} > 5000$ mg/kg

[3]LD_{50} determined using NRU IC_{50} in RC millimole regression: Log LD_{50} (mmol/kg) = 0.435 log IC_{50} (mM) + 0.625

[4]Predicted LD_{50} in mg/kg (converted from results of RC millimole regression)

[5]Combined 3T3 IC_{50} values from three laboratories

[6]Calculation to determine outliers to the RC millimole regression line

[7]Log observed LD_{50} - log predicted $LD_{50} > 0.699$ (or log 5) identifies a chemical as an "outlier"; negative=predicted value below prediction interval of RC millimole regression line; positive=predicted value above prediction interval of RC millimole regression line (Halle 1998, 2003)

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Appendix J2

NHK NRU Predictions: RC Millimole Regression

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NHK NRU Predictions: RC Millimole Regression

RC Millimole Regression: Log LD_{50} (mmol/kg) = 0.435 log IC_{50} (mM) + 0.625

Reference Substance[1]	Log Observed LD_{50} (mmol/kg)[2]	Observed LD_{50} (mg/kg)[3]	Observed LD_{50} Toxicity Category[4] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[5]	Predicted LD_{50} (mg/kg)[6]	Predicted LD_{50} Toxicity Category[4] (mg/kg)	NHK Log IC_{50} (mM)[7]	NHK IC_{50} (ug/mL)[8]	Log Observed LD_{50} - Log Predicted LD_{50} (mmol/kg)[9]	Outlier[10]
1,1,1-Trichloroethane	1.888	10298	> 5000	1.401	3361	2000-5000	1.784	4709	0.486	
2-Propanol	1.988	5843	> 5000	1.473	1788	300-2000	1.951	2635	0.514	
5-Aminosalicylic Acid	1.704	7749	> 5000	0.401	385	300-2000	-0.516	154	1.304	Positive
Acetaminophen	1.201	2404	2000-5000	0.858	1089	300-2000	0.535	934	0.344	
Acetonitrile	1.966	3798	2000-5000	1.654	1853	300-2000	2.367	3065	0.312	
Acetylsalicylic Acid	0.744	1000	300-2000	0.854	1287	300-2000	0.526	1099	-0.110	
Aminopterin	-2.167	3	< 5	0.702	2218	2000-5000	0.177	1557	-2.869	Negative
Amitriptyline HCl	0.061	361	300-2000	-0.047	282	50-300	-1.545	-13	0.107	
Arsenictrioxide	-1.000	20	5-50	-0.011	193	50-300	-1.461	-2	-0.989	Negative
Atropine Sulfate	-0.036	639	300-2000	0.221	1155	300-2000	-0.929	255	-0.257	
Boric Acid	1.634	2660	2000-5000	0.988	601	300-2000	0.833	593	0.646	
Busulfan	-2.090	2	< 5	0.635	1064	300-2000	0.024	676	-2.726	Negative
Cadmium chloride	-0.319	88	50-300	-0.249	103	50-300	-2.009	-26	-0.070	
Caffeine	-0.005	192	50-300	0.850	1374	300-2000	0.516	1167	-0.855	Negative
Carbamazepine	0.918	1957	300-2000	0.428	633	300-2000	-0.453	271	0.490	
Chloral Hydrate	0.462	479	300-2000	0.584	635	300-2000	-0.094	371	-0.122	
Chloramphenicol	1.021	3393	2000-5000	0.637	1402	300-2000	0.028	894	0.384	
Citric Acid	1.194	3000	2000-5000	0.769	1128	300-2000	0.331	867	0.425	
Colchicine	-1.82	6.00	5-50	-1.45	14.0	5-50	-4.780	0	-0.373	
Cupric Sulfate Pentahydrate	0.080	300	50-300	0.580	949	300-2000	-0.104	550	-0.500	
Cycloheximide	-2.148	2	< 5	-0.934	33	5-50	-3.584	-31	-1.214	Negative
Dibutyl Phthalate	1.635	11998	> 5000	0.196	437	300-2000	-0.987	85	1.439	Positive
Dichlorvos (DDVP)	-1.114	17	5-50	0.053	250	50-300	-1.315	13	-1.167	Negative
Diethyl Phthalate	1.588	8602	> 5000	0.509	718	300-2000	-0.266	366	1.079	Positive
Digoxin	-1.637	18	5-50	-1.937	9	5-50	-5.889	-17	0.299	
Dimethylformamide	1.583	2800	2000-5000	1.506	2345	2000-5000	2.026	3533	0.077	
Diquat Dibromide Monohydr	-0.173	243	50-300	-0.211	223	50-300	-1.922	-47	0.038	
Disulfoton	-2.137	2	< 5	0.622	1149	300-2000	-0.007	714	-2.759	Negative
Endosulfan	-1.354	18	5-50	-0.368	175	50-300	-2.282	-64	-0.987	
Epinephrine bitartrate	-1.92	4	< 5	0.372	785	300-2000	-0.581	87	-2.293	Negative

NHK NRU Predictions: RC Millimole Regression

Reference Substance[1]	Log Observed LD_{50} (mmol/kg)[2]	Observed LD_{50} (mg/kg)[3]	Observed LD_{50} Toxicity Category[4] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[5]	Predicted LD_{50} (mg/kg)[6]	Predicted LD_{50} Toxicity Category[4] (mg/kg)	NHK Log IC_{50} (mM)[7]	NHK IC_{50} (ug/mL)[8]	Log Observed LD_{50} - Log Predicted LD_{50} (mmol/kg)[9]	Outlier[10]
Ethanol	2.483	14008	> 5000	1.642	2019	2000-5000	2.337	3315	0.841	Positive
Ethyleneglycol	2.140	8567	> 5000	1.857	4462	2000-5000	2.831	8285	0.283	
Fenpropathrin	-1.288	18	5-50	-0.314	170	50-300	-2.158	-53	-0.974	Negative
Gibberellic Acid	1.260	6305	> 5000	1.024	3657	2000-5000	0.916	3743	0.237	
Glutethimide	0.441	600	300-2000	0.583	831	300-2000	-0.098	484	-0.141	
Glycerol	2.139	12691	> 5000	1.682	4424	2000-5000	2.429	7440	0.458	
Haloperidol	-0.468	128	50-300	-0.266	204	50-300	-2.049	-54	-0.202	
Hexachlorophene	-0.824	61	50-300	-1.180	27	5-50	-4.149	-32	0.356	
Lactic Acid	1.617	3730	2000-5000	1.130	1215	300-2000	1.161	1373	0.487	
Lindane	-0.585	76	50-300	0.107	372	300-2000	-1.191	40	-0.692	
Lithium carbonate	1.206	1187	300-2000	0.974	695	300-2000	0.801	677	0.232	
Meprobamate	0.561	794	300-2000	0.718	1140	300-2000	0.213	818	-0.157	
Mercury Chloride	-2.434	1	< 5	-0.102	215	50-300	-1.671	-22	-2.332	Negative
Methanol	2.609	13012	> 5000	1.355	726	300-2000	1.679	984	1.253	Positive
Nicotine	-0.511	50	5-50	0.546	570	300-2000	-0.182	311	-1.057	Negative
Paraquat	-0.509	80	50-300	0.355	582	300-2000	-0.621	207	-0.864	Negative
Parathion	-2.161	2	< 5	0.197	459	300-2000	-0.983	90	-2.358	Negative
Phenobarbital	-0.154	163	50-300	0.749	1303	300-2000	0.285	976	-0.903	Negative
Phenol	0.643	414	300-2000	0.582	360	300-2000	-0.098	209	0.061	
Phenylthiourea	-1.705	3	< 5	0.775	906	300-2000	0.344	702	-2.480	Negative
Physostigmine	-1.787	5	< 5	0.411	709	300-2000	-0.493	291	-2.197	Negative
Potassium Cyanide	-0.824	10	5-50	0.472	193	50-300	-0.352	91	-1.296	Negative
Potassium chloride	1.543	2602	2000-5000	1.268	1381	300-2000	1.477	1750	0.275	
Procainamide HCl	0.856	1950	300-2000	0.976	2571	2000-5000	0.807	2509	-0.120	
Propranolol	0.201	470	300-2000	0.228	500	300-2000	-0.912	114	-0.027	
Propylparaben	1.550	6326	> 5000	0.175	269	50-300	-1.040	16.6	1.375	Positive
Sodium Arsenite	-0.501	41	5-50	-0.434	48	5-50	-2.435	-21	-0.066	
Sodium Chloride	1.710	2998	2000-5000	1.292	1145	300-2000	1.534	1480	0.418	
Sodium Dichromate Dihydrat	-0.719	57	50-300	-0.516	91	50-300	-2.622	-47	-0.204	
Sodium Hypochlorite	2.078	8910	> 5000	1.193	1160	300-2000	1.305	1384	0.885	Positive
Sodium Oxalate	0.063	155	50-300	0.801	847	300-2000	0.404	678	-0.737	Negative

NHK NRU Predictions: RC Millimole Regression

Reference Substance[1]	Log Observed LD_{50} (mmol/kg)[2]	Observed LD_{50} (mg/kg)[3]	Observed LD_{50} Toxicity Category[4] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[5]	Predicted LD_{50} (mg/kg)[6]	Predicted LD_{50} Toxicity Category[4] (mg/kg)	NHK Log IC_{50} (mM)[7]	NHK IC_{50} (ug/mL)[8]	Log Observed LD_{50} - Log Predicted LD_{50} (mmol/kg)[9]	Outlier[10]
Sodium fluoride	0.632	180	50-300	0.654	189	50-300	0.066	124	-0.021	
Sodium selenate	-2.072	2	< 5	0.074	224	50-300	-1.267	17	-2.146	Negative
Strychnine	-2.144	2	< 5	0.302	670	300-2000	-0.743	202	-2.446	Negative
Thallium Sulfate	-1.241	29	5-50	-0.907	62	50-300	-3.522	-57	-0.333	
Trichloroacetic Acid	1.486	4999	2000-5000	0.800	1032	300-2000	0.403	826	0.685	
Triethylenemelamine	-2.310	1	< 5	-0.263	111	50-300	-2.042	-29	-2.047	Negative
Triphenyltin Hydroxide	-0.921	44	5-50	-1.360	16	5-50	-4.562	-22	0.438	
Valproic Acid	1.009	1471	300-2000	0.864	1055	300-2000	0.550	912	0.144	
Verapamil HCl	-0.658	108	50-300	0.247	868	300-2000	-0.869	214	-0.905	Negative
Xylene	1.607	4300	2000-5000	0.904	852	300-2000	0.642	770	0.703	Positive

Abbreviations: NHK=Neutral red uptake with normal human epidermal keratinocytes.

[1]Carbon tetrachloride and methanol were excluded because IC_{50} values could not be determined. Initial LD_{50} from **Table 3-2** converted to mmol/kg. Initial LD_{50} values came largely from the RC (1983/84 RTECS®) for RC susbtances and from the current Hazardous Substances Data Bank (HSDB) or RTECS® and electronic database searches for non-RC substances.

[2]Globally Harmonized System (GHS) hazard classification (UN 2005):

Abbreviation	Category	Oral LD_{50} Limits
<5	1	$LD_{50} \leq 5$ mg/kg
5-50	2	$5 < LD_{50} \leq 50$ mg/kg
50-300	3	$50 < LD_{50} \leq 300$ mg/kg
300-2000	4	$300 < LD_{50} \leq 2000$ mg/kg
2000-5000	5	$2000 < LD_{50} \leq 5000$ mg/kg
>5000	Unclassified	$LD_{50} > 5000$ mg/kg

[3]LD_{50} determined using NRU IC_{50} in RC millimole regression: Log LD_{50} (mmol/kg) = 0.435 log IC_{50} (mM) + 0.625.

[4]Predicted LD_{50} in mg/kg (converted from results of RC millimole regression).

[5]Combined NHK IC_{50} values from three laboratories.

[6]Calculation to determine outliers to the RC millimole regression line.

[7]Log observed LD_{50} - log predicted LD_{50} > 0.699 (or log 5) identifies a chemical as an "outlier"; negative=predicted value below prediction interval of RC millimole regression line; positive=predicted value above prediction interval of RC millimole regression line (Halle 1998, 2003).

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Appendix J3

3T3 NRU Predictions: RC Rat-Only Millimole Regression

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3T3 NRU Predictions: RC Rat-Only Millimole Regression
Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621

Reference Substance[1]	Log Reference LD_{50} (mmol/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	3T3 Log IC_{50} (mM)[6]
1,1,1-Trichloroethane	1.957	12078	>5000	1.580	5078	>5000	2.186
2-Propanol	1.929	5105	>5000	1.395	1494	300-2000	1.764
5-Aminosalicylic Acid	1.350	3428	2000-5000	1.076	1825	300-2000	1.037
Acetaminophen	1.155	2162	2000-5000	0.401	381	300-2000	-0.501
Acetonitrile	1.942	3595	2000-5000	1.625	1731	300-2000	2.287
Acetylsalicylic Acid	0.922	1506	300-2000	0.873	1346	300-2000	0.574
Aminopterin	-1.799	7	5-50	-1.504	14	5-50	-4.839
Amitriptyline HCl	0.046	349	300-2000	-0.103	248	50-300	-1.648
Arsenictrioxide	-0.897	25	5-50	-0.248	112	50-300	-1.980
Atropine Sulfate	0.071	819	300-2000	0.199	1099	300-2000	-0.961
Boric Acid	1.744	3426	2000-5000	1.269	1149	300-2000	1.476
Busulfan	-1.308	12	5-50	0.401	620	300-2000	-0.501
Cadmium chloride	-0.132	135	50-300	-0.498	58	50-300	-2.549
Caffeine	0.203	310	300-2000	0.575	730	300-2000	-0.105
Carbamazepine	1.075	2807	2000-5000	0.463	686	300-2000	-0.360
Chloral Hydrate	0.586	638	300-2000	0.640	723	300-2000	0.044
Chloramphenicol	1.033	3490	2000-5000	0.448	906	300-2000	-0.395
Citric Acid	1.489	5929	>5000	0.884	1472	300-2000	0.600
Cupric Sulfate Pentahydrate	0.279	475	300-2000	0.260	455	300-2000	-0.822
Cycloheximide	-2.148	2	<5	-0.774	47	5-50	-3.177
Dibutyl Phthalate	1.504	8892	>5000	0.267	514	300-2000	-0.807
Dichlorvos (DDVP)	-0.576	59	50-300	0.140	305	300-2000	-1.095
Diethyl Phthalate	1.622	9311	>5000	0.482	674	300-2000	-0.316
Digoxin	-1.441	28	5-50	0.514	2550	2000-5000	-0.244
Dimethylformamide	1.861	5305	>5000	1.435	1990	300-2000	1.854
Diquat Dibromide Monohydrate	-0.355	160	50-300	-0.105	284	50-300	-1.654
Disulfoton	-1.739	5	<5	0.693	1352	300-2000	0.163
Endosulfan	-1.165	28	5-50	-0.187	265	50-300	-1.840
Ethanol	2.391	11324	>5000	1.565	1693	300-2000	2.151
Ethylene glycol	2.062	7161	>5000	1.760	3574	2000-5000	2.595
Fenpropathrin	-0.664	76	50-300	0.105	445	300-2000	-1.175
Gibberellic Acid	1.241	6039	>5000	1.215	5683	>5000	1.353
Glutethimide	0.441	600	300-2000	0.586	838	300-2000	-0.079
Glycerol	2.332	19770	>5000	1.684	4452	2000-5000	2.422
Haloperidol	-0.057	330	300-2000	-0.164	258	50-300	-1.788
Hexachlorophene	-0.696	82	50-300	-0.251	228	50-300	-1.987
Lactic Acid	1.606	3635	2000-5000	1.292	1765	300-2000	1.529
Lindane	-0.464	100	50-300	0.438	798	300-2000	-0.416
Lithium carbonate	0.902	590	300-2000	1.008	752	300-2000	0.881
Meprobamate	0.803	1387	300-2000	0.775	1301	300-2000	0.351
Mercury Chloride	-0.830	40	5-50	-0.177	180	50-300	-1.819
Nicotine	-0.367	70	50-300	0.774	963	300-2000	0.347

3T3 NRU Predictions: RC Rat-Only Millimole Regression
Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621

Reference Substance[1]	Log Reference LD_{50} (mmol/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	3T3 Log IC_{50} (mM)[6]
Paraquat	-0.443	93	50-300	0.135	351	300-2000	-1.106
Parathion	-1.679	6	5-50	0.230	494	300-2000	-0.891
Phenobarbital	-0.016	224	50-300	0.798	1457	300-2000	0.402
Phenol	0.765	548	300-2000	0.554	337	300-2000	-0.152
Phenylthiourea	-1.705	3	<5	0.496	477	300-2000	-0.285
Physostigmine	-1.741	5	<5	0.175	412	300-2000	-1.015
Potassium Cyanide	-0.956	7	5-50	0.501	206	50-300	-0.274
Potassium chloride	1.575	2802	2000-5000	1.358	1699	300-2000	1.678
Procainamide HCl	0.856	1950	300-2000	0.713	1404	300-2000	0.210
Propranolol	0.197	466	300-2000	0.041	325	300-2000	-1.321
Sodium Arsenite	-0.474	44	5-50	-0.360	57	50-300	-2.234
Sodium Chloride	1.841	4050	2000-5000	1.459	1683	300-2000	1.910
Sodium Dichromate Dihydrate	-0.771	50	50-300	-0.567	81	50-300	-2.706
Sodium Hypochlorite	2.142	10328	>5000	1.124	990	300-2000	1.145
Sodium Oxalate	0.674	633	300-2000	0.376	319	300-2000	-0.557
Sodium fluoride	0.480	127	50-300	0.739	230	50-300	0.269
Sodium selenate	-1.799	3	<5	0.264	347	300-2000	-0.814
Strychnine	-1.725	6	5-50	0.478	1005	300-2000	-0.326
Thallium Sulfate	-1.305	25	5-50	-0.243	288	50-300	-1.968
Trichloroacetic Acid	1.505	5229	>5000	0.947	1445	300-2000	0.742
Triethylenemelamine	-1.708	4	<5	-0.641	47	5-50	-2.875
Triphenyltin Hydroxide	-0.047	329	300-2000	-1.279	19	5-50	-4.329
Valproic Acid	0.839	996	300-2000	0.954	1296	300-2000	0.758
Verapamil HCl	-0.646	111	50-300	0.117	643	300-2000	-1.148
Xylene	1.643	4665	2000-5000	0.986	1028	300-2000	0.832

[1]Three chemicals were excluded because no rat oral LD_{50} was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride and methanol were excluded because IC_{50} values could not be determined.
[2]Reference LD_{50} in mmol/kg from **Table 4-2**. Reference rat oral LD_{50} values were developed from rat acute oral LD_{50} studies located using literature searches, secondary references, and electronic database searches.
[3]Globally Harmonized System (GHS) hazard classification (UN 2005):

Abbreviation	Category	Oral LD_{50} Limits
<5	1	$LD_{50} \leq 5$ mg/kg
5-50	2	$5 < LD_{50} \leq 50$ mg/kg
50-300	3	$50 < LD_{50} \leq 300$ mg/kg
300-2000	4	$300 < LD_{50} \leq 2000$ mg/kg
2000-5000	5	$2000 < LD_{50} \leq 5000$ mg/kg
>5000	Unclassified	$LD_{50} > 5000$ mg/kg

[4]LD_{50} determined using NRU IC_{50} in RC rat-only millimole regression: Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621.
[5]LD_{50} in mg/kg (converted from results of RC rat-only millimole regression).
[6]Combined 3T3 IC_{50} values from three laboratories.

Appendix J4

NHK NRU Predictions: RC Rat-Only Millimole Regression

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NHK NRU Predictions: RC Rat-Only Millimole Regression
Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621

Reference Substance[1]	Log Reference LD_{50} (mmol/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	NHK Log IC_{50} (mM)[6]
1,1,1-Trichloroethane	1.957	12078	>5000	3.478	3009	2000-5000	1.784
2-Propanol	1.929	5105	>5000	3.411	2579	300-2000	1.951
5-Aminosalicylic Acid	1.350	3428	2000-5000	2.645	442	300-2000	-0.516
Acetaminophen	1.155	2162	2000-5000	3.034	1081	300-2000	0.535
Acetonitrile	1.942	3595	2000-5000	3.505	3196	300-2000	2.367
Acetylsalicylic Acid	0.922	1506	300-2000	3.059	1145	300-2000	0.526
Aminopterin	-1.799	7	5-50	3.073	1184	5-50	0.177
Amitriptyline HCl	0.046	349	300-2000	2.378	239	50-300	-1.545
Arsenictrioxide	-0.897	25	5-50	2.335	216	50-300	-1.461
Atropine Sulfate	0.071	819	300-2000	2.736	544	300-2000	-0.929
Boric Acid	1.744	3426	2000-5000	3.000	1001	300-2000	0.833
Busulfan	-1.308	12	5-50	2.922	836	300-2000	0.024
Cadmium chloride	-0.132	135	50-300	2.119	131	50-300	-2.009
Caffeine	0.203	310	300-2000	3.067	1168	300-2000	0.516
Carbamazepine	1.075	2807	2000-5000	2.738	547	300-2000	-0.453
Chloral Hydrate	0.586	638	300-2000	2.814	652	300-2000	-0.094
Chloramphenicol	1.033	3490	2000-5000	2.968	929	300-2000	0.028
Citric Acid	1.489	5929	>5000	2.997	992	300-2000	0.331
Cupric Sulfate Pentahydrate	0.279	475	300-2000	2.877	754	300-2000	-0.104
Cycloheximide	-2.148	2	<5	1.602	40	5-50	-3.584
Dibutyl Phthalate	1.504	8892	>5000	2.566	368	300-2000	-0.987
Dichlorvos (DDVP)	-0.576	59	50-300	2.407	255	50-300	-1.315
Diethyl Phthalate	1.622	9311	>5000	2.798	628	300-2000	-0.266
Digoxin	-1.441	28	5-50	0.909	8	5-50	-5.889
Dimethylformamide	1.861	5305	>5000	3.471	2958	2000-5000	2.026
Diquat Dibromide Monohydrate	-0.355	160	50-300	2.261	182	50-300	-1.922
Disulfoton	-1.739	5	<5	2.928	848	300-2000	-0.007
Endosulfan	-1.165	28	5-50	2.146	140	50-300	-2.282
Ethanol	2.391	11324	>5000	3.512	3253	2000-5000	2.337
Ethyleneglycol	2.062	7161	>5000	3.744	5549	2000-5000	2.831
Fenpropathrin	-0.664	76	50-300	2.167	147	50-300	-2.158
Gibberellic Acid	1.241	6039	>5000	3.310	2040	2000-5000	0.916
Glutethimide	0.441	600	300-2000	2.857	720	300-2000	-0.098
Glycerol	2.332	19770	>5000	3.658	4553	2000-5000	2.429
Haloperidol	-0.057	330	300-2000	2.220	166	50-300	-2.049
Hexachlorophene	-0.696	82	50-300	1.451	28	5-50	-4.149
Lactic Acid	1.606	3635	2000-5000	3.183	1524	300-2000	1.161
Lindane	-0.464	100	50-300	2.497	314	300-2000	-1.191
Lithium carbonate	0.902	590	300-2000	3.017	1040	300-2000	0.801
Meprobamate	0.803	1387	300-2000	2.973	941	300-2000	0.213
Mercury Chloride	-0.830	40	5-50	2.308	203	50-300	-1.671
Methanol	2.434	8710	>5000	3.209	1616	300-2000	1.679
Nicotine	-0.367	70	50-300	2.778	600	300-2000	-0.182

NHK NRU Predictions: RC Rat-Only Millimole Regression
Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621

Reference Substance[1]	Log Reference LD_{50} (mmol/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	NHK Log IC_{50} (mM)[6]
Paraquat	-0.443	93	50-300	2.690	489	300-2000	-0.621
Parathion	-1.679	6	5-50	2.575	376	300-2000	-0.983
Phenobarbital	-0.016	224	50-300	3.010	1024	300-2000	0.285
Phenol	0.765	548	300-2000	2.722	527	300-2000	-0.098
Phenylthiourea	-1.705	3	<5	2.964	920	300-2000	0.344
Physostigmine	-1.741	5	<5	2.748	560	300-2000	-0.493
Potassium Cyanide	-0.956	7	5-50	2.568	370	50-300	-0.352
Potassium chloride	1.575	2802	2000-5000	3.270	1862	300-2000	1.477
Procainamide HCl	0.856	1950	300-2000	3.230	1697	2000-5000	0.807
Propranolol	0.197	466	300-2000	2.604	402	300-2000	-0.912
Sodium Arsenite	-0.474	44	5-50	1.904	80	5-50	-2.435
Sodium Chloride	1.841	4050	2000-5000	3.252	1786	300-2000	1.534
Sodium Dichromate Dihydrate	-0.771	50	50-300	1.969	93	50-300	-2.622
Sodium Hypochlorite	2.142	10328	>5000	3.206	1606	300-2000	1.305
Sodium Oxalate	0.674	633	300-2000	2.965	923	300-2000	0.404
Sodium fluoride	0.480	127	50-300	2.652	449	50-300	0.066
Sodium selenate	-1.799	3	<5	2.399	251	50-300	-1.267
Strychnine	-1.725	6	5-50	2.687	486	300-2000	-0.743
Thallium Sulfate	-1.305	25	5-50	1.719	52	50-300	-3.522
Trichloroacetic Acid	1.505	5229	>5000	2.997	994	300-2000	0.403
Triethylenemelamine	-1.708	4	<5	2.124	133	50-300	-2.042
Triphenyltin Hydroxide	-0.047	329	300-2000	1.281	19	5-50	-4.562
Valproic Acid	0.839	996	300-2000	3.032	1076	300-2000	0.550
Verapamil HCl	-0.646	111	50-300	2.702	503	300-2000	-0.869
Xylene	1.643	4665	2000-5000	3.016	1039	300-2000	0.642

[1]Three chemicals were excluded because no rat oral LD_{50} was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride was excluded because IC_{50} values could not be determined.

[2]Reference LD_{50} in mmol/kg from **Table 4-2**. Reference rat oral LD_{50} values were developed from rat acute oral LD_{50} studies located using literature searches, secondary references, and electronic database searches.

[3]Globally Harmonized System (GHS) hazard classification (UN 2005):

Abbreviation	Category	Oral LD_{50} Limits
<5	1	$LD_{50} \leq 5$ mg/kg
5-50	2	$5 < LD_{50} \leq 50$ mg/kg
50-300	3	$50 < LD_{50} \leq 300$ mg/kg
300-2000	4	$300 < LD_{50} \leq 2000$ mg/kg
2000-5000	5	$2000 < LD_{50} \leq 5000$ mg/kg
>5000	Unclassified	$LD_{50} > 5000$ mg/kg

[4]LD_{50} determined using NRU IC_{50} in RC rat-only millimole regression: Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621

[5]LD_{50} in mg/kg (converted from results of RC rat-only millimole regression)

[6]Combined NHK IC_{50} values from three laboratories

Appendix J5

3T3 NRU Predictions: RC Rat-Only Weight Regression

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3T3 NRU Predictions: RC Rat-Only Weight Regression
Log LD_{50} (mg/kg) = 0.372 log IC_{50} (ug/mL) + 2.024

Reference Substance[1]	Log Reference LD_{50} (mg/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mg/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	3T3 Log IC_{50} (ug/mL)[6]
1,1,1-Trichloroethane	4.082	12078	>5000	3.628	5078	>5000	4.311
2-Propanol	3.708	5105	>5000	3.342	1494	300-2000	3.543
5-Aminosalicylic Acid	3.535	3428	2000-5000	3.223	1825	300-2000	3.222
Acetaminophen	3.335	2162	2000-5000	2.648	381	300-2000	1.678
Acetonitrile	3.556	3595	2000-5000	3.475	1731	300-2000	3.900
Acetylsalicylic Acid	3.178	1506	300-2000	3.077	1346	300-2000	2.830
Aminopterin	0.845	7	5-50	1.207	14	5-50	-2.195
Amitriptyline HCl	2.543	349	300-2000	2.340	248	50-300	0.848
Arsenictrioxide	1.400	25	5-50	2.142	112	50-300	0.316
Atropine Sulfate	2.913	819	300-2000	2.724	1099	300-2000	1.881
Boric Acid	3.535	3426	2000-5000	3.239	1149	300-2000	3.267
Busulfan	1.084	12	5-50	2.727	620	300-2000	1.890
Cadmium chloride	2.131	135	50-300	1.918	58	50-300	-0.286
Caffeine	2.491	310	300-2000	2.836	730	300-2000	2.183
Carbamazepine	3.448	2807	2000-5000	2.773	686	300-2000	2.014
Chloral Hydrate	2.805	638	300-2000	2.866	723	300-2000	2.263
Chloramphenicol	3.543	3490	2000-5000	2.811	906	300-2000	2.115
Citric Acid	3.773	5929	>5000	3.097	1472	300-2000	2.884
Cupric Sulfate Pentahydrate	2.677	475	300-2000	2.610	455	300-2000	1.576
Cycloheximide	0.301	2	<5	1.753	47	5-50	-0.727
Dibutyl Phthalate	3.949	8892	>5000	2.633	514	300-2000	1.637
Dichlorvos (DDVP)	1.769	59	50-300	2.489	305	300-2000	1.249
Diethyl Phthalate	3.969	9311	>5000	2.779	674	300-2000	2.031
Digoxin	1.451	28	5-50	3.009	2550	2000-5000	2.649
Dimethylformamide	3.725	5305	>5000	3.407	1990	300-2000	3.718
Diquat Dibromide Monohydrate	2.204	160	50-300	2.361	284	50-300	0.905
Disulfoton	0.699	5	<5	2.992	1352	300-2000	2.602
Endosulfan	1.444	28	5-50	2.310	265	50-300	0.770
Ethanol	4.054	11324	>5000	3.443	1693	300-2000	3.814
Ethyleneglycol	3.855	7161	>5000	3.656	3574	2000-5000	4.388
Fenpropathrin	1.879	76	50-300	2.533	445	300-2000	1.368
Gibberellic Acid	3.781	6039	>5000	3.472	5683	>5000	3.893
Glutethimide	2.778	600	300-2000	2.864	838	300-2000	2.258
Glycerol	4.296	19770	>5000	3.656	4452	2000-5000	4.386
Haloperidol	2.519	330	300-2000	2.317	258	50-300	0.787
Hexachlorophene	1.914	82	50-300	2.256	228	50-300	0.623
Lactic Acid	3.561	3635	2000-5000	3.320	1765	300-2000	3.483
Lindane	2.000	100	50-300	2.786	798	300-2000	2.047
Lithium carbonate	2.771	590	300-2000	3.047	752	300-2000	2.749
Meprobamate	3.142	1387	300-2000	3.025	1301	300-2000	2.690
Mercury Chloride	1.604	40	5-50	2.253	180	50-300	0.615
Nicotine	1.843	70	50-300	2.975	963	300-2000	2.557

3T3 NRU Predictions: RC Rat-Only Weight Regression
Log LD_{50} (mg/kg) = 0.372 log IC_{50} (ug/mL) + 2.024

Reference Substance[1]	Log Reference LD_{50} (mg/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mg/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	3T3 Log IC_{50} (ug/mL)[6]
Paraquat	1.967	93	50-300	2.509	351	300-2000	1.304
Parathion	0.785	6	5-50	2.609	494	300-2000	1.573
Phenobarbital	2.350	224	50-300	3.054	1457	300-2000	2.768
Phenol	2.739	548	300-2000	2.702	337	300-2000	1.822
Phenylthiourea	0.477	3	<5	2.730	477	300-2000	1.898
Physostigmine	0.699	5	<5	2.554	412	300-2000	1.425
Potassium Cyanide	0.857	7	5-50	2.597	206	50-300	1.540
Potassium chloride	3.447	2802	2000-5000	3.345	1699	300-2000	3.551
Procainamide HCl	3.290	1950	300-2000	3.008	1404	300-2000	2.644
Propranolol	2.668	466	300-2000	2.452	325	300-2000	1.150
Sodium Arsenite	1.639	44	5-50	1.979	57	50-300	-0.120
Sodium Chloride	3.607	4050	2000-5000	3.392	1683	300-2000	3.676
Sodium Dichromate Dihydrate	1.703	50	50-300	1.938	81	50-300	-0.232
Sodium Hypochlorite	4.014	10328	>5000	3.146	990	300-2000	3.017
Sodium Oxalate	2.801	633	300-2000	2.608	319	300-2000	1.570
Sodium fluoride	2.103	127	50-300	2.728	230	50-300	1.892
Sodium selenate	0.477	3	<5	2.568	347	300-2000	1.463
Strychnine	0.799	6	5-50	2.842	1005	300-2000	2.198
Thallium Sulfate	1.398	25	5-50	2.297	288	50-300	0.735
Trichloroacetic Acid	3.718	5229	>5000	3.123	1445	300-2000	2.955
Triethylenemelamine	0.602	4	<5	1.814	47	5-50	-0.565
Triphenyltin Hydroxide	2.517	329	300-2000	1.368	19	5-50	-1.764
Valproic Acid	2.998	996	300-2000	3.109	1296	300-2000	2.917
Verapamil HCl	2.045	111	50-300	2.598	643	300-2000	1.543
Xylene	3.669	4665	2000-5000	3.087	1028	300-2000	2.858

[1]Three chemicals were excluded because no rat oral LD_{50} was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride and methanol were excluded because IC_{50} values could not be determined.

[2]Reference LD_{50} in mmol/kg from **Table 4-2**. Reference rat oral LD_{50} values were developed from rat acute oral LD_{50} studies located using literature searches, secondary references, and electronic database searches.

[3]Globally Harmonized System (GHS) hazard classification (UN 2005):

Abbreviation	Category	Oral LD_{50} Limits
<5	1	$LD_{50} \leq 5$ mg/kg
5-50	2	$5 < LD_{50} \leq 50$ mg/kg
50-300	3	$50 < LD_{50} \leq 300$ mg/kg
300-2000	4	$300 < LD_{50} \leq 2000$ mg/kg
2000-5000	5	$2000 < LD_{50} \leq 5000$ mg/kg
>5000	Unclassified	$LD_{50} > 5000$ mg/kg

[4]LD_{50} determined using NRU IC_{50} in RC rat-only weight regression: Log LD_{50} (mg/kg) = 0.372 log IC_{50} (ug/mL) + 2.024

[5]LD_{50} in mg/kg (converted from results of RC rat-only weight regression)

[6]Combined 3T3 IC_{50} values from three laboratories

Appendix J6

NHK NRU Predictions: RC Rat-Only Weight Regression

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NHK NRU Predictions: RC Rat-Only Weight Regression
Log LD_{50} (mg/kg) = 0.372 log IC_{50} (ug/mL) + 2.024

Reference Substance[1]	Log Reference LD_{50} (mg/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mg/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	NHK Log IC_{50} (ug/mL)[6]
1,1,1-Trichloroethane	1.957	12078	>5000	3.478	3009	2000-5000	3.910
2-Propanol	1.929	5105	>5000	3.411	2579	300-2000	3.730
5-Aminosalicylic Acid	1.350	3428	2000-5000	2.645	442	300-2000	1.669
Acetaminophen	1.155	2162	2000-5000	3.034	1081	300-2000	2.714
Acetonitrile	1.942	3595	2000-5000	3.505	3196	300-2000	3.980
Acetylsalicylic Acid	0.922	1506	300-2000	3.059	1145	300-2000	2.782
Aminopterin	-1.799	7	5-50	3.073	1184	2000-5000	2.821
Amitriptyline HCl	0.046	349	300-2000	2.378	239	50-300	0.952
Arsenictrioxide	-0.897	25	5-50	2.335	216	50-300	0.835
Atropine Sulfate	0.071	819	300-2000	2.736	544	300-2000	1.913
Boric Acid	1.744	3426	2000-5000	3.000	1001	300-2000	2.625
Busulfan	-1.308	12	5-50	2.922	836	300-2000	2.415
Cadmium chloride	-0.132	135	50-300	2.119	131	50-300	0.255
Caffeine	0.203	310	300-2000	3.067	1168	300-2000	2.805
Carbamazepine	1.075	2807	2000-5000	2.738	547	300-2000	1.920
Chloral Hydrate	0.586	638	300-2000	2.814	652	300-2000	2.125
Chloramphenicol	1.033	3490	2000-5000	2.968	929	300-2000	2.538
Citric Acid	1.489	5929	>5000	2.997	992	300-2000	2.614
Cupric Sulfate Pentahydrate	0.279	475	300-2000	2.877	754	300-2000	2.293
Cycloheximide	-2.148	2	<5	1.602	40	5-50	-1.134
Dibutyl Phthalate	1.504	8892	>5000	2.566	368	300-2000	1.458
Dichlorvos (DDVP)	-0.576	59	50-300	2.407	255	50-300	1.029
Diethyl Phthalate	1.622	9311	>5000	2.798	628	300-2000	2.081
Digoxin	-1.441	28	5-50	0.909	8	5-50	-2.996
Dimethylformamide	1.861	5305	>5000	3.471	2958	2000-5000	3.890
Diquat Dibromide Monohydrate	-0.355	160	50-300	2.261	182	50-300	0.637
Disulfoton	-1.739	5	<5	2.928	848	300-2000	2.431
Endosulfan	-1.165	28	5-50	2.146	140	50-300	0.328
Ethanol	2.391	11324	>5000	3.512	3253	2000-5000	4.001
Ethyleneglycol	2.062	7161	>5000	3.744	5549	2000-5000	4.624
Fenpropathrin	-0.664	76	50-300	2.167	147	50-300	0.385
Gibberellic Acid	1.241	6039	>5000	3.310	2040	2000-5000	3.456
Glutethimide	0.441	600	300-2000	2.857	720	300-2000	2.239
Glycerol	2.332	19770	>5000	3.658	4553	2000-5000	4.393
Haloperidol	-0.057	330	300-2000	2.220	166	50-300	0.526
Hexachlorophene	-0.696	82	50-300	1.451	28	5-50	-1.540
Lactic Acid	1.606	3635	2000-5000	3.183	1524	300-2000	3.115
Lindane	-0.464	100	50-300	2.497	314	300-2000	1.272
Lithium carbonate	0.902	590	300-2000	3.017	1040	300-2000	2.670
Meprobamate	0.803	1387	300-2000	2.973	941	300-2000	2.552
Mercury Chloride	-0.830	40	5-50	2.308	203	50-300	0.763
Methanol	2.434	8710	>5000	3.209	1616	300-2000	3.184
Nicotine	-0.367	70	50-300	2.778	600	300-2000	2.028

NHK NRU Predictions: RC Rat-Only Weight Regression
Log LD_{50} (mg/kg) = 0.372 log IC_{50} (ug/mL) + 2.024

Reference Substance[1]	Log Reference LD_{50} (mg/kg)[2]	Reference LD_{50} (mg/kg)[2]	Observed Toxicity Category[3] (mg/kg)	Log Predicted LD_{50} (mg/kg)[4]	Predicted LD_{50} (mg/kg)[5]	Predicted Toxicity Category[3] (mg/kg)	NHK Log IC_{50} (ug/mL)[6]
Paraquat	-0.443	93	50-300	2.690	489	300-2000	1.790
Parathion	-1.679	6	5-50	2.575	376	300-2000	1.481
Phenobarbital	-0.016	224	50-300	3.010	1024	300-2000	2.651
Phenol	0.765	548	300-2000	2.722	527	300-2000	1.875
Phenylthiourea	-1.705	3	<5	2.964	920	300-2000	2.527
Physostigmine	-1.741	5	<5	2.748	560	300-2000	1.947
Potassium Cyanide	-0.956	7	5-50	2.568	370	50-300	1.462
Potassium chloride	1.575	2802	2000-5000	3.270	1862	300-2000	3.350
Procainamide HCl	0.856	1950	300-2000	3.230	1697	2000-5000	3.241
Propranolol	0.197	466	300-2000	2.604	402	300-2000	1.559
Sodium Arsenite	-0.474	44	5-50	1.904	80	5-50	-0.322
Sodium Chloride	1.841	4050	2000-5000	3.252	1786	300-2000	3.300
Sodium Dichromate Dihydrate	-0.771	50	50-300	1.969	93	50-300	-0.148
Sodium Hypochlorite	2.142	10328	>5000	3.206	1606	300-2000	3.177
Sodium Oxalate	0.674	633	300-2000	2.965	923	300-2000	2.531
Sodium fluoride	0.480	127	50-300	2.652	449	50-300	1.689
Sodium selenate	-1.799	3	<5	2.399	251	50-300	1.009
Strychnine	-1.725	6	5-50	2.687	486	300-2000	1.781
Thallium Sulfate	-1.305	25	5-50	1.719	52	50-300	-0.819
Trichloroacetic Acid	1.505	5229	>5000	2.997	994	300-2000	2.616
Triethylenemelamine	-1.708	4	<5	2.124	133	50-300	0.268
Triphenyltin Hydroxide	-0.047	329	300-2000	1.281	19	5-50	-1.998
Valproic Acid	0.839	996	300-2000	3.032	1076	300-2000	2.709
Verapamil HCl	-0.646	111	50-300	2.702	503	300-2000	1.823
Xylene	1.643	4665	2000-5000	3.016	1039	300-2000	2.668

[1]Three chemicals were excluded because no rat oral LD_{50} was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride was excluded because IC_{50} values could not be determined.
[2]Reference LD_{50} in mmol/kg from **Table 4-2**. Reference rat oral LD_{50} values were developed from rat acute oral LD_{50} studies located using literature searches, secondary references, and electronic database searches.
[3]Globally Harmonized System (GHS) hazard classification (UN 2005):

Abbreviation	Category	Oral LD_{50} Limits
<5	1	$LD_{50} \leq 5$ mg/kg
5-50	2	$5 < LD_{50} \leq 50$ mg/kg
50-300	3	$50 < LD_{50} \leq 300$ mg/kg
300-2000	4	$300 < LD_{50} \leq 2000$ mg/kg
2000-5000	5	$2000 < LD_{50} \leq 5000$ mg/kg
>5000	Unclassified	$LD_{50} > 5000$ mg/kg

[4]LD_{50} determined using NRU IC_{50} in RC rat-only weight regression: Log LD_{50} (mg/kg) = 0.372 log IC_{50} (ug/mL) + 2.024
[5]LD_{50} in mg/kg (converted from results of RC rat-only weight regression)
[6]Combined NHK IC_{50} values from three laboratories

Appendix J7

Comparison of Millimole Regression with Weight Regression Regarding Prediction of Toxicity (LD_{50}) for Low or High Molecular Weight Chemicals

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J.7 The Prediction of Toxicity for High and Low Molecular Weight Substances Using Millimole vs. Weight-Based Regressions

The ICCVAM Acute Toxicity Working Group expressed some concern that the RC rat-only weight regression may less accurately (than the RC rat-only millimole regression) predict the toxicity of low molecular weight substances and high molecular weight substances. Using the RC IC_{50} and LD_{50} values for the 282 RC substances with rat oral LD_{50} data, analyses were performed to

- Determine the difference in the over and under-prediction rates of acute oral toxicity (i.e., LD_{50}) from IC_{50} values for low molecular weight substances (i.e., molecular weight ≤100 g/mole) vs. substances with higher molecular weights
- Determine the difference in the over and under-prediction rates of acute oral toxicity from IC_{50} values for high molecular weight substances (i.e., molecular weight ≥400 g/mole) vs. substances with lower molecular weights
- Compare the RC rat-only millimole regression with the RC rat-only weight regression with respect to the over and under-prediction rates of the toxicity of low and high molecular weight substances

J.7.1 Methods

The data used for to evaluate the over- and under-prediction rates of toxicity of low or high molecular weight chemicals were the RC data rather than the NICEATM/ECVAM validation study data because the RC contains data for many more substances. The RC IC_{50} and LD_{50} values for the 282 RC substances with rat oral LD_{50} data were used since substances with rat data are the focus of the BRD with respect to the prediction of oral LD_{50} (and starting dose for acute oral toxicity testing) from IC_{50} (see **Appendix K-3** for the data used). Over- or under-prediction of toxicity was determined by subtracting the predicted LD_{50} in mg/kg (i.e., the rat oral LD_{50} calculated using the RC IC_{50} in the regression equation) from the observed LD_{50} in mg/kg (i.e., the *in vivo* rat oral LD_{50} from the RC that was used to develop the regression). Negative values indicated that toxicity was underpredicted by the regression (i.e., predicted LD_{50} was greater than observed LD_{50}) and positive values indicated that toxicity was overpredicted by the regression (i.e., predicted LD_{50} was less than observed LD_{50}). This analysis assumed that the regressions either underpredicted or overpredicted the toxicity of all of the substances evaluated. In other words, there was a difference between the LD_{50} predicted by the regression and the *in vivo* LD_{50} used to calculate the regression even if it was a tiny fraction (i.e., no substances fit the regression exactly).

The proportion of low or high molecular weight chemicals that were under- and over-predicted in terms of acute oral toxicity (i.e., predicted LD_{50} values were higher or lower than reported *in vivo* LD_{50} values, respectively) using a millimole regression were calculated. These proportions were compared with those for chemicals that did not have low or high molecular weights. The same calculations were then performed for a weight-based regression. The proportions of under- and over-prediction of the toxicity for the millimole and weight-based regressions were compared to determine whether the weight regression increased the proportion of low molecular weight chemicals for which toxicity was underpredicted or the proportion of high molecular weight chemicals for which toxicity was overpredicted.

The millimole regression used was the RC rat-only millimole regression. The RC rat-only regression in millimole units, was calculated using the IC_{50} and oral LD_{50} data from the 282 RC chemicals with rat oral LD_{50} values and is strikingly similar in slope and intercept to the original RC millimole regression, which was based on 347 chemicals (282 chemicals with rat LD_{50} data and 65 chemicals with mouse LD_{50} data) (see **Table J7-1**). The weight-based regression used was the RC rat-only weight regression calculated using the IC_{50} and oral LD_{50} values from the 282 RC chemicals with rat oral LD_{50} values (see **Table J7-1**).

Table J7-1 IC_{50}-LD_{50} Linear Regressions

Moniker	Data Used	Slope	Intercept	R^2
RC millimole regression	347 RC substances with oral rat and mouse LD_{50} data – millimole units[1]	0.435	0.625	0.452
RC rat-only millimole regression	282 RC substances with rat oral LD_{50} data – millimole units[1]	0.439	0.621	0.452
RC rat-only weight regression	282 RC substances with rat oral LD_{50} data – weight units[2]	0.372	2.024	0.325

Abbreviations: RC=Registry of Cytotoxicity; R^2=coefficient of determination
[1]IC_{50} in mM; LD_{50} in mmol/kg.
[2]IC_{50} in µg/mL; LD_{50} in mg/kg.

J.7.2 Results

Figures J7-1 and **J7-2** show either the low molecular weight or high molecular weight chemicals plotted with either the RC rat-only millimole regression or the RC rat-only weight regression. Since LD_{50} is inversely related to toxicity, low LD_{50} values indicate high toxicity and high LD_{50} values indicate low toxicity. The regression lines show the predicted LD_{50} for each IC_{50}. The regression lines underpredict the toxicity of chemicals that are plotted below the lines (i.e., predicted LD_{50} > *in vivo* LD_{50} and predicted toxicity < *in vivo* toxicity). The regression lines overpredict the toxicity of chemicals that are plotted above the lines (i.e., predicted LD_{50} < *in vivo* LD_{50} and predicted toxicity > *in vivo* toxicity).

Of the 282 RC substances with rat oral LD_{50} values, there were 51 substances with molecular weights ≤100 g/mole and 231 substances with molecular weights >100 g/mole. **Figure J7-1** shows the 51 low molecular weight chemicals (i.e., with molecular weight ≤100 g/mole) graphed with both the RC rat-only millimole regression (**Figure J7-1a**) and the RC rat-only weight regression (**Figure J7-1b**). The RC rat-only millimole regression underestimated the toxicity of 20/51 (39%) substances and overestimated the toxicity of 31/51 (61%) substances (see **Table J7-2**). The RC rat-only weight regression underestimated the toxicity of 24/51 (47%) substances and overestimated the toxicity of 27/51 (53%) substances. Fisher's exact test indicated that there was no difference between the millimole and weight regressions for the under and over-prediction rates of toxicity for the 51 low molecular weight substances (two-tailed p=0.549) (see **Table J7-3**).

Figure J7-1 Rat-only Regressions Graphed with 51 Chemicals with Molecular Weight ≤100 g/mole

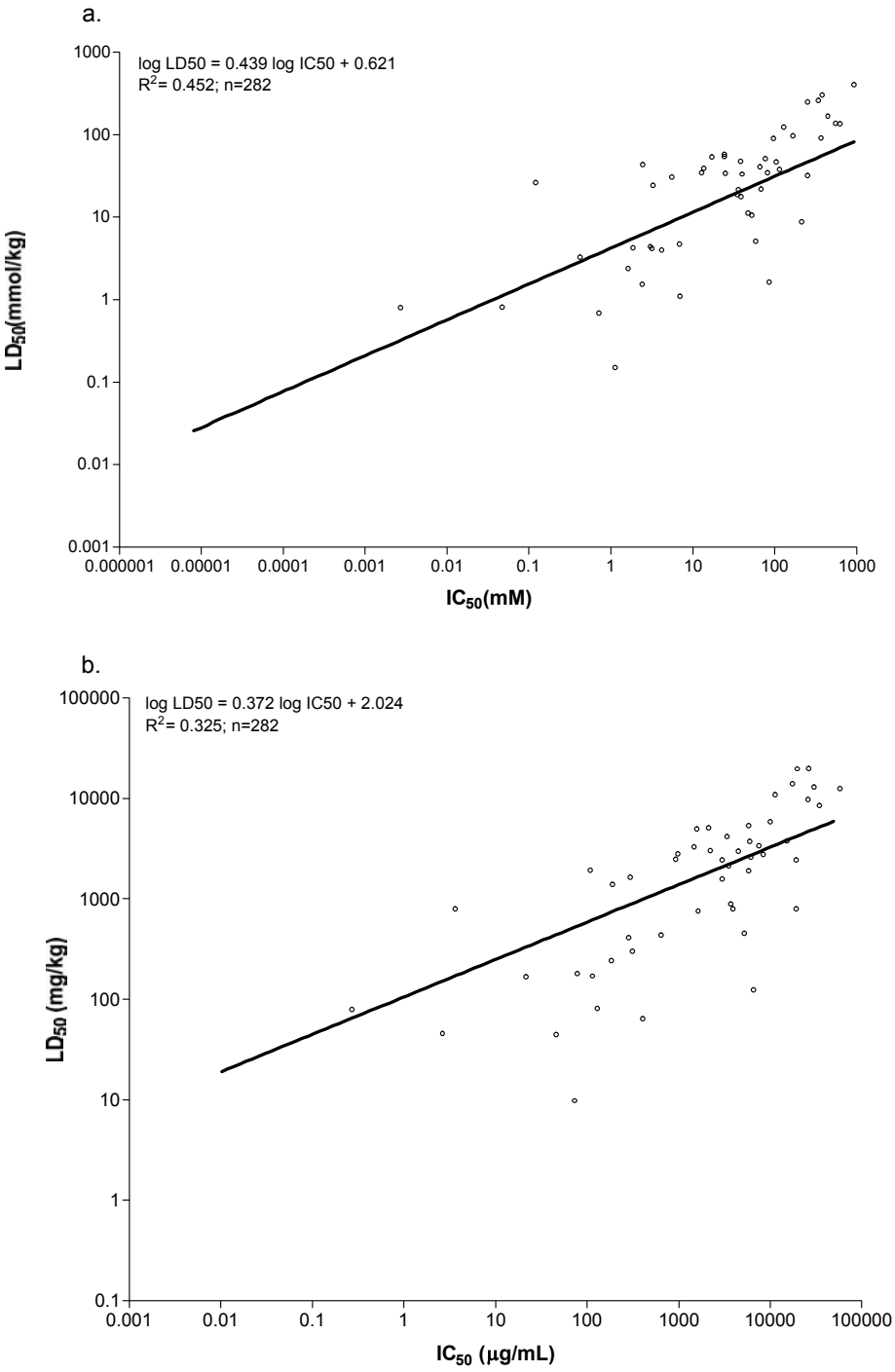

Figure J7-1a shows the RC rat-only millimole regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 20/51 (39%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 31/51 (61%) chemicals. **Figure J7-1b** shows the RC rat-only weight regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 24/51 (47%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 27/51 (53%) chemicals.

Table J7-2 Over- and Under Prediction of Toxicity for Low and High Molecular Weight Chemicals Using RC Rat-only Weight and Millimole Regressions

Regression	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted
	51 Chemicals with Molecular Weight ≤100 g/mole		231 Chemicals with Molecular Weight >100 g/mole	
RC Rat-only Weight	24/51 (47%)	27/51 (53%)	101/231 (44%)	130/231 (57%)
RC Rat-only Millimole	20/51 (39%)	31/51 (61%)	108/231 (47%)	123/231 (53%)
	20 Chemicals with Molecular Weight ≥400 g/mole		262 Chemicals with Molecular Weight <400 g/mole	
RC Rat-only Weight	4/20 (20%)	16/20 (80%)	121/262 (46%)	141/262 (54%)
RC Rat-only Millimole	7/20 (35%)	13/20 (65%)	121/262 (46%)	141/262 (54%)

Table J7-3 Over- and Under Prediction of Toxicity for Low and High Molecular Weight Substances Using RC Rat-Only Weight and Millimole Regressions

Comparison	For	Fisher's Exact Test[1]
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 51 substances with molecular weight ≤100 g/mole	0.549
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 231 substances with molecular weight >100 g/mole	0.575
51 Low molecular weight (≤100 g/mole) substances vs. 231 other substances (>100 g/mole)	RC rat-only millimole regression	0.355
51 Low molecular weight (≤100 g/mole) substances vs. 231 other substances (>100 g/mole)	RC rat-only weight regression	0.756
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 20 substances with molecular weight ≥400 g/mole	0.480
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 262 substances with molecular weight <400 g/mole	NT
20 High molecular weight substances (≥400 g/mole) vs. 262 other substances (<400 g/mole)	RC rat-only millimole regression	0.362
20 High molecular weight substances (≥400 g/mole) vs. 262 other substances (<400 g/mole)	RC rat-only weight regression	0.033

Abbreviations: RC=Registry of Cytotoxicity; NT=Not tested since the proportions were the same.
Toxicity was underpredicted for 121/262 (46%) substances and overpredicted for 141/262 (54%) substances.
[1]P-values.

For the 231 substances with molecular weights >100 g/mole, the RC rat-only millimole regression underestimated the toxicity of 108/231 (47%) substances and overestimated the toxicity of 123/231 (53%) substances (see **Table J7-2**). The RC rat-only weight regression underestimated the toxicity of 101/231 (44%) substances and overestimated the toxicity of 130/231 (57%) substances. Fisher's exact test indicated that there was no difference between the millimole and weight regressions for the under- and over-prediction rates for the 231 substances with molecular weight >100 g/mole (two-tailed p=0.575; see **Table J7-3**). Additionally, Fisher's exact test also showed that there was no difference in the under- and over-prediction rates for the 51 substances with molecular weight ≤100 g/mole compared to the under- and over-prediction of the toxicity of the 231 substances with molecular weight >100 g/mole (two-tailed p=0.756 for the RC rat-only weight regression and two-tailed p=0.355 for the RC rat-only millimole regression).

Of the 282 RC substances with rat oral LD_{50} values, there were 20 substances with molecular weights ≥400 g/mole and 262 substances with molecular weights <400 g/mole (see **Table J7-2**). **Figure J7-2** shows the 20 chemicals with molecular weights ≥400 g/mole plotted with the RC rat-only milllimole regression (**Figure J7-2a**) and the RC rat-only weight regression (**Figure J7-2b**). The RC rat-only millimole regression underestimated the toxicity of 7/20 (35%) substances and overestimated the toxicity of 13/20 (65%) substances (see **Table J7-2**). The RC rat-only weight regression underestimated the toxicity of 4/20 (20%) substances and overestimated the toxicity of 16/20 (80%) substances. Fisher's exact test indicated that there was no difference between the millimole and weight regressions for the under- and over-prediction of toxicity for the 20 high molecular weight substances (two-tailed p=0.480; see **Table J7-3**).

For the remaining 262 substances with molecular weights <400 g/mole, the RC rat-only millimole and the RC rat-only weight regressions both underestimated the toxicity of 121/262 (46%) substances and overestimated toxicity of 141/262 (54%) substances (see **Table J7-2**). Thus, there was no difference in the two regressions in the rates of under- and over-estimation of toxicity for the 262 substances with molecular weights <400 g/mole. Fisher's exact test also showed that there was no difference in the rates for under- and over-prediction of the toxicity of substances with high molecular weight (≥400 g/mole) compared with the under- and over-prediction of the toxicity of substances with lower molecular weight for the RC rat-only millimole regression (two-tailed p=0.362; see **Table J7-3**). For the RC rat-only weight regression, however, there was a significant difference in the under- and over-prediction rates for substances with high molecular weight (>400 g/mole) compared with the under- and over-prediction rates for substances with lower molecular weight (two-tailed p =0.033). Thus, the weight-based regression overestimated the toxicity of the high molecular weight substances (compared with substances with lower molecular weight) while the millimole regression did not.

Figure J7-2 Rat-only Regressions Graphed with 20 Chemicals with Molecular Weight ≥400 g/mole

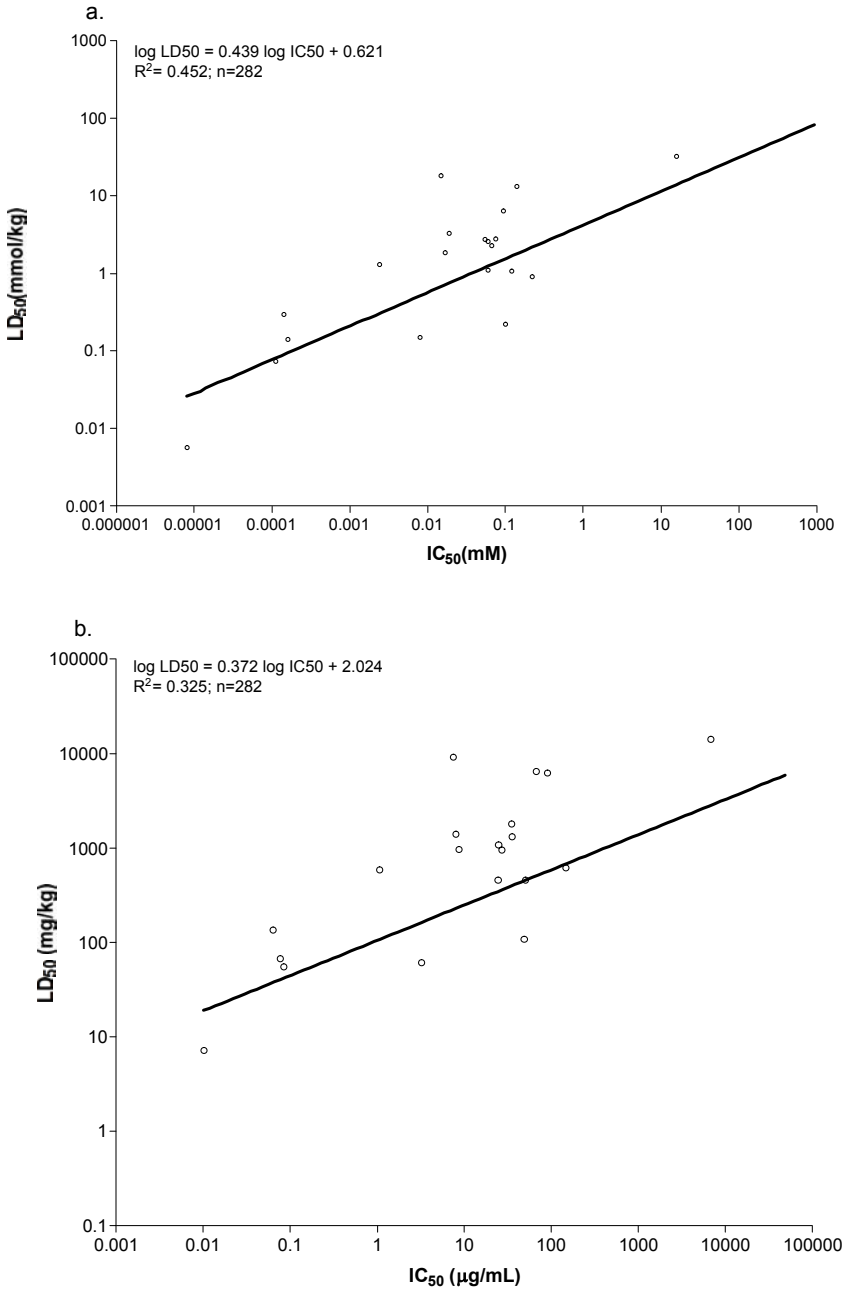

Figure J7-2a shows the RC rat-only millimole regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 7/20 (35%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 13/20 (65%) chemicals. **Figure J7-2b** shows the RC rat-only weight regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 4/20 (20%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 16/20 (80%) chemicals.

Appendix K

IC_{50} and LD_{50} Data for Regressions

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Appendix K1

IC_{50} and LD_{50} Values Used for Laboratory-Specific Regressions

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IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	1,1,1-Trichloroethane	ECBC	2.489	1.957	308.185	90.534	133.410	41114.974	12078
3T3	1,1,1-Trichloroethane	FAL	2.200	1.957	158.512	90.534	133.410	21147.061	12078
3T3	1,1,1-Trichloroethane	IIVS	1.868	1.957	73.758	90.534	133.410	9840.111	12078
3T3	2-Propanol	ECBC	1.637	1.929	43.328	84.928	60.110	2604.458	5105
3T3	2-Propanol	FAL	1.820	1.929	66.019	84.928	60.110	3968.400	5105
3T3	2-Propanol	IIVS	1.835	1.929	68.340	84.928	60.110	4107.888	5105
3T3	5-Aminosalicylic acid	ECBC	0.979	1.350	9.529	22.391	153.100	1458.814	3428
3T3	5-Aminosalicylic acid	FAL	1.127	1.350	13.387	22.391	153.100	2049.588	3428
3T3	5-Aminosalicylic acid	IIVS	1.005	1.350	10.116	22.391	153.100	1548.817	3428
3T3	Acetaminophen	ECBC	-0.577	1.155	0.265	14.299	151.200	40.087	2162
3T3	Acetaminophen	FAL	-0.375	1.155	0.421	14.299	151.200	63.728	2162
3T3	Acetaminophen	IIVS	-0.553	1.155	0.280	14.299	151.200	42.364	2162
3T3	Acetonitrile	ECBC	2.195	1.942	156.682	87.576	41.050	6431.812	3595
3T3	Acetonitrile	FAL	2.312	1.942	204.968	87.576	41.050	8413.951	3595
3T3	Acetonitrile	IIVS	2.355	1.942	226.301	87.576	41.050	9289.664	3595
3T3	Acetylsalicylic acid	ECBC	0.553	0.922	3.572	8.357	180.200	643.675	1506
3T3	Acetylsalicylic acid	FAL	0.827	0.922	6.708	8.357	180.200	1208.741	1506
3T3	Acetylsalicylic acid	IIVS	0.344	0.922	2.208	8.357	180.200	397.802	1506
3T3	Aminopterin	ECBC	-4.926	-1.799	0.000012	0.016	440.470	0.0052	7.00
3T3	Aminopterin	FAL	-4.612	-1.799	0.000024	0.016	440.470	0.011	7.00
3T3	Aminopterin	IIVS	-4.980	-1.799	0.000010	0.016	440.470	0.005	7.00
3T3	Amitriptyline HCl	ECBC	-1.724	0.046	0.019	1.112	313.900	5.920	349
3T3	Amitriptyline HCl	FAL	-1.611	0.046	0.024	1.112	313.900	7.681	349
3T3	Amitriptyline HCl	IIVS	-1.609	0.046	0.025	1.112	313.900	7.719	349
3T3	Arsenic III trioxide	ECBC	-1.937	-0.897	0.012	0.127	197.840	2.285	25.1
3T3	Arsenic III trioxide	FAL	-2.278	-0.897	0.005	0.127	197.840	1.042	25.1
3T3	Arsenic III trioxide	IIVS	-1.724	-0.897	0.019	0.127	197.840	3.731	25.1
3T3	Atropine sulfate	ECBC	-1.151	0.071	0.071	1.179	694.800	49.128	819
3T3	Atropine sulfate	FAL	-0.734	0.071	0.184	1.179	694.800	128.135	819
3T3	Atropine sulfate	IIVS	-0.998	0.071	0.100	1.179	694.800	69.823	819
3T3	Boric acid	ECBC	1.370	1.744	23.432	55.410	61.830	1448.772	3426
3T3	Boric acid	FAL	1.804	1.744	63.748	55.410	61.830	3941.547	3426
3T3	Boric acid	IIVS	1.254	1.744	17.939	55.410	61.830	1109.175	3426
3T3	Busulfan	ECBC	-0.827	-1.308	0.149	0.049	246.310	36.700	12.1
3T3	Busulfan	FAL	0.075	-1.308	1.187	0.049	246.310	292.415	12.1
3T3	Busulfan	IIVS	-0.751	-1.308	0.177	0.049	246.310	43.685	12.1
3T3	Cadmium II chloride	ECBC	-2.585	-0.132	0.0026	0.738	183.300	0.477	135
3T3	Cadmium II chloride	FAL	-2.675	-0.132	0.0021	0.738	183.300	0.387	135
3T3	Cadmium II chloride	IIVS	-2.387	-0.132	0.0041	0.738	183.300	0.752	135

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	Caffeine	ECBC	-0.165	0.203	0.684	1.596	194.200	132.841	310
3T3	Caffeine	FAL	-0.143	0.203	0.720	1.596	194.200	139.744	310
3T3	Caffeine	IIVS	-0.007	0.203	0.984	1.596	194.200	191.132	310
3T3	Carbamazepine	ECBC	-0.457	1.075	0.349	11.879	236.300	82.414	2807
3T3	Carbamazepine	FAL	-0.209	1.075	0.617	11.879	236.300	145.881	2807
3T3	Carbamazepine	IIVS	-0.412	1.075	0.387	11.879	236.300	91.411	2807
3T3	Chloral hydrate	ECBC	-0.041	0.586	0.910	3.857	165.400	150.545	638
3T3	Chloral hydrate	FAL	0.162	0.586	1.454	3.857	165.400	240.436	638
3T3	Chloral hydrate	IIVS	0.011	0.586	1.025	3.857	165.400	169.564	638
3T3	Chloramphenicol	ECBC	-0.776	1.033	0.168	10.800	323.150	54.147	3490
3T3	Chloramphenicol	FAL	-0.088	1.033	0.817	10.800	323.150	264.039	3490
3T3	Chloramphenicol	IIVS	-0.321	1.033	0.478	10.800	323.150	154.402	3490
3T3	Citric acid	ECBC	0.378	1.489	2.389	30.864	192.100	458.846	5929
3T3	Citric acid	FAL	0.774	1.489	5.943	30.864	192.100	1141.563	5929
3T3	Citric acid	IIVS	0.648	1.489	4.444	30.864	192.100	853.755	5929
3T3	Colchicine	ECBC	-4.292	-1.425	0.000051	0.038	399.480	0.020	15.0
3T3	Colchicine	FAL	-3.671	-1.425	0.000213	0.038	399.480	0.085	15.0
3T3	Colchicine	IIVS	-4.158	-1.425	0.000070	0.038	399.480	0.028	15.0
3T3	Cupric sulfate pentahydrate	ECBC	-0.480	0.279	0.331	1.902	249.700	82.667	475
3T3	Cupric sulfate pentahydrate	FAL	-0.333	0.279	0.465	1.902	249.700	116.078	475
3T3	Cupric sulfate pentahydrate	IIVS	-1.653	0.279	0.022	1.902	249.700	5.556	475
3T3	Cycloheximide	ECBC	-3.384	-2.148	0.00041	0.007	281.400	0.116	2.0
3T3	Cycloheximide	FAL	-2.726	-2.148	0.00188	0.007	281.400	0.529	2.0
3T3	Cycloheximide	IIVS	-3.420	-2.148	0.00038	0.007	281.400	0.107	2.0
3T3	Dibutyl phthalate	ECBC	-1.079	1.504	0.083	31.951	278.300	23.227	8892
3T3	Dibutyl phthalate	FAL	-0.214	1.504	0.611	31.951	278.300	169.922	8892
3T3	Dibutyl phthalate	IIVS	-1.129	1.504	0.074	31.951	278.300	20.670	8892
3T3	Dichlorvos	ECBC	-1.373	-0.576	0.042	0.266	220.980	9.358	58.7
3T3	Dichlorvos	FAL	-0.829	-0.576	0.148	0.266	220.980	32.759	58.7
3T3	Dichlorvos	IIVS	-1.084	-0.576	0.082	0.266	220.980	18.225	58.7
3T3	Diethyl phthalate	ECBC	-0.430	1.622	0.372	41.904	222.200	82.604	9311
3T3	Diethyl phthalate	FAL	-0.191	1.622	0.644	41.904	222.200	143.109	9311
3T3	Diethyl phthalate	IIVS	-0.328	1.622	0.470	41.904	222.200	104.472	9311
3T3	Digoxin	ECBC	-0.373	-1.441	0.424	0.036	780.900	330.877	28.3
3T3	Digoxin	FAL	0.039	-1.441	1.093	0.036	780.900	853.755	28.3
3T3	Digoxin	IIVS	-0.397	-1.441	0.401	0.036	780.900	312.968	28.3
3T3	Dimethylformamide	ECBC	1.862	1.861	72.848	72.572	73.100	5325.168	5305
3T3	Dimethylformamide	FAL	1.874	1.861	74.774	72.572	73.100	5465.962	5305
3T3	Dimethylformamide	IIVS	1.826	1.861	67.001	72.572	73.100	4897.788	5305

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	Diquat dibromide monohydra	ECBC	-1.978	-0.355	0.011	0.442	362.100	3.811	160
3T3	Diquat dibromide monohydra	FAL	-1.146	-0.355	0.071	0.442	362.100	25.882	160
3T3	Diquat dibromide monohydra	IIVS	-1.837	-0.355	0.015	0.442	362.100	5.268	160
3T3	Disulfoton	ECBC	-0.361	-1.739	0.435	0.018	274.420	119.402	5.0
3T3	Disulfoton	FAL	1.611	-1.739	40.796	0.018	274.420	11195.195	5.0
3T3	Disulfoton	IIVS	-0.760	-1.739	0.174	0.018	274.420	47.728	5.0
3T3	Endosulfan	ECBC	-1.933	-1.165	0.012	0.068	406.910	4.751	27.8
3T3	Endosulfan	FAL	-1.511	-1.165	0.031	0.068	406.910	12.554	27.8
3T3	Endosulfan	IIVS	-2.076	-1.165	0.008	0.068	406.910	3.416	27.8
3T3	Epinephrine bitartrate	ECBC	-0.813	-1.921	0.154	0.012	333.300	51.286	4.0
3T3	Epinephrine bitartrate	FAL	-0.723	-1.921	0.189	0.012	333.300	63.144	4.0
3T3	Epinephrine bitartrate	IIVS	-0.721	-1.921	0.190	0.012	333.300	63.338	4.0
3T3	Ethanol	ECBC	2.051	2.391	112.439	245.800	46.070	5180.043	11324
3T3	Ethanol	FAL	2.259	2.391	181.516	245.800	46.070	8362.446	11324
3T3	Ethanol	IIVS	2.143	2.391	139.075	245.800	46.070	6407.176	11324
3T3	Ethylene glycol	ECBC	2.469	2.062	294.295	115.351	62.080	18269.837	7161
3T3	Ethylene glycol	FAL	2.698	2.062	499.068	115.351	62.080	30982.150	7161
3T3	Ethylene glycol	IIVS	2.618	2.062	415.216	115.351	62.080	25776.589	7161
3T3	Fenpropathrin	ECBC	-1.191	-0.664	0.064	0.217	349.430	22.491	75.7
3T3	Fenpropathrin	FAL	-1.012	-0.664	0.097	0.217	349.430	33.982	75.7
3T3	Fenpropathrin	IIVS	-1.322	-0.664	0.048	0.217	349.430	16.647	75.7
3T3	Gibberellic acid	ECBC	1.363	1.241	23.074	17.436	346.380	7992.206	6039
3T3	Gibberellic acid	IIVS	1.343	1.241	22.035	17.436	346.380	7632.497	6039
3T3	Glutethimide	ECBC	-0.115	0.441	0.767	2.761	217.300	166.725	600
3T3	Glutethimide	FAL	0.117	0.441	1.308	2.761	217.300	284.228	600
3T3	Glutethimide	IIVS	-0.240	0.441	0.576	2.761	217.300	125.098	600
3T3	Glycerol	ECBC	2.334	2.332	215.835	214.681	92.090	19876.198	19770
3T3	Glycerol	FAL	2.477	2.332	299.942	214.681	92.090	27621.673	19770
3T3	Glycerol	IIVS	2.455	2.332	285.400	214.681	92.090	26282.499	19770
3T3	Haloperidol	ECBC	-1.851	-0.057	0.014	0.878	375.900	5.297	330
3T3	Haloperidol	FAL	-1.673	-0.057	0.021	0.878	375.900	7.977	330
3T3	Haloperidol	IIVS	-1.840	-0.057	0.014	0.878	375.900	5.440	330
3T3	Hexachlorophene	ECBC	-1.939	-0.696	0.012	0.202	406.910	4.684	82.0
3T3	Hexachlorophene	FAL	-1.896	-0.696	0.013	0.202	406.910	5.172	82.0
3T3	Hexachlorophene	IIVS	-2.126	-0.696	0.007	0.202	406.910	3.046	82.0
3T3	Lactic acid	ECBC	1.513	1.606	32.562	40.353	90.080	2933.144	3635
3T3	Lactic acid	FAL	1.584	1.606	38.374	40.353	90.080	3456.740	3635
3T3	Lactic acid	IIVS	1.490	1.606	30.882	40.353	90.080	2781.848	3635
3T3	Lindane	ECBC	-0.496	-0.464	0.319	0.344	290.800	92.754	100

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	Lindane	FAL	-0.067	-0.464	0.856	0.344	290.800	249.029	100
3T3	Lindane	IIVS	-0.685	-0.464	0.207	0.344	290.800	60.090	100
3T3	Lithium I carbonate	ECBC	0.881	0.902	7.601	7.985	73.890	561.606	590
3T3	Meprobamate	ECBC	0.207	0.803	1.609	6.353	218.300	351.291	1387
3T3	Meprobamate	FAL	0.600	0.803	3.981	6.353	218.300	868.960	1387
3T3	Meprobamate	IIVS	0.247	0.803	1.766	6.353	218.300	385.478	1387
3T3	Mercury II chloride	ECBC	-1.897	-0.830	0.013	0.148	271.500	3.446	40.2
3T3	Mercury II chloride	FAL	-1.670	-0.830	0.021	0.148	271.500	5.801	40.2
3T3	Mercury II chloride	IIVS	-1.889	-0.830	0.013	0.148	271.500	3.505	40.2
3T3	Nicotine	ECBC	0.216	-0.367	1.643	0.430	162.200	266.481	69.7
3T3	Nicotine	FAL	0.386	-0.367	2.430	0.430	162.200	394.155	69.7
3T3	Nicotine	IIVS	0.441	-0.367	2.760	0.430	162.200	447.713	69.7
3T3	Paraquat	ECBC	-1.103	-0.443	0.079	0.360	257.200	20.308	92.7
3T3	Paraquat	FAL	-1.109	-0.443	0.078	0.360	257.200	19.991	92.7
3T3	Paraquat	IIVS	-1.107	-0.443	0.078	0.360	257.200	20.116	92.7
3T3	Parathion	ECBC	-1.147	-1.679	0.071	0.021	291.300	20.750	6.1
3T3	Parathion	FAL	-0.398	-1.679	0.400	0.021	291.300	116.413	6.1
3T3	Parathion	IIVS	-1.128	-1.679	0.074	0.021	291.300	21.695	6.1
3T3	Phenobarbital	ECBC	0.429	-0.016	2.688	0.965	232.230	624.214	224.0
3T3	Phenobarbital	FAL	0.473	-0.016	2.975	0.965	232.230	690.770	224.0
3T3	Phenobarbital	IIVS	0.303	-0.016	2.011	0.965	232.230	466.928	224.0
3T3	Phenol	ECBC	-0.280	0.908	0.524	8.097	94.110	49.355	762.0
3T3	Phenol	FAL	0.036	0.908	1.086	8.097	94.110	102.172	762.0
3T3	Phenol	IIVS	-0.211	0.908	0.615	8.097	94.110	57.854	762.0
3T3	Phenylthiourea	ECBC	-0.795	-1.705	0.160	0.020	152.200	24.389	3.0
3T3	Phenylthiourea	FAL	0.183	-1.705	1.523	0.020	152.200	231.739	3.0
3T3	Phenylthiourea	IIVS	-0.242	-1.705	0.573	0.020	152.200	87.163	3.0
3T3	Physostigmine	ECBC	-1.038	-1.741	0.092	0.018	275.400	25.235	5.0
3T3	Physostigmine	FAL	-0.863	-1.741	0.137	0.018	275.400	37.786	5.0
3T3	Physostigmine	IIVS	-1.145	-1.741	0.072	0.018	275.400	19.702	5.0
3T3	Potassium cyanide	ECBC	-0.637	-0.956	0.231	0.111	65.120	15.031	7.2
3T3	Potassium cyanide	FAL	0.353	-0.956	2.254	0.111	65.120	146.780	7.2
3T3	Potassium cyanide	IIVS	-0.538	-0.956	0.289	0.111	65.120	18.851	7.2
3T3	Potassium I chloride	ECBC	1.650	1.575	44.667	37.586	74.550	3329.953	2802.0
3T3	Potassium I chloride	FAL	1.690	1.575	48.972	37.586	74.550	3650.893	2802.0
3T3	Potassium I chloride	IIVS	1.695	1.575	49.557	37.586	74.550	3694.501	2802.0
3T3	Procainamide HCl	ECBC	0.168	0.856	1.473	7.175	271.790	400.252	1950.0
3T3	Procainamide HCl	FAL	0.200	0.856	1.585	7.175	271.790	430.857	1950.0
3T3	Procainamide HCl	IIVS	0.261	0.856	1.826	7.175	271.790	496.211	1950.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	Propranolol	ECBC	-1.354	0.197	0.044	1.575	295.840	13.089	466.0
3T3	Propranolol	FAL	-1.378	0.197	0.042	1.575	295.840	12.377	466.0
3T3	Propranolol	IIVS	-1.232	0.197	0.059	1.575	295.840	17.352	466.0
3T3	Propylparaben	ECBC	-0.940	1.546	0.115	35.139	180.200	20.686	6332.0
3T3	Propylparaben	FAL	-0.553	1.546	0.280	35.139	180.200	50.466	6332.0
3T3	Propylparaben	IIVS	-1.026	1.546	0.094	35.139	180.200	16.982	6332.0
3T3	Sodium arsenite	ECBC	-2.419	-0.474	0.00381	0.336	129.900	0.495	43.6
3T3	Sodium arsenite	FAL	-1.998	-0.474	0.01005	0.336	129.900	1.305	43.6
3T3	Sodium arsenite	IIVS	-2.284	-0.474	0.00520	0.336	129.900	0.676	43.6
3T3	Sodium chloride	ECBC	1.913	1.841	81.901	69.302	58.440	4786.301	4050.0
3T3	Sodium chloride	FAL	1.896	1.841	78.621	69.302	58.440	4594.624	4050.0
3T3	Sodium chloride	IIVS	1.920	1.841	83.168	69.302	58.440	4860.340	4050.0
3T3	Sodium dichromate dihydrate	ECBC	-2.697	-0.771	0.00201	0.169	298.000	0.599	50.5
3T3	Sodium dichromate dihydrate	FAL	-2.680	-0.771	0.00209	0.169	298.000	0.622	50.5
3T3	Sodium dichromate dihydrate	IIVS	-2.740	-0.771	0.00182	0.169	298.000	0.542	50.5
3T3	Sodium hypochlorite	ECBC	1.041	2.142	10.995	138.737	74.440	818.465	10327.6
3T3	Sodium hypochlorite	FAL	0.996	2.142	9.898	138.737	74.440	736.772	10327.6
3T3	Sodium hypochlorite	IIVS	1.399	2.142	25.058	138.737	74.440	1865.306	10327.6
3T3	Sodium oxalate	ECBC	-0.535	0.674	0.292	4.724	134.000	39.114	633.0
3T3	Sodium oxalate	FAL	-0.649	0.674	0.224	4.724	134.000	30.061	633.0
3T3	Sodium oxalate	IIVS	-0.488	0.674	0.325	4.724	134.000	43.576	633.0
3T3	Sodium I fluoride	ECBC	0.163	0.480	1.456	3.020	41.990	61.136	126.8
3T3	Sodium I fluoride	FAL	0.354	0.480	2.260	3.020	41.990	94.911	126.8
3T3	Sodium I fluoride	IIVS	0.290	0.480	1.950	3.020	41.990	81.860	126.8
3T3	Sodium selenate	ECBC	-1.176	-1.799	0.067	0.016	188.940	12.594	3.0
3T3	Sodium selenate	FAL	-0.548	-1.799	0.283	0.016	188.940	53.487	3.0
3T3	Sodium selenate	IIVS	-0.717	-1.799	0.192	0.016	188.940	36.291	3.0
3T3	Strychnine	ECBC	0.059	-1.725	1.146	0.019	334.400	383.119	6.3
3T3	Strychnine	FAL	-0.434	-1.725	0.368	0.019	334.400	123.121	6.3
3T3	Strychnine	IIVS	-0.603	-1.725	0.249	0.019	334.400	83.432	6.3
3T3	Thallium II sulfate	ECBC	-2.263	-1.305	0.005	0.050	504.800	2.756	25.0
3T3	Thallium II sulfate	FAL	-1.726	-1.305	0.019	0.050	504.800	9.483	25.0
3T3	Thallium II sulfate	IIVS	-1.916	-1.305	0.012	0.050	504.800	6.124	25.0
3T3	Trichloroacetic acid	ECBC	0.666	1.282	4.639	19.137	163.400	757.996	3127.0
3T3	Trichloroacetic acid	FAL	0.872	1.282	7.443	19.137	163.400	1216.186	3127.0
3T3	Trichloroacetic acid	IIVS	0.687	1.282	4.869	19.137	163.400	795.548	3127.0
3T3	Triethylenemelamine	ECBC	-3.378	-1.708	0.000419	0.020	204.230	0.086	4.0
3T3	Triethylenemelamine	FAL	-2.153	-1.708	0.00703	0.020	204.230	1.436	4.0
3T3	Triethylenemelamine	IIVS	-3.095	-1.708	0.000804	0.020	204.230	0.164	4.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	Triphenyltin hydroxide	ECBC	-4.161	-0.047	0.000069	0.896	367.020	0.025	329.0
3T3	Triphenyltin hydroxide	FAL	-4.366	-0.047	0.000043	0.896	367.020	0.016	329.0
3T3	Triphenyltin hydroxide	IIVS	-4.459	-0.047	0.000035	0.896	367.020	0.013	329.0
3T3	Valproic acid	ECBC	0.577	0.839	3.776	6.907	144.200	544.503	996.0
3T3	Valproic acid	FAL	1.097	0.839	12.494	6.907	144.200	1801.634	996.0
3T3	Valproic acid	IIVS	0.600	0.839	3.981	6.907	144.200	574.116	996.0
3T3	Verapamil HCl	ECBC	-1.188	-0.646	0.065	0.226	491.080	31.842	111.0
3T3	Verapamil HCl	FAL	-1.153	-0.646	0.070	0.226	491.080	34.514	111.0
3T3	Verapamil HCl	IIVS	-1.103	-0.646	0.079	0.226	491.080	38.726	111.0
3T3	Xylene	IIVS	0.832	1.643	6.787	43.939	106.170	720.554	4665.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	1,1,1-Trichloroethane	ECBC	1.784	1.957	60.881	90.534	133.410	8122.069	12078.1
NHK	2-Propanol	ECBC	1.940	1.929	87.191	84.928	60.110	5241.038	5105.0
NHK	2-Propanol	FAL	1.840	1.929	69.247	84.928	60.110	4162.458	5105.0
NHK	2-Propanol	IIVS	2.071	1.929	117.715	84.928	60.110	7075.821	5105.0
NHK	5-Aminosalicylic acid	ECBC	-0.717	1.350	0.192	22.391	153.100	29.399	3428.0
NHK	5-Aminosalicylic acid	FAL	-0.330	1.350	0.468	22.391	153.100	71.669	3428.0
NHK	5-Aminosalicylic acid	IIVS	-0.501	1.350	0.316	22.391	153.100	48.343	3428.0
NHK	Acetaminophen	ECBC	0.564	1.155	3.663	14.299	151.200	553.775	2162.0
NHK	Acetaminophen	FAL	0.466	1.155	2.925	14.299	151.200	442.249	2162.0
NHK	Acetaminophen	IIVS	0.574	1.155	3.754	14.299	151.200	567.545	2162.0
NHK	Acetonitrile	ECBC	2.357	1.942	227.608	87.576	41.050	9343.293	3595.0
NHK	Acetonitrile	FAL	2.388	1.942	244.542	87.576	41.050	10038.450	3595.0
NHK	Acetonitrile	IIVS	2.354	1.942	226.128	87.576	41.050	9282.536	3595.0
NHK	Acetylsalicylic acid	ECBC	0.544	0.922	3.501	8.357	180.200	630.957	1506.0
NHK	Acetylsalicylic acid	FAL	0.583	0.922	3.827	8.357	180.200	689.710	1506.0
NHK	Acetylsalicylic acid	IIVS	0.452	0.922	2.831	8.357	180.200	510.113	1506.0
NHK	Aminopterin	ECBC	0.299	-1.799	1.990	0.016	440.470	876.710	7.0
NHK	Aminopterin	FAL	0.091	-1.799	1.234	0.016	440.470	543.604	7.0
NHK	Aminopterin	IIVS	0.141	-1.799	1.383	0.016	440.470	608.951	7.0
NHK	Amitriptyline HCl	ECBC	-1.480	0.046	0.033	1.112	313.900	10.402	349.0
NHK	Amitriptyline HCl	FAL	-1.696	0.046	0.020	1.112	313.900	6.328	349.0
NHK	Amitriptyline HCl	IIVS	-1.458	0.046	0.035	1.112	313.900	10.923	349.0
NHK	Arsenic III trioxide	ECBC	-1.426	-0.897	0.038	0.127	197.840	7.425	25.1
NHK	Arsenic III trioxide	FAL	-1.968	-0.897	0.011	0.127	197.840	2.132	25.1
NHK	Arsenic III trioxide	IIVS	-0.991	-0.897	0.102	0.127	197.840	20.216	25.1
NHK	Atropine sulfate	ECBC	-0.912	0.071	0.122	1.179	694.800	85.049	819.0
NHK	Atropine sulfate	FAL	-0.943	0.071	0.114	1.179	694.800	79.189	819.0
NHK	Atropine sulfate	IIVS	-0.932	0.071	0.117	1.179	694.800	81.345	819.0
NHK	Boric acid	ECBC	0.839	1.744	6.899	55.410	61.830	426.580	3426.0
NHK	Boric acid	FAL	0.786	1.744	6.111	55.410	61.830	377.862	3426.0
NHK	Boric acid	IIVS	0.875	1.744	7.501	55.410	61.830	463.803	3426.0
NHK	Busulfan	ECBC	0.003	-1.308	1.006	0.049	246.310	247.742	12.1
NHK	Busulfan	FAL	-0.033	-1.308	0.926	0.049	246.310	228.034	12.1
NHK	Busulfan	IIVS	0.102	-1.308	1.265	0.049	246.310	311.650	12.1
NHK	Cadmium II chloride	ECBC	-1.948	-0.132	0.011	0.738	183.300	2.066	135.2
NHK	Cadmium II chloride	FAL	-2.083	-0.132	0.008	0.738	183.300	1.514	135.2
NHK	Cadmium II chloride	IIVS	-1.995	-0.132	0.010	0.738	183.300	1.856	135.2
NHK	Caffeine	ECBC	0.609	0.203	4.062	1.596	194.200	788.860	310.0
NHK	Caffeine	FAL	0.469	0.203	2.947	1.596	194.200	572.357	310.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	Caffeine	IIVS	0.471	0.203	2.956	1.596	194.200	574.116	310.0
NHK	Carbamazepine	ECBC	-0.555	1.075	0.278	11.879	236.300	65.766	2807.0
NHK	Carbamazepine	FAL	-0.235	1.075	0.582	11.879	236.300	137.615	2807.0
NHK	Carbamazepine	IIVS	-0.569	1.075	0.270	11.879	236.300	63.728	2807.0
NHK	Chloral hydrate	ECBC	-0.082	0.586	0.829	3.857	165.400	137.088	638.0
NHK	Chloral hydrate	FAL	-0.031	0.586	0.931	3.857	165.400	153.934	638.0
NHK	Chloral hydrate	IIVS	-0.169	0.586	0.677	3.857	165.400	112.030	638.0
NHK	Chloramphenicol	ECBC	-0.033	1.033	0.927	10.800	323.150	299.663	3490.0
NHK	Chloramphenicol	FAL	0.070	1.033	1.175	10.800	323.150	379.588	3490.0
NHK	Chloramphenicol	IIVS	0.048	1.033	1.117	10.800	323.150	361.049	3490.0
NHK	Citric acid	ECBC	0.434	1.489	2.715	30.864	192.100	521.595	5929.0
NHK	Citric acid	FAL	0.206	1.489	1.608	30.864	192.100	308.852	5929.0
NHK	Citric acid	IIVS	0.352	1.489	2.250	30.864	192.100	432.182	5929.0
NHK	Colchicine	ECBC	-4.918	-1.425	0.0000121	0.038	399.480	0.005	15.0
NHK	Colchicine	FAL	-4.720	-1.425	0.0000190	0.038	399.480	0.008	15.0
NHK	Colchicine	IIVS	-4.699	-1.425	0.0000200	0.038	399.480	0.008	15.0
NHK	Cupric sulfate pentahydrate	ECBC	-0.121	0.279	0.757	1.902	249.700	188.944	475.0
NHK	Cupric sulfate pentahydrate	FAL	-0.109	0.279	0.778	1.902	249.700	194.387	475.0
NHK	Cupric sulfate pentahydrate	IIVS	-0.082	0.279	0.828	1.902	249.700	206.697	475.0
NHK	Cycloheximide	ECBC	-3.732	-2.148	0.000185	0.007	281.400	0.052	2.0
NHK	Cycloheximide	FAL	-3.418	-2.148	0.000382	0.007	281.400	0.108	2.0
NHK	Cycloheximide	IIVS	-3.601	-2.148	0.000251	0.007	281.400	0.071	2.0
NHK	Dibutyl phthalate	ECBC	-1.005	1.504	0.099	31.951	278.300	27.521	8892.0
NHK	Dibutyl phthalate	FAL	-0.854	1.504	0.140	31.951	278.300	38.964	8892.0
NHK	Dibutyl phthalate	IIVS	-1.102	1.504	0.079	31.951	278.300	22.012	8892.0
NHK	Dichlorvos	ECBC	-1.423	-0.576	0.038	0.266	220.980	8.348	58.7
NHK	Dichlorvos	FAL	-1.265	-0.576	0.054	0.266	220.980	11.991	58.7
NHK	Dichlorvos	IIVS	-1.258	-0.576	0.055	0.266	220.980	12.199	58.7
NHK	Diethyl phthalate	ECBC	-0.108	1.622	0.779	41.904	222.200	173.114	9311.0
NHK	Diethyl phthalate	FAL	-0.615	1.622	0.243	41.904	222.200	53.910	9311.0
NHK	Diethyl phthalate	IIVS	-0.074	1.622	0.843	41.904	222.200	187.212	9311.0
NHK	Digoxin	ECBC	-5.164	-1.441	0.00000685	0.036	780.900	0.0053	28.3
NHK	Digoxin	FAL	-7.209	-1.441	0.00000006	0.036	780.900	0.000048	28.3
NHK	Digoxin	IIVS	-5.293	-1.441	0.00000509	0.036	780.900	0.0040	28.3
NHK	Dimethylformamide	ECBC	2.107	1.861	127.962	72.572	73.100	9354.057	5305.0
NHK	Dimethylformamide	FAL	2.029	1.861	106.926	72.572	73.100	7816.278	5305.0
NHK	Dimethylformamide	IIVS	1.942	1.861	87.448	72.572	73.100	6392.440	5305.0
NHK	Diquat dibromide monohydra	ECBC	-2.012	-0.355	0.010	0.442	362.100	3.525	160.0
NHK	Diquat dibromide monohydra	FAL	-1.779	-0.355	0.017	0.442	362.100	6.028	160.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	Diquat dibromide monohydra	IIVS	-1.976	-0.355	0.011	0.442	362.100	3.829	160.0
NHK	Disulfoton	ECBC	-0.298	-1.739	0.504	0.018	274.420	138.250	5.0
NHK	Disulfoton	FAL	0.458	-1.739	2.872	0.018	274.420	788.255	5.0
NHK	Disulfoton	IIVS	-0.182	-1.739	0.657	0.018	274.420	180.302	5.0
NHK	Endosulfan	ECBC	-2.077	-1.165	0.0084	0.068	406.910	3.411	27.8
NHK	Endosulfan	FAL	-2.493	-1.165	0.0032	0.068	406.910	1.307	27.8
NHK	Endosulfan	IIVS	-2.276	-1.165	0.0053	0.068	406.910	2.157	27.8
NHK	Epinephrine bitartrate	ECBC	-0.464	-1.921	0.343	0.012	333.300	114.463	4.0
NHK	Epinephrine bitartrate	FAL	-0.628	-1.921	0.236	0.012	333.300	78.584	4.0
NHK	Epinephrine bitartrate	IIVS	-0.652	-1.921	0.223	0.012	333.300	74.245	4.0
NHK	Ethanol	ECBC	2.255	2.391	179.852	245.800	46.070	8285.779	11324.0
NHK	Ethanol	FAL	2.411	2.391	257.780	245.800	46.070	11875.904	11324.0
NHK	Ethanol	IIVS	2.346	2.391	221.776	245.800	46.070	10217.234	11324.0
NHK	Ethylene glycol	ECBC	2.785	2.062	609.021	115.351	62.080	37808.041	7161.0
NHK	Ethylene glycol	FAL	2.903	2.062	800.306	115.351	62.080	49683.006	7161.0
NHK	Ethylene glycol	IIVS	2.806	2.062	639.741	115.351	62.080	39715.137	7161.0
NHK	Fenpropathrin	ECBC	-1.982	-0.664	0.0104	0.217	349.430	3.645	75.7
NHK	Fenpropathrin	FAL	-2.207	-0.664	0.0062	0.217	349.430	2.171	75.7
NHK	Fenpropathrin	IIVS	-2.287	-0.664	0.0052	0.217	349.430	1.806	75.7
NHK	Gibberellic acid	ECBC	0.912	1.241	8.174	17.436	346.380	2831.392	6039.5
NHK	Gibberellic acid	FAL	0.927	1.241	8.461	17.436	346.380	2930.893	6039.5
NHK	Gibberellic acid	IIVS	0.909	1.241	8.106	17.436	346.380	2807.588	6039.5
NHK	Glutethimide	ECBC	-0.087	0.441	0.819	2.761	217.300	177.964	600.0
NHK	Glutethimide	FAL	-0.110	0.441	0.776	2.761	217.300	168.655	600.0
NHK	Glutethimide	IIVS	-0.096	0.441	0.802	2.761	217.300	174.181	600.0
NHK	Glycerol	ECBC	2.542	2.332	348.180	214.681	92.090	32063.852	19770.0
NHK	Glycerol	FAL	2.250	2.332	177.877	214.681	92.090	16380.733	19770.0
NHK	Glycerol	IIVS	2.495	2.332	312.695	214.681	92.090	28796.077	19770.0
NHK	Haloperidol	ECBC	-2.019	-0.057	0.00958	0.878	375.900	3.601	330.0
NHK	Haloperidol	FAL	-2.053	-0.057	0.00886	0.878	375.900	3.329	330.0
NHK	Haloperidol	IIVS	-2.076	-0.057	0.00840	0.878	375.900	3.157	330.0
NHK	Hexachlorophene	ECBC	-4.179	-0.696	0.000066	0.202	406.910	0.027	82.0
NHK	Hexachlorophene	FAL	-3.984	-0.696	0.000104	0.202	406.910	0.042	82.0
NHK	Hexachlorophene	IIVS	-4.285	-0.696	0.000052	0.202	406.910	0.021	82.0
NHK	Lactic acid	ECBC	1.155	1.606	14.274	40.353	90.080	1285.822	3635.0
NHK	Lactic acid	FAL	1.166	1.606	14.646	40.353	90.080	1319.269	3635.0
NHK	Lactic acid	IIVS	1.162	1.606	14.511	40.353	90.080	1307.174	3635.0
NHK	Lindane	ECBC	-1.188	-0.464	0.065	0.344	290.800	18.880	100.0
NHK	Lindane	FAL	-1.113	-0.464	0.077	0.344	290.800	22.439	100.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	Lindane	IIVS	-1.273	-0.464	0.053	0.344	290.800	15.500	100.0
NHK	Lithium I carbonate	ECBC	0.733	0.902	5.413	7.985	73.890	399.969	590.0
NHK	Lithium I carbonate	FAL	0.812	0.902	6.482	7.985	73.890	478.918	590.0
NHK	Lithium I carbonate	IIVS	0.859	0.902	7.228	7.985	73.890	534.059	590.0
NHK	Meprobamate	ECBC	0.539	0.803	3.459	6.353	218.300	755.092	1386.8
NHK	Meprobamate	FAL	-0.353	0.803	0.444	6.353	218.300	96.902	1386.8
NHK	Meprobamate	IIVS	0.454	0.803	2.842	6.353	218.300	620.393	1386.8
NHK	Mercury II chloride	ECBC	-1.600	-0.830	0.025	0.148	271.500	6.815	40.2
NHK	Mercury II chloride	FAL	-1.706	-0.830	0.020	0.148	271.500	5.339	40.2
NHK	Mercury II chloride	IIVS	-1.705	-0.830	0.020	0.148	271.500	5.352	40.2
NHK	Methanol	FAL	1.543	2.434	34.885	271.835	32.040	1117.721	8709.6
NHK	Methanol	IIVS	1.815	2.434	65.259	271.835	32.040	2090.900	8709.6
NHK	Nicotine	ECBC	-0.246	-0.367	0.568	0.430	162.200	92.116	69.7
NHK	Nicotine	FAL	-0.129	-0.367	0.742	0.430	162.200	120.411	69.7
NHK	Nicotine	IIVS	-0.172	-0.367	0.673	0.430	162.200	109.144	69.7
NHK	Paraquat	ECBC	-0.729	-0.443	0.187	0.360	257.200	48.010	92.7
NHK	Paraquat	FAL	-0.449	-0.443	0.356	0.360	257.200	91.482	92.7
NHK	Paraquat	IIVS	-0.684	-0.443	0.207	0.360	257.200	53.211	92.7
NHK	Parathion	ECBC	-0.945	-1.679	0.114	0.021	291.300	33.090	6.1
NHK	Parathion	FAL	-0.993	-1.679	0.102	0.021	291.300	29.582	6.1
NHK	Parathion	IIVS	-1.012	-1.679	0.097	0.021	291.300	28.316	6.1
NHK	Phenobarbital	ECBC	0.466	-0.016	2.922	0.965	232.230	678.683	224.0
NHK	Phenobarbital	FAL	0.179	-0.016	1.512	0.965	232.230	351.021	224.0
NHK	Phenobarbital	IIVS	0.210	-0.016	1.622	0.965	232.230	376.704	224.0
NHK	Phenol	ECBC	-0.224	0.908	0.598	8.097	94.110	56.234	762.0
NHK	Phenol	FAL	-0.005	0.908	0.989	8.097	94.110	93.111	762.0
NHK	Phenol	IIVS	-0.067	0.908	0.857	8.097	94.110	80.662	762.0
NHK	Phenylthiourea	ECBC	0.374	-1.705	2.367	0.020	152.200	360.302	3.0
NHK	Phenylthiourea	FAL	0.415	-1.705	2.600	0.020	152.200	395.670	3.0
NHK	Phenylthiourea	IIVS	0.244	-1.705	1.754	0.020	152.200	266.891	3.0
NHK	Physostigmine	ECBC	-0.226	-1.741	0.594	0.018	275.400	163.682	5.0
NHK	Physostigmine	FAL	-0.954	-1.741	0.111	0.018	275.400	30.617	5.0
NHK	Physostigmine	IIVS	-0.299	-1.741	0.502	0.018	275.400	138.250	5.0
NHK	Potassium cyanide	ECBC	-0.356	-0.956	0.441	0.111	65.120	28.708	7.2
NHK	Potassium cyanide	FAL	-0.112	-0.956	0.773	0.111	65.120	50.350	7.2
NHK	Potassium cyanide	IIVS	-0.589	-0.956	0.258	0.111	65.120	16.788	7.2
NHK	Potassium I chloride	ECBC	1.531	1.575	33.999	37.586	74.550	2534.622	2802.0
NHK	Potassium I chloride	FAL	1.475	1.575	29.837	37.586	74.550	2224.317	2802.0
NHK	Potassium I chloride	IIVS	1.425	1.575	26.634	37.586	74.550	1985.553	2802.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	Procainamide HCl	ECBC	0.733	0.856	5.413	7.175	271.790	1471.183	1950.0
NHK	Procainamide HCl	FAL	0.816	0.856	6.543	7.175	271.790	1778.280	1950.0
NHK	Procainamide HCl	IIVS	0.871	0.856	7.426	7.175	271.790	2018.366	1950.0
NHK	Propranolol	ECBC	-0.890	0.197	0.129	1.575	295.840	38.084	466.0
NHK	Propranolol	FAL	-0.830	0.197	0.148	1.575	295.840	43.758	466.0
NHK	Propranolol	IIVS	-1.017	0.197	0.096	1.575	295.840	28.465	466.0
NHK	Propylparaben	ECBC	-1.000	1.546	0.100	35.139	180.200	18.016	6332.0
NHK	Propylparaben	FAL	-0.991	1.546	0.102	35.139	180.200	18.394	6332.0
NHK	Propylparaben	IIVS	-1.115	1.546	0.077	35.139	180.200	13.825	6332.0
NHK	Sodium arsenite	ECBC	-2.231	-0.474	0.0059	0.336	129.900	0.763	43.6
NHK	Sodium arsenite	FAL	-2.631	-0.474	0.0023	0.336	129.900	0.304	43.6
NHK	Sodium arsenite	IIVS	-2.444	-0.474	0.0036	0.336	129.900	0.467	43.6
NHK	Sodium chloride	ECBC	1.787	1.841	61.229	69.302	58.440	3578.217	4050.0
NHK	Sodium chloride	FAL	1.042	1.841	11.014	69.302	58.440	643.675	4050.0
NHK	Sodium chloride	IIVS	1.772	1.841	59.196	69.302	58.440	3459.394	4050.0
NHK	Sodium dichromate dihydrate	ECBC	-2.583	-0.771	0.0026	0.169	298.000	0.779	50.5
NHK	Sodium dichromate dihydrate	FAL	-2.565	-0.771	0.0027	0.169	298.000	0.811	50.5
NHK	Sodium dichromate dihydrate	IIVS	-2.718	-0.771	0.0019	0.169	298.000	0.571	50.5
NHK	Sodium hypochlorite	ECBC	1.384	2.142	24.203	138.737	74.440	1801.634	10327.6
NHK	Sodium hypochlorite	FAL	1.192	2.142	15.543	138.737	74.440	1157.000	10327.6
NHK	Sodium hypochlorite	IIVS	1.340	2.142	21.854	138.737	74.440	1626.797	10327.6
NHK	Sodium oxalate	ECBC	0.420	0.674	2.632	4.724	134.000	352.641	633.0
NHK	Sodium oxalate	FAL	0.373	0.674	2.360	4.724	134.000	316.228	633.0
NHK	Sodium oxalate	IIVS	0.418	0.674	2.616	4.724	134.000	350.483	633.0
NHK	Sodium I fluoride	ECBC	0.061	0.480	1.152	3.020	41.990	48.363	126.8
NHK	Sodium I fluoride	FAL	0.032	0.480	1.078	3.020	41.990	45.250	126.8
NHK	Sodium I fluoride	IIVS	0.105	0.480	1.272	3.020	41.990	53.423	126.8
NHK	Sodium selenate	ECBC	-1.405	-1.799	0.039	0.016	188.940	7.439	3.0
NHK	Sodium selenate	FAL	-1.117	-1.799	0.076	0.016	188.940	14.440	3.0
NHK	Sodium selenate	IIVS	-1.279	-1.799	0.053	0.016	188.940	9.935	3.0
NHK	Strychnine	ECBC	-0.601	-1.725	0.251	0.019	334.400	83.898	6.3
NHK	Strychnine	FAL	-0.844	-1.725	0.143	0.019	334.400	47.863	6.3
NHK	Strychnine	IIVS	-0.784	-1.725	0.164	0.019	334.400	54.996	6.3
NHK	Thallium II sulfate	ECBC	-3.440	-1.305	0.00036	0.050	504.800	0.183	25.0
NHK	Thallium II sulfate	FAL	-3.525	-1.305	0.00030	0.050	504.800	0.151	25.0
NHK	Thallium II sulfate	IIVS	-3.602	-1.305	0.00025	0.050	504.800	0.126	25.0
NHK	Trichloroacetic acid	ECBC	0.323	1.282	2.103	19.137	163.400	343.558	3127.0
NHK	Trichloroacetic acid	FAL	0.507	1.282	3.214	19.137	163.400	525.210	3127.0
NHK	Trichloroacetic acid	IIVS	0.379	1.282	2.394	19.137	163.400	391.141	3127.0

IC50 and LD50 Values Used for Laboratory-Specific Regressions

NRU Test Method	Substance	Lab	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	Triethylenemelamine	ECBC	-2.126	-1.708	0.0075	0.020	204.230	1.527	4.0
NHK	Triethylenemelamine	FAL	-2.012	-1.708	0.0097	0.020	204.230	1.986	4.0
NHK	Triethylenemelamine	IIVS	-1.988	-1.708	0.0103	0.020	204.230	2.097	4.0
NHK	Triphenyltin hydroxide	ECBC	-4.250	-0.047	0.000056	0.896	367.020	0.021	329.0
NHK	Triphenyltin hydroxide	FAL	-4.885	-0.047	0.000013	0.896	367.020	0.0048	329.0
NHK	Triphenyltin hydroxide	IIVS	-4.552	-0.047	0.000028	0.896	367.020	0.010	329.0
NHK	Valproic acid	ECBC	0.501	0.839	3.172	6.907	144.200	457.439	996.0
NHK	Valproic acid	FAL	0.679	0.839	4.779	6.907	144.200	689.181	996.0
NHK	Valproic acid	IIVS	0.470	0.839	2.954	6.907	144.200	425.925	996.0
NHK	Verapamil HCl	ECBC	-0.917	-0.646	0.121	0.226	491.080	59.384	111.0
NHK	Verapamil HCl	FAL	-0.817	-0.646	0.152	0.226	491.080	74.874	111.0
NHK	Verapamil HCl	IIVS	-0.871	-0.646	0.134	0.226	491.080	66.019	111.0
NHK	Xylene	IIVS	0.642	1.643	4.385	43.939	106.170	465.586	4665.0

Abbreviations: 3T3=Neutral red uptake with mouse fibroblast 3T3 cell line; NHK=Neutral red uptake with normal human epidermal keratinocytes; ECBC=US Army Chemical Biological Center; FAL=FRAME Alternatives Lab; IIVS=Institute for *In Vitro* Sciences.

[1] IC_{50} values are the geometric mean IC_{50} values for each substance in each lab.

[2] Reference rat oral LD_{50} values from **Table 4-2**. Reference values were developed from rat acute oral LD_{50} studies located using literature searches, secondary references, and electronic database searches.

Appendix K2

IC_{50} and LD_{50} Values Used for Combined-Laboratory Regressions

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IC50 and LD50 Values Used for Combined-Laboratory Regressions

NRU Test Method	Substance	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	1,1,1-Trichloroethane	2.186	1.957	133.41	153.307	90.534	20453	12078.1
3T3	2-Propanol	1.764	1.929	60.11	58.037	84.928	3489	5105.0
3T3	5-Aminosalicylic Acid	1.037	1.350	153.10	10.887	22.391	1667	3428.0
3T3	Acetaminophen	-0.501	1.155	151.20	0.315	14.299	47.7	2162.0
3T3	Acetonitrile	2.287	1.942	41.05	193.701	87.576	7951	3595.0
3T3	Acetylsalicylic Acid	0.574	0.922	180.20	3.754	8.357	676	1506.0
3T3	Aminopterin	-4.839	-1.799	440.47	0.000	0.016	0.006	7.0
3T3	Amitriptyline HCl	-1.648	0.046	313.90	0.022	1.112	7.05	349.0
3T3	Arsenictrioxide	-1.980	-0.897	197.84	0.010	0.127	2.07	25.1
3T3	Atropine Sulfate	-0.961	0.071	694.80	0.109	1.179	76.0	819.0
3T3	Boric Acid	1.476	1.744	61.83	29.924	55.410	1850	3426.0
3T3	Busulfan	-0.501	-1.308	246.31	0.315	0.049	77.7	12.1
3T3	Cadmium chloride	-2.549	-0.132	183.30	0.003	0.738	0.518	135.2
3T3	Caffeine	-0.105	0.203	194.20	0.785	1.596	153	310.0
3T3	Carbamazepine	-0.360	1.075	236.30	0.437	11.879	103	2807.0
3T3	Chloral Hydrate	0.044	0.586	165.40	1.107	3.857	183	638.0
3T3	Chloramphenicol	-0.395	1.033	323.15	0.403	10.800	130	3490.0
3T3	Citric Acid	0.600	1.489	192.10	3.981	30.864	765	5929.0
3T3	Cupric Sulfate Pentahydrate	-0.822	0.279	249.70	0.151	1.902	37.6	475.0
3T3	Cycloheximide	-3.177	-2.148	281.40	0.001	0.007	0.187	2.0
3T3	Dibutyl Phthalate	-0.807	1.504	278.30	0.156	31.951	43.4	8892.0
3T3	Dichlorvos (DDVP)	-1.095	-0.576	220.98	0.080	0.266	17.7	58.7
3T3	Diethyl Phthalate	-0.316	1.622	222.20	0.483	41.904	107	9311.0
3T3	Digoxin	-0.244	-1.441	780.90	0.570	0.036	445	28.3
3T3	Dimethylformamide	1.854	1.861	73.10	71.463	72.572	5224	5305.0
3T3	Diquat Dibromide Monohydrate	-1.654	-0.355	362.10	0.022	0.442	8.04	160.0
3T3	Disulfoton	0.163	-1.739	274.42	1.456	0.018	400	5.0
3T3	Endosulfan	-1.840	-1.165	406.91	0.014	0.068	5.88	27.8
3T3	Ethanol	2.151	2.391	46.07	141.588	245.800	6523	11324.0
3T3	Ethyleneglycol	2.595	2.062	62.08	393.615	115.351	24436	7161.0
3T3	Fenpropathrin	-1.175	-0.664	349.43	0.067	0.217	23.3	75.7
3T3	Gibberellic Acid	1.353	1.241	346.38	22.548	17.436	7810	6039.5
3T3	Glutethimide	-0.079	0.441	217.30	0.833	2.761	181	600.0
3T3	Glycerol	2.422	2.332	92.09	264.365	214.681	24345	19770.0
3T3	Haloperidol	-1.788	-0.057	375.90	0.016	0.878	6.13	330.0
3T3	Hexachlorophene	-1.987	-0.696	406.91	0.010	0.202	4.19	82.0
3T3	Lactic Acid	1.529	1.606	90.08	33.792	40.353	3044	3635.0

IC50 and LD50 Values Used for Combined-Laboratory Regressions

NRU Test Method	Substance	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
3T3	Lindane	-0.416	-0.464	290.80	0.384	0.344	112	100.0
3T3	Lithium carbonate	0.881	0.902	73.89	7.601	7.985	562	590.0
3T3	Meprobamate	0.351	0.803	218.30	2.245	6.353	490	1386.8
3T3	Mercury Chloride	-1.819	-0.830	271.50	0.015	0.148	4.12	40.2
3T3	Nicotine	0.347	-0.367	162.20	2.225	0.430	361	69.7
3T3	Paraquat	-1.106	-0.443	257.20	0.078	0.360	20.1	92.7
3T3	Parathion	-0.891	-1.679	291.30	0.128	0.021	37.4	6.1
3T3	Phenobarbital	0.402	-0.016	232.23	2.524	0.965	586	224.0
3T3	Phenol	-0.152	0.765	94.11	0.705	5.823	66.3	548.0
3T3	Phenylthiourea	-0.285	-1.705	152.20	0.519	0.020	79.0	3.0
3T3	Physostigmine	-1.015	-1.741	275.40	0.097	0.018	26.6	5.0
3T3	Potassium Cyanide	-0.274	-0.956	65.12	0.532	0.111	34.6	7.2
3T3	Potassium chloride	1.678	1.575	74.55	47.682	37.586	3555	2802.0
3T3	Procainamide HCl	0.210	0.856	271.79	1.621	7.175	441	1950.0
3T3	Propranolol	-1.321	0.197	295.84	0.048	1.575	14.1	466.0
3T3	Sodium Arsenite	-2.234	-0.474	129.90	0.006	0.336	0.759	43.6
3T3	Sodium Chloride	1.910	1.841	58.44	81.207	69.302	4746	4050.0
3T3	Sodium Dichromate Dihydrate	-2.706	-0.771	298.00	0.002	0.169	0.587	50.5
3T3	Sodium Hypochlorite	1.145	2.142	74.44	13.971	138.737	1040	10327.6
3T3	Sodium Oxalate	-0.557	0.674	134.00	0.277	4.724	37.1	633.0
3T3	Sodium fluoride	0.269	0.480	41.99	1.858	3.020	78.0	126.8
3T3	Sodium selenate	-0.814	-1.799	188.94	0.154	0.016	29.0	3.0
3T3	Strychnine	-0.326	-1.725	334.40	0.472	0.019	158	6.3
3T3	Thallium Sulfate	-1.968	-1.305	504.80	0.011	0.050	5.43	25.0
3T3	Trichloroacetic Acid	0.742	1.505	163.40	5.519	32.001	902	5229.0
3T3	Triethylenemelamine	-2.875	-1.708	204.23	0.001	0.020	0.272	4.0
3T3	Triphenyltin Hydroxide	-4.329	-0.047	367.02	0.000	0.896	0.017	329.0
3T3	Valproic Acid	0.758	0.839	144.20	5.727	6.907	826	996.0
3T3	Verapamil HCl	-1.148	-0.646	491.08	0.071	0.226	34.9	111.0
3T3	Xylene	0.832	1.643	106.17	6.787	43.939	721	4665.0

IC50 and LD50 Values Used for Combined-Laboratory Regressions

NRU Test Method	Substance	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	1,1,1-Trichloroethane	1.784	1.957	133.41	60.881	90.534	8122	12078.1
NHK	2-Propanol	1.951	1.929	60.11	89.242	84.928	5364	5105.0
NHK	5-Aminosalicylic Acid	-0.516	1.350	153.10	0.305	22.391	46.7	3428.0
NHK	Acetaminophen	0.535	1.155	151.20	3.426	14.299	518	2162.0
NHK	Acetonitrile	2.367	1.942	41.05	232.612	87.576	9549	3595.0
NHK	Acetylsalicylic Acid	0.526	0.922	180.20	3.360	8.357	605	1506.0
NHK	Aminopterin	0.177	-1.799	440.47	1.503	0.016	662	7.0
NHK	Amitriptyline HCl	-1.545	0.046	313.90	0.029	1.112	8.96	349.0
NHK	Arsenictrioxide	-1.461	-0.897	197.84	0.035	0.127	6.84	25.1
NHK	Atropine Sulfate	-0.929	0.071	694.80	0.118	1.179	81.8	819.0
NHK	Boric Acid	0.833	1.744	61.83	6.813	55.410	421	3426.0
NHK	Busulfan	0.024	-1.308	246.31	1.056	0.049	260	12.1
NHK	Cadmium chloride	-2.009	-0.132	183.30	0.010	0.738	1.80	135.2
NHK	Caffeine	0.516	0.203	194.20	3.283	1.596	638	310.0
NHK	Carbamazepine	-0.453	1.075	236.30	0.352	11.879	83.2	2807.0
NHK	Chloral Hydrate	-0.094	0.586	165.40	0.805	3.857	133	638.0
NHK	Chloramphenicol	0.028	1.033	323.15	1.068	10.800	345	3490.0
NHK	Citric Acid	0.331	1.489	192.10	2.142	30.864	411	5929.0
NHK	Cupric Sulfate Pentahydrate	-0.104	0.279	249.70	0.787	1.902	197	475.0
NHK	Cycloheximide	-3.584	-2.148	281.40	0.000	0.007	0.073	2.0
NHK	Dibutyl Phthalate	-0.987	1.504	278.30	0.103	31.951	28.7	8892.0
NHK	Dichlorvos (DDVP)	-1.315	-0.576	220.98	0.048	0.266	10.7	58.7
NHK	Diethyl Phthalate	-0.266	1.622	222.20	0.542	41.904	120	9311.0
NHK	Digoxin	-5.889	-1.441	780.90	0.000	0.036	0.001	28.3
NHK	Dimethylformamide	2.026	1.861	73.10	106.163	72.572	7760	5305.0
NHK	Diquat Dibromide Monohydrate	-1.922	-0.355	362.10	0.012	0.442	4.33	160.0
NHK	Disulfoton	-0.007	-1.739	274.42	0.983	0.018	270	5.0
NHK	Endosulfan	-2.282	-1.165	406.91	0.005	0.068	2.13	27.8
NHK	Ethanol	2.337	2.391	46.07	217.450	245.800	10018	11324.0
NHK	Ethyleneglycol	2.831	2.062	62.08	678.106	115.351	42097	7161.0
NHK	Fenpropathrin	-2.158	-0.664	349.43	0.007	0.217	2.43	75.7
NHK	Gibberellic Acid	0.916	1.241	346.38	8.246	17.436	2856	6039.5
NHK	Glutethimide	-0.098	0.441	217.30	0.799	2.761	174	600.0
NHK	Glycerol	2.429	2.332	92.09	268.544	214.681	24730	19770.0
NHK	Haloperidol	-2.049	-0.057	375.90	0.009	0.878	3.36	330.0
NHK	Hexachlorophene	-4.149	-0.696	406.91	0.000	0.202	0.029	82.0
NHK	Lactic Acid	1.161	1.606	90.08	14.476	40.353	1304	3635.0

IC50 and LD50 Values Used for Combined-Laboratory Regressions

NRU Test Method	Substance	Log IC_{50} (mM)[1]	Log Reference LD_{50} (mmol/kg)[2]	Molecular Weight (g/mole)	IC_{50} (mM)[1]	Reference LD_{50} (mmol/kg)[2]	IC_{50} (µg/mL)[1]	Reference LD_{50} (mg/kg)[2]
NHK	Lindane	-1.191	-0.464	290.80	0.064	0.344	18.7	100.0
NHK	Lithium carbonate	0.801	0.902	73.89	6.330	7.985	468	590.0
NHK	Meprobamate	0.213	0.803	218.30	1.634	6.353	357	1386.8
NHK	Mercury Chloride	-1.671	-0.830	271.50	0.021	0.148	5.80	40.2
NHK	Methanol	1.679	2.434	32.04	47.713	271.835	1529	8709.6
NHK	Nicotine	-0.182	-0.367	162.20	0.657	0.430	107	69.7
NHK	Paraquat	-0.621	-0.443	257.20	0.239	0.360	61.6	92.7
NHK	Parathion	-0.983	-1.679	291.30	0.104	0.021	30.3	6.1
NHK	Phenobarbital	0.285	-0.016	232.23	1.928	0.965	448	224.0
NHK	Phenol	-0.098	0.765	94.11	0.797	5.823	75.0	548.0
NHK	Phenylthiourea	0.344	-1.705	152.20	2.210	0.020	336	3.0
NHK	Physostigmine	-0.493	-1.741	275.40	0.321	0.018	88.5	5.0
NHK	Potassium Cyanide	-0.352	-0.956	65.12	0.445	0.111	29.0	7.2
NHK	Potassium chloride	1.477	1.575	74.55	30.007	37.586	2237	2802.0
NHK	Procainamide HCl	0.807	0.856	271.79	6.407	7.175	1741	1950.0
NHK	Propranolol	-0.912	0.197	295.84	0.122	1.575	36.2	466.0
NHK	Sodium Arsenite	-2.435	-0.474	129.90	0.004	0.336	0.477	43.6
NHK	Sodium Chloride	1.534	1.841	58.44	34.177	69.302	1997	4050.0
NHK	Sodium Dichromate Dihydrate	-2.622	-0.771	298.00	0.002	0.169	0.712	50.5
NHK	Sodium Hypochlorite	1.305	2.142	74.44	20.182	138.737	1502	10327.6
NHK	Sodium Oxalate	0.404	0.674	134.00	2.533	4.724	339	633.0
NHK	Sodium fluoride	0.066	0.480	41.99	1.165	3.020	48.9	126.8
NHK	Sodium selenate	-1.267	-1.799	188.94	0.054	0.016	10.2	3.0
NHK	Strychnine	-0.743	-1.725	334.40	0.181	0.019	60.4	6.3
NHK	Thallium Sulfate	-3.522	-1.305	504.80	0.000	0.050	0.152	25.0
NHK	Trichloroacetic Acid	0.403	1.505	163.40	2.529	32.001	413	5229.0
NHK	Triethylenemelamine	-2.042	-1.708	204.23	0.009	0.020	1.85	4.0
NHK	Triphenyltin Hydroxide	-4.562	-0.047	367.02	0.000	0.896	0.010	329.0
NHK	Valproic Acid	0.550	0.839	144.20	3.551	6.907	512	996.0
NHK	Verapamil HCl	-0.869	-0.646	491.08	0.135	0.226	66.5	111.0
NHK	Xylene	0.642	1.643	106.17	4.385	43.939	466	4665.0

Abbreviations: 3T3=Neutral red uptake with mouse fibroblast 3T3 cell line; NHK=Neutral red uptake with normal human epidermal keratinocytes; ECBC=US Army Chemical Biological Center; FAL=FRAME Alternatives Lab; IIVS=Institute for *In Vitro* Sciences.

[1]IC_{50} values are the geometric mean IC_{50} values for each substance in each lab.

[2]Reference rat oral LD_{50} values from **Table 4-2**. Reference values were developed from rat acute oral LD_{50} studies located using literature searches, secondary references, and electronic database searches.

Appendix K3

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

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RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
Formaldehyde	30.03	0.12	3.60	26.6	798.8
Methanol	32.05	930	29806.5	406	13012.3
Acetonitrile	41.06	368	15110.1	92.5	3798.1
Sodium I fluoride	41.99	1.85	77.68	4.29	180.1
Lithium I chloride	42.39	38.6	1636.3	17.9	758.8
Acetaldehyde	44.06	2.45	107.95	43.8	1929.8
Ethanol	46.08	379	17464.3	304	14008.3
Ammonium sulfide	51.12	0.42	21.47	3.29	168.2
Acrylonitrile	53.07	2.42	128.43	1.54	81.7
Ammonium chloride	53.5	5.52	295.32	30.8	1647.8
Acrolein	56.07	0.047	2.64	0.82	46.0
Propionaldehyde	58.09	3.25	188.79	24.3	1411.6
Allylalcohol	58.09	6.94	403.14	1.1	63.9
Acetone	58.09	444	25792.0	168	9759.1
Potassium I fluoride	58.1	3.13	181.85	4.22	245.2
Sodium chloride	58.44	75.9	4435.6	51.3	2998.0
Acetic acid	60.06	24.3	1459.5	55.1	3309.3
1-Propanol	60.11	96.5	5800.6	89.8	5397.9
2-Propanol	60.11	167	10038.4	97.2	5842.7
Ethylene glycol	62.08	555	34454.4	138	8567.0
Sodium azide	65.02	0.71	46.16	0.69	44.9
Potassium cyanide	65.12	1.12	72.93	0.15	9.8
Acrylamide	71.09	1.61	114.45	2.39	169.9
n-Butanal	72.12	12.8	923.1	34.5	2488.1
Isobutanal	72.12	13.5	973.62	39	2812.7
Ethyl methyl ketone	72.12	104	7500.5	47.1	3396.9
Dimethylformamide	73.11	114	8334.5	38.3	2800.1
Isobutanol	74.14	40.1	2973.0	33.2	2461.4
1-Butanol	74.14	52.5	3892.4	10.7	793.3
Potassium I chloride	74.55	82	6113.1	34.9	2601.8
Thioacetamide	75.14	4.17	313.33	4.01	301.3
2-Methoxyethanol	76.11	251	19103.6	32.3	2458.4
Propylene glycol	76.11	342	26029.6	263	20016.9
Thiourea	76.13	86	6547.2	1.64	124.9
Dimethyl sulfoxide	78.14	252	19691.3	252	19691.3
Pyridine	79.11	46.9	3710.3	11.3	893.9
Dichloromethane	84.93	34.9	2964.1	18.8	1596.7
Piperazine	86.16	67.2	5790.0	22.1	1904.1
N,N-Dimethylacetamide	87.14	24.2	2108.8	58.4	5089.0
1,4-Dioxane	88.12	38.1	3357.4	47.7	4203.3

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
Ethyl acetate	88.12	128	11279.4	125	11015.0
1-Pentanol	88.17	24.9	2195.4	34.4	3033.0
1-Nitropropane	89.11	57.9	5159.5	5.11	455.4
Lactic acid	90.09	66	5945.9	41.4	3729.7
1,3,5-Trioxane	90.09	213	19189.2	8.88	800.0
Glycerol	92.11	624	57476.6	137	12619.1
Toluene	92.15	17.1	1575.8	54.3	5003.7
Aniline	93.14	6.9	642.67	4.72	439.6
Phenol	94.12	3.01	283.30	4.4	414.1
Sulfuric acid	98.08	36	3530.9	21.8	2138.1
Chromium VI trioxide	100	0.0027	0.27	0.8	80.0
2-Ethylbutanal	100.18	13.2	1322.4	39.7	3977.1
Cyclohexanol	100.18	26.3	2634.7	20.6	2063.7
Tetrahydrofurfuryl alcohol	102.15	111	11338.7	24.5	2502.7
1-Hexanol	102.2	15.4	1573.9	7.04	719.5
Styrene	104.16	3.3	343.73	48	4999.7
Sodium I bromide	104.92	77.4	8120.8	33.4	3504.3
Beryllium II sulfate	105.07	0.61	64.09	0.78	82.0
Diethylene glycol	106.14	62.1	6591.3	139	14753.5
Xylene	106.18	12	1274.2	40.5	4300.3
p-Cresol	108.15	0.22	23.79	1.91	206.6
o-Cresol	108.15	0.52	56.24	1.12	121.1
m-Cresol	108.15	0.66	71.38	2.24	242.3
Benzylalcohol	108.15	5.81	628.35	11.4	1232.9
Anisole	108.15	13.2	1427.6	34.2	3698.7
p-Phenylenediamine	108.16	0.05	5.41	0.74	80.0
o-Phenylenediamine	108.16	0.31	33.53	9.89	1069.7
p-Aminophenol	109.14	0.062	6.77	15.2	1658.9
m-Aminophenol	109.14	0.86	93.86	15.2	1658.9
Catechol	110.12	0.2	22.02	35.3	3887.2
Resorcinol	110.12	0.8	88.10	2.73	300.6
Calcium II chloride	110.98	12.4	1376.2	9.01	999.9
Trifluoroacetic acid	114.03	20.5	2337.6	1.75	199.6
2,5-Hexanedione	114.16	8.45	964.65	23.7	2705.6
1-Heptanol	116.23	6.25	726.44	28	3254.4
Sodium monochloroacetate	116.48	1.45	168.90	0.65	75.7
2-Butoxyethanol	118.2	26	3073.2	12.5	1477.5
Chloroform	119.37	13.4	1599.6	7.61	908.4
Benzoic acid	122.13	15.7	1917.4	20.7	2528.1
Nicotinamide	122.14	44.4	5423.0	28.7	3505.4

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
p-Toluylendiamine	122.19	0.094	11.49	0.83	101.4
Nitrobenzene	123.12	12.2	1502.1	5.2	640.2
p-Anisidine	123.17	0.73	89.91	11.4	1404.1
2-Thiouracil	128.16	0.32	41.01	7.8	999.6
Dichloroacetic acid	128.94	11.5	1482.8	21.9	2823.8
Nickel II chloride	129.61	0.27	34.99	0.81	105.0
Cobalt II chloride	129.83	0.16	20.77	0.62	80.5
5-Fluorouracil	130.09	0.0026	0.34	1.77	230.3
1,1,1-Trichloroethane	133.4	10.3	1374.0	77.2	10298.5
Sodium oxalate	134	0.44	58.96	1.16	155.4
1,2,6-Hexanetriol	134.2	123	16506.6	119	15969.8
Cupric chloride	134.44	0.11	14.79	1.04	139.8
Zinc II chloride	136.27	0.13	17.72	2.57	350.2
Salicylamide	137.15	1.08	148.12	13.8	1892.7
Isoniazid	137.16	7.49	1027.3	4.74	650.1
Salicylic acid	138.13	3.38	466.9	6.45	890.9
p-Nitrophenol	139.12	0.2	27.8	2.52	350.6
Isononylaldehyde	142.27	1.52	216.3	22.8	3243.8
8-Hydroxyquinoline	145.17	0.0033	0.48	8.27	1200.6
Coumarin	146.15	1.71	249.9	2	292.3
N-Methyl-N'-nitro-N-nitroso-guanidine	147.12	0.012	1.8	0.61	89.7
Isobenzoic furanodione	148.12	17	2518.0	27.1	4014.1
Thymol	150.24	0.23	34.6	6.52	979.6
Acetaminophen	151.18	2.71	409.7	15.9	2403.8
Ferrous sulfate	151.91	1.85	281.0	2.1	319.0
Methyl salicylate	152.16	1.7	258.7	5.83	887.1
Phenylthiourea	152.23	0.54	82.2	0.02	3.0
2-Nitro-p-phenylenediamine	153.16	0.39	59.7	20.1	3078.5
Carbon tetrachloride	153.81	8.51	1308.9	18.2	2799.3
Menthol	156.3	0.95	148.5	20.3	3172.9
Bromobenzene	157.02	3.46	543.3	17.2	2700.7
Dimethylaminoethyl methacrylate (polymer)	157.24	0.11	17.3	11.1	1745.4
Strontium II chloride	158.52	36.4	5770.1	14.2	2251.0
Sodium salicylate	160.11	4.33	693.3	9.99	1599.5
6-Methylcoumarin	160.18	0.31	49.7	10.5	1681.9
Hydralazine	160.2	0.33	52.9	0.56	89.7
Nicotine	162.26	1.79	290.4	0.31	50.3
2,4-Dichlorophenol	163	0.055	9.0	3.56	580.3
Trichloroacetic acid	163.38	8.19	1338.1	30.6	4999.4

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
Chloral hydrate	165.4	2.65	438.3	2.9	479.7
Tetrachloroethene	165.82	6.54	1084.5	53.4	8854.8
t-Butyl hydroquinone	166.24	0.069	11.5	4.81	799.6
(-)-Phenylephrine	167.23	4.45	744.2	2.09	349.5
m-Dinitrobenzene	168.12	0.39	65.6	0.49	82.4
Azaserine	173.15	0.002	0.35	0.98	169.7
1,2-Dibromomethane	173.85	4.2	730.2	0.62	107.8
L-Ascorbic acid	176.14	1.52	267.7	67.6	11907.1
n-Butyl benzoate	178.25	0.41	73.1	28.8	5133.6
Phenacetin	179.24	1.27	227.6	9.21	1650.8
Iproniazid	179.25	0.79	141.6	2.04	365.7
Acetylsalicylic acid	180.17	2.27	409.0	5.55	999.9
D-Glucose	180.18	226	40720.7	143	25765.7
Butylated hydoxyanisole	180.27	0.24	43.3	12.2	2199.3
1,2,4-Trichlorobenzene	181.44	0.71	128.8	4.17	756.6
Cadmium II chloride	183.3	0.0064	1.2	0.48	88.0
2,4-Dinitrophenol	184.12	0.21	38.7	0.16	29.5
Undecylenic acid	184.31	0.18	33.2	13.6	2506.6
Tributylamine	185.4	15.4	2855.2	2.91	539.5
Paraquat	186.25	0.54	100.6	0.31	57.7
Amrinone	187.22	0.28	52.4	0.54	101.1
Antipyrine	188.25	11.6	2183.7	9.56	1799.7
Tin II chloride	189.59	1.51	286.3	3.69	699.6
Nitrilotriacetic acid	191.16	3.61	690.1	7.69	1470.0
Nitrogen mustard * HCl	192.53	0.0026	0.50	0.052	10.0
Dimethyl phthalate	194.2	23.4	4544.3	35.5	6894.1
Caffeine	194.22	2.64	512.7	0.99	192.3
4-Hexylresorcinol	194.3	0.064	12.4	2.83	549.9
L-Dopa	197.21	0.13	25.6	9.03	1780.8
Halothane	197.39	31.1	6138.8	28.8	5684.8
Arsenic III trioxide	197.84	0.0042	0.8	0.1	19.8
Manganese II chloride *4 H2O	197.92	0.13	25.7	7.5	1484.4
Carbaryl	201.24	0.26	52.3	1.24	249.5
Sodium cyclamate	201.24	35.4	7123.9	75.8	15254.0
Magnesium II chloride * 6 H2O	203.33	70.4	14314.4	39.8	8092.5
Phenylephrine * HCl	203.69	4.16	847.4	1.72	350.3
Triethylene melamine	204.27	0.00078	0.16	0.005	1.0
Ibuprofen	206.31	0.52	107.3	4.89	1008.9
Milrinone	211.24	4.77	1007.6	0.43	90.8
1,3-Bis(2-chloroethyl)- 1-	214.07	0.078	16.7	0.093	19.9

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
nitrosourea					
Clofibric acid	214.66	2.61	560.3	5.82	1249.3
Glutethimide	217.29	1.56	339.0	2.76	599.7
Butylated hydroxytoluene	220.39	0.056	12.3	4.04	890.4
2,4-Dichlorophenoxyacetic acid	221.04	0.77	170.2	1.67	369.1
Diethyl phthalate	222.26	5.52	1226.9	38.7	8601.5
Bendiocarb	223.25	0.18	40.2	0.8	178.6
Diethyldithiocarbamate sodium* 3H20	225.33	0.00039	0.088	6.66	1500.7
Ammonium persulfate	228.22	0.23	52.5	3.59	819.3
Cygon	229.27	1.24	284.3	0.66	151.3
Aminophenazone	231.33	5.39	1246.9	4.32	999.3
Nalidixic acid	232.26	1.5	348.4	5.81	1349.4
Phenobarbital	232.26	3.81	884.9	0.7	162.6
Ambazone	237.32	0.038	9.0	3.16	749.9
Mefenamic acid	241.31	0.087	21.0	3.27	789.1
Triethyltin chloride	241.35	0.00046	0.11	0.021	5.1
Busulphan	246.32	0.046	11.3	0.0076	1.9
Isoproterenol * HCl	247.75	0.022	5.5	8.96	2219.8
Pentobarbital sodium	248.29	0.71	176.3	0.81	201.1
Cupric sulfate * 5 H2O	249.7	0.33	82.4	1.2	299.6
2,4,5-Trichlorophenoxyacetic acid	255.48	0.44	112.4	1.17	298.9
Nabam	256.34	0.035	9.0	1.54	394.8
Trichlorfon	257.44	0.27	69.5	1.75	450.5
Natulan * HCl	257.8	2.74	706.4	3.04	783.7
Diethyl sebacate	258.4	1.63	421.2	56	14470.4
Versalide	258.44	0.15	38.8	1.22	315.3
Secobarbital sodium	260.3	0.21	54.7	0.48	124.9
Barium II nitrate	261.36	0.81	211.7	1.36	355.4
Sodium bichromate VI	261.98	0.00093	0.24	0.19	49.8
Theophylline sodium acetate	262.23	4.19	1098.7	2.22	582.2
Maneb	266.31	0.0042	1.1	16.9	4500.6
3-Cyano-2-morpholino-5-(pyrid-4-yl)-pyridine (Chemical 122)	266.31	0.96	255.7	1.3	346.2
Pentachlorophenol	266.32	0.036	9.6	0.19	50.6
Isoxepac	268.28	1.33	356.8	0.74	198.5
Dichlorophene	269.13	0.0083	2.2	10	2691.3
Mercury II chloride	271.49	0.015	4.1	0.0037	1.0
Hexachlorocyclopentadiene	272.75	0.0031	0.85	0.41	111.8
Disulfoton	274.42	0.11	30.2	0.0073	2.0
Zineb	275.73	0.059	16.3	18.9	5211.3

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
Triethyl citrate	276.32	14.7	4061.9	25.3	6990.9
Azathioprine	277.29	0.14	38.8	1.93	535.2
Amitriptyline	277.44	0.056	15.5	1.15	319.1
Imidazolidinyl urea	278.26	0.36	100.2	9.34	2598.9
Dibutyl phthalate	278.38	0.76	211.6	43.1	11998.2
Cyclophosphamide * H2O	279.13	3.12	870.9	0.34	94.9
Flufenamic acid	281.25	0.029	8.2	0.97	272.8
Cycloheximide	281.39	0.00059	0.17	0.0071	2.0
Diazepam	284.76	0.16	45.6	2.49	709.1
Retinol	286.5	0.00054	0.15	6.98	1999.8
Dihydralazine sulfate	288.32	0.14	40.4	2.84	818.8
Sodium dodecyl sulfate	289.43	0.27	78.1	4.45	1288.0
Lindane	290.82	0.41	119.2	0.26	75.6
Parathion	291.28	0.093	27.1	0.0069	2.0
Diphenhydramine * HCl	291.85	0.24	70.0	2.93	855.1
Naftipramide	298.47	0.084	25.1	3.45	1029.7
Cis-platinum	300.07	0.0028	0.84	0.086	25.8
all-trans-Retinoic acid	300.48	0.11	33.1	6.66	2001.2
Captan	300.59	0.0039	1.2	33.3	10009.6
Chlorambucil	304.24	0.076	23.1	0.25	76.1
Orphenadrine * HCl	305.88	0.49	149.9	1.39	425.2
Buflomedil	307.43	1.35	415.0	1.19	365.8
Warfarin	308.35	0.67	206.6	1.05	323.8
Phenylbutazone	308.41	0.32	98.7	1.22	376.3
Aflatoxin B1	312.29	0.034	10.6	0.016	5.0
Refortan	313.1	0.25	78.3	10.1	3162.3
Imipramine * HCl	316.91	0.054	17.1	0.96	304.2
p,p'-DDE	318.02	0.1	31.8	2.77	880.9
Chlorpromazine	318.89	0.014	4.5	0.44	140.3
p,p'-DDD	320.04	0.024	7.7	0.35	112.0
Chloramphenicol	323.15	0.79	255.3	10.5	3393.1
Oxyphenbutazone	324.41	0.19	61.6	3.08	999.2
Tributyltin chloride	325.53	0.00054	0.18	0.37	120.4
Malathion	330.38	0.2	66.1	2.68	885.4
Frusemide	330.76	2.33	770.7	7.86	2599.8
Mitomycin C	334.37	0.00084	0.28	0.042	14.0
Metamizol	334.38	0.58	193.9	21.5	7189.2
Dicoumarol	336.31	0.027	9.1	2.11	709.6
Caffeine sodium benzoate	338.33	5.67	1918.3	2.54	859.4
Papaverine	339.42	0.045	15.3	0.96	325.8

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
Diquat dibromide	344.08	0.16	55.1	0.67	230.5
Gibberellic acid	346.41	2.3	796.7	18.2	6304.7
Dodecylbenzene sodiumsulfonate	348.52	0.42	146.4	3.62	1261.6
Triisooctylamine	353.76	0.023	8.1	4.58	1620.2
p,p'-DDT	354.48	0.16	56.7	0.32	113.4
Benzylpenicillin sodium	356.4	5.73	2042.2	19.4	6914.2
Indomethacin	357.81	0.16	57.2	0.034	12.2
Quinine * HCl	360.92	0.075	27.1	1.72	620.8
Cetyltrimethylammonium chloride	362.16	0.021	7.6	1.31	474.4
Hexadecyltrimethylammonium bromide	364.53	0.0089	3.2	1.12	408.3
Aldrin	364.9	0.067	24.4	0.11	40.1
Benzalkonium chloride	365	0.0052	1.9	1.1	401.5
Triphenyltin hydroxide	367.03	0.000049	0.0180	0.12	44.0
Potassium hexacyanoferrate II	368.37	42.3	15582.1	17.4	6409.6
Amphetamine sulfate	368.54	1.97	726.0	0.15	55.3
Homatropine methylbromide	370.33	9	3333.0	3.24	1199.9
Kelthane	370.48	0.012	4.4	1.55	574.2
Di(2-ethylhexyl)adipate	370.64	3.15	1167.5	24.6	9117.7
Ioxynil	370.91	0.11	40.8	0.3	111.3
Heptachlor	373.3	0.059	22.0	0.11	41.1
Dextropropoxyphene * HCl	375.98	0.49	184.2	0.22	82.7
Dieldrin	380.9	0.18	68.6	0.12	45.7
Scopolamine * HBr	384.31	1.08	415.1	3.3	1268.2
Di(2-ethylhexyl)phthalate	390.62	0.84	328.1	79.4	31015.2
Rotenone	394.45	0.00013	0.051	0.33	130.2
Hexachlorophene	406.89	0.0079	3.2	0.15	61.0
Chlordan	409.76	0.06	24.6	1.12	458.9
Hydroxyzine * HCl	411.41	0.067	27.6	2.31	950.4
Chloroquine sulfate	418	0.06	25.1	2.6	1086.8
Quinidine sulfate	422.54	0.12	50.7	1.08	456.3
Oxatomide	426.61	0.019	8.1	3.31	1412.1
Xanthinol nicotinate	434.51	15.8	6865.3	32.5	14121.6
Mitoxantrone	444.54	0.0024	1.07	1.32	586.8
Amethopterin	454.5	0.00014	0.064	0.3	136.4
Dimenhydrinate	470.02	0.076	35.7	2.81	1320.8
Emetine	480.71	0.00016	0.077	0.14	67.3
Tetracycline * HCl	480.94	0.14	67.3	13.4	6444.6
VerapamilHCl	491.13	0.1	49.1	0.22	108.0
Chlorhexidine	505.52	0.015	7.6	18.2	9200.5

RC IC_{50} and LD_{50} Values for RC Substances with Rat Oral LD_{50} Data

Substance	Molecular Weight (g/mole)	IC_{50x} (mM)[1]	IC_{50x} (mg/mL)[1]	Rat Oral LD_{50} (mmol/kg)[2]	Rat Oral LD_{50} (mg/kg)[2]
Chloroquine diphosphate	515.92	0.017	8.8	1.88	969.9
Triton X-100	647	0.055	35.6	2.78	1798.7
Atropine sulfate	676.9	0.22	148.9	0.92	622.7
Digitoxin	765.05	0.00011	0.0842	0.073	55.8
Trypan blue	964.88	0.095	91.7	6.43	6204.2
Actinomycin D	1255.6	0.0000081	0.0102	0.0057	7.2

Abbreviations: RC=Registry of Cytotoxicity.
[1]Geometric mean of the IC_{50} values collected from the literature for various *in vitro* basal cytotoxicity endpoints and cell types (from the RC [Halle 1998, 2003]).
[2]Rat oral LD_{50} values used in the RC (Halle 1998, 2003), which generally came from the 1983/1984 Registry of Toxic Effects for Chemical Substances®.

Appendix K4

Individual Laboratory LD_{50} Predictions: RC Rat-Only Millimole Regression

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Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
3T3	1,1,1-Trichloroethane	ECBC	1.957	12078	> 5000	51.712	6899	> 5000	2.489
3T3	1,1,1-Trichloroethane	FAL	1.957	12078	> 5000	38.621	5152	> 5000	2.200
3T3	1,1,1-Trichloroethane	IIVS	1.957	12078	> 5000	27.604	3683	2000-5000	1.868
3T3	2-Propanol	ECBC	1.929	5105	> 5000	21.855	1314	300-2000	1.637
3T3	2-Propanol	FAL	1.929	5105	> 5000	26.293	1580	300-2000	1.820
3T3	2-Propanol	IIVS	1.929	5105	> 5000	26.694	1605	300-2000	1.835
3T3	5-Aminosalicylic acid	ECBC	1.350	3428	2000-5000	11.241	1721	300-2000	0.979
3T3	5-Aminosalicylic acid	FAL	1.350	3428	2000-5000	13.050	1998	300-2000	1.127
3T3	5-Aminosalicylic acid	IIVS	1.350	3428	2000-5000	11.540	1767	300-2000	1.005
3T3	Acetaminophen	ECBC	1.155	2162	2000-5000	2.333	353	300-2000	-0.577
3T3	Acetaminophen	FAL	1.155	2162	2000-5000	2.859	432	300-2000	-0.375
3T3	Acetaminophen	IIVS	1.155	2162	2000-5000	2.390	361	300-2000	-0.553
3T3	Acetonitrile	ECBC	1.942	3595	2000-5000	38.425	1577	300-2000	2.195
3T3	Acetonitrile	FAL	1.942	3595	2000-5000	43.235	1775	300-2000	2.312
3T3	Acetonitrile	IIVS	1.942	3595	2000-5000	45.155	1854	300-2000	2.355
3T3	Acetylsalicylic acid	ECBC	0.922	1506	300-2000	7.307	1317	300-2000	0.553
3T3	Acetylsalicylic acid	FAL	0.922	1506	300-2000	9.635	1736	300-2000	0.827
3T3	Acetylsalicylic acid	IIVS	0.922	1506	300-2000	5.915	1066	300-2000	0.344
3T3	Aminopterin	ECBC	-1.799	7	5-50	0.029	13	5-50	-4.926
3T3	Aminopterin	FAL	-1.799	7	5-50	0.039	17	5-50	-4.612
3T3	Aminopterin	IIVS	-1.799	7	5-50	0.027	12	5-50	-4.980
3T3	Amitriptyline HCl	ECBC	0.046	349	300-2000	0.731	229	50-300	-1.724
3T3	Amitriptyline HCl	FAL	0.046	349	300-2000	0.820	257	50-300	-1.611
3T3	Amitriptyline HCl	IIVS	0.046	349	300-2000	0.821	258	50-300	-1.609
3T3	Arsenic III trioxide	ECBC	-0.897	25	5-50	0.589	117	50-300	-1.937
3T3	Arsenic III trioxide	FAL	-0.897	25	5-50	0.418	83	50-300	-2.278
3T3	Arsenic III trioxide	IIVS	-0.897	25	5-50	0.731	145	50-300	-1.724
3T3	Atropine sulfate	ECBC	0.071	819	300-2000	1.306	907	300-2000	-1.151
3T3	Atropine sulfate	FAL	0.071	819	300-2000	1.989	1382	300-2000	-0.734
3T3	Atropine sulfate	IIVS	0.071	819	300-2000	1.524	1059	300-2000	-0.998
3T3	Boric acid	ECBC	1.744	3426	2000-5000	16.686	1032	300-2000	1.370
3T3	Boric acid	FAL	1.744	3426	2000-5000	25.892	1601	300-2000	1.804
3T3	Boric acid	IIVS	1.744	3426	2000-5000	14.839	918	300-2000	1.254
3T3	Busulfan	ECBC	-1.308	12	5-50	1.811	446	300-2000	-0.827
3T3	Busulfan	FAL	-1.308	12	5-50	4.505	1110	300-2000	0.075
3T3	Busulfan	IIVS	-1.308	12	5-50	1.955	482	300-2000	-0.751
3T3	Cadmium II chloride	ECBC	-0.132	135	50-300	0.306	56	50-300	-2.585
3T3	Cadmium II chloride	FAL	-0.132	135	50-300	0.280	51	50-300	-2.675
3T3	Cadmium II chloride	IIVS	-0.132	135	50-300	0.374	69	50-300	-2.387
3T3	Caffeine	ECBC	0.203	310	300-2000	3.537	687	300-2000	-0.165
3T3	Caffeine	FAL	0.203	310	300-2000	3.616	702	300-2000	-0.143

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
3T3	Caffeine	IIVS	0.203	310	300-2000	4.149	806	300-2000	-0.007
3T3	Carbamazepine	ECBC	1.075	2807	2000-5000	2.631	622	300-2000	-0.457
3T3	Carbamazepine	FAL	1.075	2807	2000-5000	3.381	799	300-2000	-0.209
3T3	Carbamazepine	IIVS	1.075	2807	2000-5000	2.754	651	300-2000	-0.412
3T3	Carbon Tetrachloride	ECBC	1.391	3783	2000-5000	NA	NA	NA	NA
3T3	Carbon Tetrachloride	FAL	1.391	3783	2000-5000	NA	NA	NA	NA
3T3	Carbon Tetrachloride	IIVS	1.391	3783	2000-5000	NA	NA	NA	NA
3T3	Chloral hydrate	ECBC	0.586	638	300-2000	4.009	663	300-2000	-0.041
3T3	Chloral hydrate	FAL	0.586	638	300-2000	4.924	814	300-2000	0.162
3T3	Chloral hydrate	IIVS	0.586	638	300-2000	4.224	699	300-2000	0.011
3T3	Chloramphenicol	ECBC	1.033	3490	2000-5000	1.907	616	300-2000	-0.776
3T3	Chloramphenicol	FAL	1.033	3490	2000-5000	3.824	1236	300-2000	-0.088
3T3	Chloramphenicol	IIVS	1.033	3490	2000-5000	3.021	976	300-2000	-0.321
3T3	Citric acid	ECBC	1.489	5929	> 5000	6.124	1176	300-2000	0.378
3T3	Citric acid	FAL	1.489	5929	> 5000	9.136	1755	300-2000	0.774
3T3	Citric acid	IIVS	1.489	5929	> 5000	8.042	1545	300-2000	0.648
3T3	Colchicine	ECBC	-1.425	15	5-50	0.055	22	5-50	-4.292
3T3	Colchicine	FAL	-1.425	15	5-50	0.102	41	5-50	-3.671
3T3	Colchicine	IIVS	-1.425	15	5-50	0.062	25	5-50	-4.158
3T3	Cupric sulfate pentahydrate	ECBC	0.279	475	300-2000	2.572	642	300-2000	-0.480
3T3	Cupric sulfate pentahydrate	FAL	0.279	475	300-2000	2.985	745	300-2000	-0.333
3T3	Cupric sulfate pentahydrate	IIVS	0.279	475	300-2000	0.786	196	50-300	-1.653
3T3	Cycloheximide	ECBC	-2.148	2	< 5	0.137	38	5-50	-3.384
3T3	Cycloheximide	FAL	-2.148	2	< 5	0.266	75	50-300	-2.726
3T3	Cycloheximide	IIVS	-2.148	2	< 5	0.132	37	5-50	-3.420
3T3	Dibutyl phthalate	ECBC	1.504	8892	> 5000	1.405	391	300-2000	-1.079
3T3	Dibutyl phthalate	FAL	1.504	8892	> 5000	3.365	936	300-2000	-0.214
3T3	Dibutyl phthalate	IIVS	1.504	8892	> 5000	1.334	371	300-2000	-1.129
3T3	Dichlorvos	ECBC	-0.576	59	50-300	1.043	230	50-300	-1.373
3T3	Dichlorvos	FAL	-0.576	59	50-300	1.807	399	300-2000	-0.829
3T3	Dichlorvos	IIVS	-0.576	59	50-300	1.397	309	300-2000	-1.084
3T3	Diethyl phthalate	ECBC	1.622	9311	> 5000	2.706	601	300-2000	-0.430
3T3	Diethyl phthalate	FAL	1.622	9311	> 5000	3.444	765	300-2000	-0.191
3T3	Diethyl phthalate	IIVS	1.622	9311	> 5000	3.000	667	300-2000	-0.328
3T3	Digoxin	ECBC	-1.441	28	5-50	2.866	2238	2000-5000	-0.373
3T3	Digoxin	FAL	-1.441	28	5-50	4.345	3393	2000-5000	0.039
3T3	Digoxin	IIVS	-1.441	28	5-50	2.797	2184	2000-5000	-0.397
3T3	Dimethylformamide	ECBC	1.861	5305	> 5000	27.454	2007	2000-5000	1.862
3T3	Dimethylformamide	FAL	1.861	5305	> 5000	27.770	2030	2000-5000	1.874
3T3	Dimethylformamide	IIVS	1.861	5305	> 5000	26.464	1934	300-2000	1.826
3T3	Diquat dibromide monohydrate	ECBC	-0.355	160	50-300	0.566	205	50-300	-1.978
3T3	Diquat dibromide monohydrate	FAL	-0.355	160	50-300	1.312	475	300-2000	-1.146

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
3T3	Diquat dibromide monohydrate	IIVS	-0.355	160	50-300	0.652	236	50-300	-1.837
3T3	Disulfoton	ECBC	-1.739	5	< 5	2.900	796	300-2000	-0.361
3T3	Disulfoton	FAL	-1.739	5	< 5	21.284	5841	> 5000	1.611
3T3	Disulfoton	IIVS	-1.739	5	< 5	1.939	532	300-2000	-0.760
3T3	Endosulfan	ECBC	-1.165	28	5-50	0.592	241	50-300	-1.933
3T3	Endosulfan	FAL	-1.165	28	5-50	0.907	369	300-2000	-1.511
3T3	Endosulfan	IIVS	-1.165	28	5-50	0.512	209	50-300	-2.076
3T3	Epinephrine bitartrate	ECBC	-1.921	4	< 5	1.837	612	300-2000	-0.813
3T3	Epinephrine bitartrate	FAL	-1.921	4	< 5	2.013	671	300-2000	-0.723
3T3	Epinephrine bitartrate	IIVS	-1.921	4	< 5	2.016	672	300-2000	-0.721
3T3	Ethanol	ECBC	2.391	11324	> 5000	33.216	1530	300-2000	2.051
3T3	Ethanol	FAL	2.391	11324	> 5000	40.989	1888	300-2000	2.259
3T3	Ethanol	IIVS	2.391	11324	> 5000	36.466	1680	300-2000	2.143
3T3	Ethylene glycol	ECBC	2.062	7161	> 5000	50.675	3146	2000-5000	2.469
3T3	Ethylene glycol	FAL	2.062	7161	> 5000	63.899	3967	2000-5000	2.698
3T3	Ethylene glycol	IIVS	2.062	7161	> 5000	58.942	3659	2000-5000	2.618
3T3	Fenpropathrin	ECBC	-0.664	76	50-300	1.253	438	300-2000	-1.191
3T3	Fenpropathrin	FAL	-0.664	76	50-300	1.502	525	300-2000	-1.012
3T3	Fenpropathrin	IIVS	-0.664	76	50-300	1.098	384	300-2000	-1.322
3T3	Gibberellic acid	ECBC	1.241	6039	> 5000	16.573	5741	> 5000	1.363
3T3	Gibberellic acid	FAL	1.241	6039	> 5000	NA	NA	NA	NA
3T3	Gibberellic acid	IIVS	1.241	6039	> 5000	16.242	5626	> 5000	1.343
3T3	Glutethimide	ECBC	0.441	600	300-2000	3.720	808	300-2000	-0.115
3T3	Glutethimide	FAL	0.441	600	300-2000	4.701	1022	300-2000	0.117
3T3	Glutethimide	IIVS	0.441	600	300-2000	3.279	712	300-2000	-0.240
3T3	Glycerol	ECBC	2.332	19770	> 5000	44.226	4073	2000-5000	2.334
3T3	Glycerol	FAL	2.332	19770	> 5000	51.100	4706	2000-5000	2.477
3T3	Glycerol	IIVS	2.332	19770	> 5000	49.997	4604	2000-5000	2.455
3T3	Haloperidol	ECBC	-0.057	330	300-2000	0.643	242	50-300	-1.851
3T3	Haloperidol	FAL	-0.057	330	300-2000	0.770	289	50-300	-1.673
3T3	Haloperidol	IIVS	-0.057	330	300-2000	0.651	245	50-300	-1.840
3T3	Hexachlorophene	ECBC	-0.696	82	50-300	0.589	240	50-300	-1.939
3T3	Hexachlorophene	FAL	-0.696	82	50-300	0.615	250	50-300	-1.896
3T3	Hexachlorophene	IIVS	-0.696	82	50-300	0.487	198	50-300	-2.126
3T3	Lactic acid	ECBC	1.606	3635	2000-5000	19.279	1737	300-2000	1.513
3T3	Lactic acid	FAL	1.606	3635	2000-5000	20.720	1866	300-2000	1.584
3T3	Lactic acid	IIVS	1.606	3635	2000-5000	18.836	1697	300-2000	1.490
3T3	Lindane	ECBC	-0.464	100	50-300	2.530	736	300-2000	-0.496
3T3	Lindane	FAL	-0.464	100	50-300	3.903	1135	300-2000	-0.067
3T3	Lindane	IIVS	-0.464	100	50-300	2.091	608	300-2000	-0.685
3T3	Lithium I carbonate	ECBC	0.902	590	300-2000	10.179	752	300-2000	0.881
3T3	Lithium I carbonate	FAL	0.902	590	300-2000	NA	NA	NA	NA

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
3T3	Lithium I carbonate	IIVS	0.902	590	300-2000	NA	NA	NA	NA
3T3	Meprobamate	ECBC	0.803	1387	300-2000	5.149	1124	300-2000	0.207
3T3	Meprobamate	FAL	0.803	1387	300-2000	7.663	1673	300-2000	0.600
3T3	Meprobamate	IIVS	0.803	1387	300-2000	5.363	1171	300-2000	0.247
3T3	Mercury II chloride	ECBC	-0.830	40	5-50	0.614	167	50-300	-1.897
3T3	Mercury II chloride	FAL	-0.830	40	5-50	0.772	210	50-300	-1.670
3T3	Mercury II chloride	IIVS	-0.830	40	5-50	0.619	168	50-300	-1.889
3T3	Methanol	ECBC	2.430	8710	> 5000	NA	NA	NA	NA
3T3	Methanol	FAL	2.430	8710	> 5000	NA	NA	NA	NA
3T3	Methanol	IIVS	2.430	8710	> 5000	NA	NA	NA	NA
3T3	Nicotine	ECBC	-0.367	70	50-300	5.196	843	300-2000	0.216
3T3	Nicotine	FAL	-0.367	70	50-300	6.170	1001	300-2000	0.386
3T3	Nicotine	IIVS	-0.367	70	50-300	6.525	1058	300-2000	0.441
3T3	Paraquat	ECBC	-0.443	93	50-300	1.371	353	300-2000	-1.103
3T3	Paraquat	FAL	-0.443	93	50-300	1.361	350	300-2000	-1.109
3T3	Paraquat	IIVS	-0.443	93	50-300	1.365	351	300-2000	-1.107
3T3	Parathion	ECBC	-1.679	6	5-50	1.310	382	300-2000	-1.147
3T3	Parathion	FAL	-1.679	6	5-50	2.793	814	300-2000	-0.398
3T3	Parathion	IIVS	-1.679	6	5-50	1.336	389	300-2000	-1.128
3T3	Phenobarbital	ECBC	-0.016	224	50-300	6.449	1498	300-2000	0.429
3T3	Phenobarbital	FAL	-0.016	224	50-300	6.743	1566	300-2000	0.473
3T3	Phenobarbital	IIVS	-0.016	224	50-300	5.678	1319	300-2000	0.303
3T3	Phenol	ECBC	0.908	762	300-2000	3.147	296	50-300	-0.280
3T3	Phenol	FAL	0.908	762	300-2000	4.332	408	300-2000	0.036
3T3	Phenol	IIVS	0.908	762	300-2000	3.375	318	300-2000	-0.211
3T3	Phenylthiourea	ECBC	-1.705	3	< 5	1.870	285	50-300	-0.795
3T3	Phenylthiourea	FAL	-1.705	3	< 5	5.025	765	300-2000	0.183
3T3	Phenylthiourea	IIVS	-1.705	3	< 5	3.271	498	300-2000	-0.242
3T3	Physostigmine	ECBC	-1.741	5	< 5	1.463	403	300-2000	-1.038
3T3	Physostigmine	FAL	-1.741	5	< 5	1.747	481	300-2000	-0.863
3T3	Physostigmine	IIVS	-1.741	5	< 5	1.313	361	300-2000	-1.145
3T3	Potassium cyanide	ECBC	-0.956	7	5-50	2.195	143	50-300	-0.637
3T3	Potassium cyanide	FAL	-0.956	7	5-50	5.970	389	300-2000	0.353
3T3	Potassium cyanide	IIVS	-0.956	7	5-50	2.425	158	50-300	-0.538
3T3	Potassium I chloride	ECBC	1.575	2802	2000-5000	22.149	1651	300-2000	1.650
3T3	Potassium I chloride	FAL	1.575	2802	2000-5000	23.062	1719	300-2000	1.690
3T3	Potassium I chloride	IIVS	1.575	2802	2000-5000	23.182	1728	300-2000	1.695
3T3	Procainamide HCl	ECBC	0.856	1950	300-2000	4.952	1346	300-2000	0.168
3T3	Procainamide HCl	FAL	0.856	1950	300-2000	5.115	1390	300-2000	0.200
3T3	Procainamide HCl	IIVS	0.856	1950	300-2000	5.442	1479	300-2000	0.261
3T3	Propranolol	ECBC	0.197	466	300-2000	1.063	314	300-2000	-1.354
3T3	Propranolol	FAL	0.197	466	300-2000	1.037	307	300-2000	-1.378

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
3T3	Propranolol	IIVS	0.197	466	300-2000	1.203	356	300-2000	-1.232
3T3	Propylparaben	ECBC	1.546	6332	> 5000	1.615	291	50-300	-0.940
3T3	Propylparaben	FAL	1.546	6332	> 5000	2.390	431	300-2000	-0.553
3T3	Propylparaben	IIVS	1.546	6332	> 5000	1.481	267	50-300	-1.026
3T3	Sodium arsenite	ECBC	-0.474	44	5-50	0.362	47	5-50	-2.419
3T3	Sodium arsenite	FAL	-0.474	44	5-50	0.554	72	50-300	-1.998
3T3	Sodium arsenite	IIVS	-0.474	44	5-50	0.415	54	50-300	-2.284
3T3	Sodium chloride	ECBC	1.841	4050	2000-5000	28.902	1689	300-2000	1.913
3T3	Sodium chloride	FAL	1.841	4050	2000-5000	28.388	1659	300-2000	1.896
3T3	Sodium chloride	IIVS	1.841	4050	2000-5000	29.098	1700	300-2000	1.920
3T3	Sodium dichromate dihydrate	ECBC	-0.771	50	50-300	0.274	82	50-300	-2.697
3T3	Sodium dichromate dihydrate	FAL	-0.771	50	50-300	0.278	83	50-300	-2.680
3T3	Sodium dichromate dihydrate	IIVS	-0.771	50	50-300	0.262	78	50-300	-2.740
3T3	Sodium hypochlorite	ECBC	2.142	10328	> 5000	11.970	891	300-2000	1.041
3T3	Sodium hypochlorite	FAL	2.142	10328	> 5000	11.430	851	300-2000	0.996
3T3	Sodium hypochlorite	IIVS	2.142	10328	> 5000	17.184	1279	300-2000	1.399
3T3	Sodium oxalate	ECBC	0.674	633	300-2000	2.434	326	300-2000	-0.535
3T3	Sodium oxalate	FAL	0.674	633	300-2000	2.168	291	50-300	-0.649
3T3	Sodium oxalate	IIVS	0.674	633	300-2000	2.552	342	300-2000	-0.488
3T3	Sodium I fluoride	ECBC	0.480	127	50-300	4.927	207	50-300	0.163
3T3	Sodium I fluoride	FAL	0.480	127	50-300	5.977	251	50-300	0.354
3T3	Sodium I fluoride	IIVS	0.480	127	50-300	5.601	235	50-300	0.290
3T3	Sodium selenate	ECBC	-1.799	3	< 5	1.273	240	50-300	-1.176
3T3	Sodium selenate	FAL	-1.799	3	< 5	2.401	454	300-2000	-0.548
3T3	Sodium selenate	IIVS	-1.799	3	< 5	2.025	383	300-2000	-0.717
3T3	Strychnine	ECBC	-1.725	6	5-50	4.435	1483	300-2000	0.059
3T3	Strychnine	FAL	-1.725	6	5-50	2.695	901	300-2000	-0.434
3T3	Strychnine	IIVS	-1.725	6	5-50	2.271	760	300-2000	-0.603
3T3	Thallium II sulfate	ECBC	-1.305	25	5-50	0.424	214	50-300	-2.263
3T3	Thallium II sulfate	FAL	-1.305	25	5-50	0.730	368	300-2000	-1.726
3T3	Thallium II sulfate	IIVS	-1.305	25	5-50	0.602	304	300-2000	-1.916
3T3	Trichloroacetic acid	ECBC	1.505	5229	> 5000	8.195	1339	300-2000	0.666
3T3	Trichloroacetic acid	FAL	1.505	5229	> 5000	10.085	1648	300-2000	0.872
3T3	Trichloroacetic acid	IIVS	1.505	5229	> 5000	8.371	1368	300-2000	0.687
3T3	Triethylenemelamine	ECBC	-1.708	4	< 5	0.137	28	5-50	-3.378
3T3	Triethylenemelamine	FAL	-1.708	4	< 5	0.474	97	50-300	-2.153
3T3	Triethylenemelamine	IIVS	-1.708	4	< 5	0.183	37	5-50	-3.095
3T3	Triphenyltin hydroxide	ECBC	-0.047	329	300-2000	0.062	23	5-50	-4.161
3T3	Triphenyltin hydroxide	FAL	-0.047	329	300-2000	0.051	19	5-50	-4.366
3T3	Triphenyltin hydroxide	IIVS	-0.047	329	300-2000	0.046	17	5-50	-4.459
3T3	Valproic acid	ECBC	0.839	996	300-2000	7.487	1080	300-2000	0.577
3T3	Valproic acid	FAL	0.839	996	300-2000	12.661	1826	300-2000	1.097

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
3T3	Valproic acid	IIVS	0.839	996	300-2000	7.663	1105	300-2000	0.600
3T3	Verapamil HCl	ECBC	-0.646	111	50-300	1.257	617	300-2000	-1.188
3T3	Verapamil HCl	FAL	-0.646	111	50-300	1.302	640	300-2000	-1.153
3T3	Verapamil HCl	IIVS	-0.646	111	50-300	1.370	673	300-2000	-1.103
3T3	Xylene	ECBC	1.643	4667	2000-5000	NA	NA	NA	NA
3T3	Xylene	FAL	1.643	4667	2000-5000	NA	NA	NA	NA
3T3	Xylene	IIVS	1.643	4667	2000-5000	9.685	1028	300-2000	0.832

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
NHK	1,1,1-Trichloroethane	ECBC	1.957	12078	> 5000	25.374	3385	2000-5000	1.784
NHK	1,1,1-Trichloroethane	FAL	1.957	12078	> 5000	NA	NA	NA	NA
NHK	1,1,1-Trichloroethane	IIVS	1.957	12078	> 5000	NA	NA	NA	NA
NHK	2-Propanol	ECBC	1.929	5105	> 5000	29.708	1786	300-2000	1.940
NHK	2-Propanol	FAL	1.929	5105	> 5000	26.850	1614	300-2000	1.840
NHK	2-Propanol	IIVS	1.929	5105	> 5000	33.892	2037	2000-5000	2.071
NHK	5-Aminosalicylic acid	ECBC	1.350	3428	2000-5000	2.025	310	300-2000	-0.717
NHK	5-Aminosalicylic acid	FAL	1.350	3428	2000-5000	2.994	458	300-2000	-0.330
NHK	5-Aminosalicylic acid	IIVS	1.350	3428	2000-5000	2.519	386	300-2000	-0.501
NHK	Acetaminophen	ECBC	1.155	2162	2000-5000	7.388	1117	300-2000	0.564
NHK	Acetaminophen	FAL	1.155	2162	2000-5000	6.693	1012	300-2000	0.466
NHK	Acetaminophen	IIVS	1.155	2162	2000-5000	7.468	1129	300-2000	0.574
NHK	Acetonitrile	ECBC	1.942	3595	2000-5000	45.269	1858	300-2000	2.357
NHK	Acetonitrile	FAL	1.942	3595	2000-5000	46.718	1918	300-2000	2.388
NHK	Acetonitrile	IIVS	1.942	3595	2000-5000	45.140	1853	300-2000	2.354
NHK	Acetylsalicylic acid	ECBC	0.922	1506	300-2000	7.243	1305	300-2000	0.544
NHK	Acetylsalicylic acid	FAL	0.922	1506	300-2000	7.532	1357	300-2000	0.583
NHK	Acetylsalicylic acid	IIVS	0.922	1506	300-2000	6.598	1189	300-2000	0.452
NHK	Aminopterin	ECBC	-1.799	7	5-50	5.652	2490	2000-5000	0.299
NHK	Aminopterin	FAL	-1.799	7	5-50	4.583	2018	2000-5000	0.091
NHK	Aminopterin	IIVS	-1.799	7	5-50	4.817	2122	2000-5000	0.141
NHK	Amitriptyline HCl	ECBC	0.046	349	300-2000	0.936	294	50-300	-1.480
NHK	Amitriptyline HCl	FAL	0.046	349	300-2000	0.753	236	50-300	-1.696
NHK	Amitriptyline HCl	IIVS	0.046	349	300-2000	0.957	300	50-300	-1.458
NHK	Arsenic III trioxide	ECBC	-0.897	25	5-50	0.989	196	50-300	-1.426
NHK	Arsenic III trioxide	FAL	-0.897	25	5-50	0.572	113	50-300	-1.968
NHK	Arsenic III trioxide	IIVS	-0.897	25	5-50	1.535	304	300-2000	-0.991
NHK	Atropine sulfate	ECBC	0.071	819	300-2000	1.662	1155	300-2000	-0.912
NHK	Atropine sulfate	FAL	0.071	819	300-2000	1.610	1119	300-2000	-0.943
NHK	Atropine sulfate	IIVS	0.071	819	300-2000	1.630	1132	300-2000	-0.932
NHK	Boric acid	ECBC	1.744	3426	2000-5000	9.755	603	300-2000	0.839
NHK	Boric acid	FAL	1.744	3426	2000-5000	9.249	572	300-2000	0.786
NHK	Boric acid	IIVS	1.744	3426	2000-5000	10.120	626	300-2000	0.875
NHK	Busulfan	ECBC	-1.308	12	5-50	4.189	1032	300-2000	0.003
NHK	Busulfan	FAL	-1.308	12	5-50	4.039	995	300-2000	-0.033
NHK	Busulfan	IIVS	-1.308	12	5-50	4.633	1141	300-2000	0.102
NHK	Cadmium II chloride	ECBC	-0.132	135	50-300	0.583	107	50-300	-1.948
NHK	Cadmium II chloride	FAL	-0.132	135	50-300	0.509	93	50-300	-2.083
NHK	Cadmium II chloride	IIVS	-0.132	135	50-300	0.556	102	50-300	-1.995
NHK	Caffeine	ECBC	0.203	310	300-2000	7.731	1501	300-2000	0.609
NHK	Caffeine	FAL	0.203	310	300-2000	6.715	1304	300-2000	0.469
NHK	Caffeine	IIVS	0.203	310	300-2000	6.724	1306	300-2000	0.471

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
NHK	Carbamazepine	ECBC	1.075	2807	2000-5000	2.383	563	300-2000	-0.555
NHK	Carbamazepine	FAL	1.075	2807	2000-5000	3.296	779	300-2000	-0.235
NHK	Carbamazepine	IIVS	1.075	2807	2000-5000	2.350	555	300-2000	-0.569
NHK	Carbon tetrachloride	ECBC	1.391	3783	2000-5000	NA	NA	NA	NA
NHK	Carbon tetrachloride	FAL	1.391	3783	2000-5000	NA	NA	NA	NA
NHK	Carbon tetrachloride	IIVS	1.391	3783	2000-5000	NA	NA	NA	NA
NHK	Chloral hydrate	ECBC	0.586	638	300-2000	3.848	636	300-2000	-0.082
NHK	Chloral hydrate	FAL	0.586	638	300-2000	4.049	670	300-2000	-0.031
NHK	Chloral hydrate	IIVS	0.586	638	300-2000	3.521	582	300-2000	-0.169
NHK	Chloramphenicol	ECBC	1.033	3490	2000-5000	4.042	1306	300-2000	-0.033
NHK	Chloramphenicol	FAL	1.033	3490	2000-5000	4.484	1449	300-2000	0.070
NHK	Chloramphenicol	IIVS	1.033	3490	2000-5000	4.387	1418	300-2000	0.048
NHK	Citric acid	ECBC	1.489	5929	> 5000	6.478	1244	300-2000	0.434
NHK	Citric acid	FAL	1.489	5929	> 5000	5.147	989	300-2000	0.206
NHK	Citric acid	IIVS	1.489	5929	> 5000	5.965	1146	300-2000	0.352
NHK	Colchicine	ECBC	-1.425	15	5-50	0.029	12	5-50	-4.918
NHK	Colchicine	FAL	-1.425	15	5-50	0.035	14	5-50	-4.720
NHK	Colchicine	IIVS	-1.425	15	5-50	0.036	14	5-50	-4.699
NHK	Cupric sulfate pentahydrate	ECBC	0.279	475	300-2000	3.697	923	300-2000	-0.121
NHK	Cupric sulfate pentahydrate	FAL	0.279	475	300-2000	3.743	935	300-2000	-0.109
NHK	Cupric sulfate pentahydrate	IIVS	0.279	475	300-2000	3.846	960	300-2000	-0.082
NHK	Cycloheximide	ECBC	-2.148	2	< 5	0.096	27	5-50	-3.732
NHK	Cycloheximide	FAL	-2.148	2	< 5	0.132	37	5-50	-3.418
NHK	Cycloheximide	IIVS	-2.148	2	< 5	0.110	31	5-50	-3.601
NHK	Dibutyl phthalate	ECBC	1.504	8892	> 5000	1.513	421	300-2000	-1.005
NHK	Dibutyl phthalate	FAL	1.504	8892	> 5000	1.763	491	300-2000	-0.854
NHK	Dibutyl phthalate	IIVS	1.504	8892	> 5000	1.372	382	300-2000	-1.102
NHK	Dichlorvos	ECBC	-0.576	59	50-300	0.992	219	50-300	-1.423
NHK	Dichlorvos	FAL	-0.576	59	50-300	1.163	257	50-300	-1.265
NHK	Dichlorvos	IIVS	-0.576	59	50-300	1.171	259	50-300	-1.258
NHK	Diethyl phthalate	ECBC	1.622	9311	> 5000	3.745	832	300-2000	-0.108
NHK	Diethyl phthalate	FAL	1.622	9311	> 5000	2.244	499	300-2000	-0.615
NHK	Diethyl phthalate	IIVS	1.622	9311	> 5000	3.876	861	300-2000	-0.074
NHK	Digoxin	ECBC	-1.441	28	5-50	0.023	18	5-50	-5.164
NHK	Digoxin	FAL	-1.441	28	5-50	0.003	2	< 5	-7.209
NHK	Digoxin	IIVS	-1.441	28	5-50	0.020	15	5-50	-5.293
NHK	Dimethylformamide	ECBC	1.861	5305	> 5000	35.157	2570	2000-5000	2.107
NHK	Dimethylformamide	FAL	1.861	5305	> 5000	32.491	2375	2000-5000	2.029
NHK	Dimethylformamide	IIVS	1.861	5305	> 5000	29.746	2174	2000-5000	1.942
NHK	Diquat dibromide monohydrate	ECBC	-0.355	160	50-300	0.547	198	50-300	-2.012
NHK	Diquat dibromide monohydrate	FAL	-0.355	160	50-300	0.692	251	50-300	-1.779
NHK	Diquat dibromide monohydrate	IIVS	-0.355	160	50-300	0.567	205	50-300	-1.976

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
NHK	Disulfoton	ECBC	-1.739	5	< 5	3.092	849	300-2000	-0.298
NHK	Disulfoton	FAL	-1.739	5	< 5	6.640	1822	300-2000	0.458
NHK	Disulfoton	IIVS	-1.739	5	< 5	3.475	954	300-2000	-0.182
NHK	Endosulfan	ECBC	-1.165	28	5-50	0.512	208	50-300	-2.077
NHK	Endosulfan	FAL	-1.165	28	5-50	0.336	137	50-300	-2.493
NHK	Endosulfan	IIVS	-1.165	28	5-50	0.419	170	50-300	-2.276
NHK	Epinephrine bitartrate	ECBC	-1.921	4	< 5	2.614	871	300-2000	-0.464
NHK	Epinephrine bitartrate	FAL	-1.921	4	< 5	2.216	739	300-2000	-0.628
NHK	Epinephrine bitartrate	IIVS	-1.921	4	< 5	2.161	720	300-2000	-0.652
NHK	Ethanol	ECBC	2.391	11324	> 5000	40.823	1881	300-2000	2.255
NHK	Ethanol	FAL	2.391	11324	> 5000	47.812	2203	2000-5000	2.411
NHK	Ethanol	IIVS	2.391	11324	> 5000	44.757	2062	2000-5000	2.346
NHK	Ethylene glycol	ECBC	2.062	7161	> 5000	69.735	4329	2000-5000	2.785
NHK	Ethylene glycol	FAL	2.062	7161	> 5000	78.619	4881	2000-5000	2.903
NHK	Ethylene glycol	IIVS	2.062	7161	> 5000	71.258	4424	2000-5000	2.806
NHK	Fenpropathrin	ECBC	-0.664	76	50-300	0.564	197	50-300	-1.982
NHK	Fenpropathrin	FAL	-0.664	76	50-300	0.449	157	50-300	-2.207
NHK	Fenpropathrin	IIVS	-0.664	76	50-300	0.414	145	50-300	-2.287
NHK	Gibberellic acid	ECBC	1.241	6039	> 5000	10.509	3640	2000-5000	0.912
NHK	Gibberellic acid	FAL	1.241	6039	> 5000	10.670	3696	2000-5000	0.927
NHK	Gibberellic acid	IIVS	1.241	6039	> 5000	10.470	3627	2000-5000	0.909
NHK	Glutethimide	ECBC	0.441	600	300-2000	3.828	832	300-2000	-0.087
NHK	Glutethimide	FAL	0.441	600	300-2000	3.738	812	300-2000	-0.110
NHK	Glutethimide	IIVS	0.441	600	300-2000	3.792	824	300-2000	-0.096
NHK	Glycerol	ECBC	2.332	19770	> 5000	54.557	5024	> 5000	2.542
NHK	Glycerol	FAL	2.332	19770	> 5000	40.626	3741	2000-5000	2.250
NHK	Glycerol	IIVS	2.332	19770	> 5000	52.042	4793	2000-5000	2.495
NHK	Haloperidol	ECBC	-0.057	330	300-2000	0.543	204	50-300	-2.019
NHK	Haloperidol	FAL	-0.057	330	300-2000	0.525	197	50-300	-2.053
NHK	Haloperidol	IIVS	-0.057	330	300-2000	0.513	193	50-300	-2.076
NHK	Hexachlorophene	ECBC	-0.696	82	50-300	0.061	25	5-50	-4.179
NHK	Hexachlorophene	FAL	-0.696	82	50-300	0.074	30	5-50	-3.984
NHK	Hexachlorophene	IIVS	-0.696	82	50-300	0.055	22	5-50	-4.285
NHK	Lactic acid	ECBC	1.606	3635	2000-5000	13.423	1209	300-2000	1.155
NHK	Lactic acid	FAL	1.606	3635	2000-5000	13.575	1223	300-2000	1.166
NHK	Lactic acid	IIVS	1.606	3635	2000-5000	13.520	1218	300-2000	1.162
NHK	Lindane	ECBC	-0.464	100	50-300	1.258	366	300-2000	-1.188
NHK	Lindane	FAL	-0.464	100	50-300	1.357	395	300-2000	-1.113
NHK	Lindane	IIVS	-0.464	100	50-300	1.154	335	300-2000	-1.273
NHK	Lithium I carbonate	ECBC	0.902	590	300-2000	8.770	648	300-2000	0.733
NHK	Lithium I carbonate	FAL	0.902	590	300-2000	9.491	701	300-2000	0.812
NHK	Lithium I carbonate	IIVS	0.902	590	300-2000	9.956	736	300-2000	0.859

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
NHK	Meprobamate	ECBC	0.803	1387	300-2000	7.204	1573	300-2000	0.539
NHK	Meprobamate	FAL	0.803	1387	300-2000	2.925	639	300-2000	-0.353
NHK	Meprobamate	IIVS	0.803	1387	300-2000	6.609	1443	300-2000	0.454
NHK	Mercury II chloride	ECBC	-0.830	40	5-50	0.829	225	50-300	-1.600
NHK	Mercury II chloride	FAL	-0.830	40	5-50	0.745	202	50-300	-1.706
NHK	Mercury II chloride	IIVS	-0.830	40	5-50	0.745	202	50-300	-1.705
NHK	Methanol	ECBC	2.434	8710	> 5000	NA	NA	NA	NA
NHK	Methanol	FAL	2.434	8710	> 5000	19.871	637	300-2000	1.543
NHK	Methanol	IIVS	2.434	8710	> 5000	26.159	838	300-2000	1.815
NHK	Nicotine	ECBC	-0.367	70	50-300	3.259	529	300-2000	-0.246
NHK	Nicotine	FAL	-0.367	70	50-300	3.666	595	300-2000	-0.129
NHK	Nicotine	IIVS	-0.367	70	50-300	3.511	570	300-2000	-0.172
NHK	Paraquat	ECBC	-0.443	93	50-300	2.000	514	300-2000	-0.729
NHK	Paraquat	FAL	-0.443	93	50-300	2.654	683	300-2000	-0.449
NHK	Paraquat	IIVS	-0.443	93	50-300	2.092	538	300-2000	-0.684
NHK	Parathion	ECBC	-1.679	6	5-50	1.608	468	300-2000	-0.945
NHK	Parathion	FAL	-1.679	6	5-50	1.531	446	300-2000	-0.993
NHK	Parathion	IIVS	-1.679	6	5-50	1.502	437	300-2000	-1.012
NHK	Phenobarbital	ECBC	-0.016	224	50-300	6.691	1554	300-2000	0.466
NHK	Phenobarbital	FAL	-0.016	224	50-300	5.009	1163	300-2000	0.179
NHK	Phenobarbital	IIVS	-0.016	224	50-300	5.167	1200	300-2000	0.210
NHK	Phenol	ECBC	0.908	762	300-2000	3.333	314	300-2000	-0.224
NHK	Phenol	FAL	0.908	762	300-2000	4.159	391	300-2000	-0.005
NHK	Phenol	IIVS	0.908	762	300-2000	3.905	367	300-2000	-0.067
NHK	Phenylthiourea	ECBC	-1.705	3	< 5	6.100	928	300-2000	0.374
NHK	Phenylthiourea	FAL	-1.705	3	< 5	6.355	967	300-2000	0.415
NHK	Phenylthiourea	IIVS	-1.705	3	< 5	5.347	814	300-2000	0.244
NHK	Physostigmine	ECBC	-1.741	5	< 5	3.325	916	300-2000	-0.226
NHK	Physostigmine	FAL	-1.741	5	< 5	1.593	439	300-2000	-0.954
NHK	Physostigmine	IIVS	-1.741	5	< 5	3.088	850	300-2000	-0.299
NHK	Potassium cyanide	ECBC	-0.956	7	5-50	2.916	190	50-300	-0.356
NHK	Potassium cyanide	FAL	-0.956	7	5-50	3.732	243	50-300	-0.112
NHK	Potassium cyanide	IIVS	-0.956	7	5-50	2.304	150	50-300	-0.589
NHK	Potassium I chloride	ECBC	1.575	2802	2000-5000	19.648	1465	300-2000	1.531
NHK	Potassium I chloride	FAL	1.575	2802	2000-5000	18.553	1383	300-2000	1.475
NHK	Potassium I chloride	IIVS	1.575	2802	2000-5000	17.651	1316	300-2000	1.425
NHK	Procainamide HCl	ECBC	0.856	1950	300-2000	8.770	2383	2000-5000	0.733
NHK	Procainamide HCl	FAL	0.856	1950	300-2000	9.531	2590	2000-5000	0.816
NHK	Procainamide HCl	IIVS	0.856	1950	300-2000	10.075	2738	2000-5000	0.871
NHK	Propranolol	ECBC	0.197	466	300-2000	1.699	503	300-2000	-0.890
NHK	Propranolol	FAL	0.197	466	300-2000	1.806	534	300-2000	-0.830
NHK	Propranolol	IIVS	0.197	466	300-2000	1.495	442	300-2000	-1.017

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
NHK	Propylparaben	ECBC	1.546	6332	> 5000	1.520	274	50-300	-1.000
NHK	Propylparaben	FAL	1.546	6332	> 5000	1.534	276	50-300	-0.991
NHK	Propylparaben	IIVS	1.546	6332	> 5000	1.354	244	50-300	-1.115
NHK	Sodium arsenite	ECBC	-0.474	44	5-50	0.438	57	50-300	-2.231
NHK	Sodium arsenite	FAL	-0.474	44	5-50	0.292	38	5-50	-2.631
NHK	Sodium arsenite	IIVS	-0.474	44	5-50	0.353	46	5-50	-2.444
NHK	Sodium chloride	ECBC	1.841	4050	2000-5000	25.437	1487	300-2000	1.787
NHK	Sodium chloride	FAL	1.841	4050	2000-5000	11.979	700	300-2000	1.042
NHK	Sodium chloride	IIVS	1.841	4050	2000-5000	25.063	1465	300-2000	1.772
NHK	Sodium dichromate dihydrate	ECBC	-0.771	50	50-300	0.307	92	50-300	-2.583
NHK	Sodium dichromate dihydrate	FAL	-0.771	50	50-300	0.312	93	50-300	-2.565
NHK	Sodium dichromate dihydrate	IIVS	-0.771	50	50-300	0.268	80	50-300	-2.718
NHK	Sodium hypochlorite	ECBC	2.142	10328	> 5000	16.924	1260	300-2000	1.384
NHK	Sodium hypochlorite	FAL	2.142	10328	> 5000	13.934	1037	300-2000	1.192
NHK	Sodium hypochlorite	IIVS	2.142	10328	> 5000	16.183	1205	300-2000	1.340
NHK	Sodium oxalate	ECBC	0.674	633	300-2000	6.390	856	300-2000	0.420
NHK	Sodium oxalate	FAL	0.674	633	300-2000	6.091	816	300-2000	0.373
NHK	Sodium oxalate	IIVS	0.674	633	300-2000	6.372	854	300-2000	0.418
NHK	Sodium I fluoride	ECBC	0.480	127	50-300	4.446	187	50-300	0.061
NHK	Sodium I fluoride	FAL	0.480	127	50-300	4.318	181	50-300	0.032
NHK	Sodium I fluoride	IIVS	0.480	127	50-300	4.644	195	50-300	0.105
NHK	Sodium selenate	ECBC	-1.799	3	< 5	1.010	191	50-300	-1.405
NHK	Sodium selenate	FAL	-1.799	3	< 5	1.351	255	50-300	-1.117
NHK	Sodium selenate	IIVS	-1.799	3	< 5	1.147	217	50-300	-1.279
NHK	Strychnine	ECBC	-1.725	6	5-50	2.277	761	300-2000	-0.601
NHK	Strychnine	FAL	-1.725	6	5-50	1.780	595	300-2000	-0.844
NHK	Strychnine	IIVS	-1.725	6	5-50	1.892	633	300-2000	-0.784
NHK	Thallium II sulfate	ECBC	-1.305	25	5-50	0.129	65	50-300	-3.440
NHK	Thallium II sulfate	FAL	-1.305	25	5-50	0.118	60	50-300	-3.525
NHK	Thallium II sulfate	IIVS	-1.305	25	5-50	0.110	55	50-300	-3.602
NHK	Trichloroacetic acid	ECBC	1.505	5229	> 5000	5.790	946	300-2000	0.323
NHK	Trichloroacetic acid	FAL	1.505	5229	> 5000	6.976	1140	300-2000	0.507
NHK	Trichloroacetic acid	IIVS	1.505	5229	> 5000	6.129	1002	300-2000	0.379
NHK	Triethylenemelamine	ECBC	-1.708	4	< 5	0.487	99	50-300	-2.126
NHK	Triethylenemelamine	FAL	-1.708	4	< 5	0.547	112	50-300	-2.012
NHK	Triethylenemelamine	IIVS	-1.708	4	< 5	0.560	114	50-300	-1.988
NHK	Triphenyltin hydroxide	ECBC	-0.047	329	300-2000	0.057	21	5-50	-4.250
NHK	Triphenyltin hydroxide	FAL	-0.047	329	300-2000	0.030	11	5-50	-4.885
NHK	Triphenyltin hydroxide	IIVS	-0.047	329	300-2000	0.042	15	5-50	-4.552
NHK	Valproic acid	ECBC	0.839	996	300-2000	6.936	1000	300-2000	0.501
NHK	Valproic acid	FAL	0.839	996	300-2000	8.303	1197	300-2000	0.679
NHK	Valproic acid	IIVS	0.839	996	300-2000	6.722	969	300-2000	0.470

Individual Laboratory LD50 Predictions: RC Rat-Only Millimole Regression

NRU Test Method	Substance	Lab	Log Reference LD_{50} (mmol/kg)[1]	Reference LD_{50} (mg/kg)[1]	Observed LD_{50} Toxicity Category[2] (mg/kg)	Log Predicted LD_{50} (mmol/kg)[3]	Predicted LD_{50} (mg/kg)[3]	Predicted LD_{50} Toxicity Category[2] (mg/kg)	Log IC_{50} (mM)[4]
NHK	Verapamil HCl	ECBC	-0.646	111	50-300	1.653	812	300-2000	-0.917
NHK	Verapamil HCl	FAL	-0.646	111	50-300	1.830	899	300-2000	-0.817
NHK	Verapamil HCl	IIVS	-0.646	111	50-300	1.731	850	300-2000	-0.871
NHK	Xylene	ECBC	1.643	4665	2000-5000	NA	NA	NA	NA
NHK	Xylene	FAL	1.643	4665	2000-5000	NA	NA	NA	NA
NHK	Xylene	IIVS	1.643	4665	2000-5000	7.995	849	300-2000	0.642

Abbreviations: 3T3=Mouse fibroblast 3T3 cell line; NHK=Normal human epidermal keratinocytes; NRU=Neutral red uptake; ECBC=U.S. Army Edgewood Chemical Biological Center; FAL=Fund for the Replacement of Animals in Medical Experiments Alternatives Laboratory; IIVS=Institute for In Vitro Sciences.

[1]Reference rat oral LD_{50} values from **Table 4-2**. Reference values were developed from rat acute oral LD_{50} studies located using literature searches, secondary references, and electronic database searches.

[2]Globally Harmonized System (GHS) hazard classification (UN 2005):

Abbreviation	Category	Oral LD_{50} Limits
<5	1	$LD_{50} \leq 5$ mg/kg
5-50	2	$5 < LD_{50} \leq 50$ mg/kg
50-300	3	$50 < LD_{50} \leq 300$ mg/kg
300-2000	4	$300 < LD_{50} \leq 2000$ mg/kg
2000-5000	5	$2000 < LD_{50} \leq 5000$ mg/kg
>5000	Unclassified	$LD_{50} > 5000$ mg/kg

[3]LD_{50} determined using NRU IC_{50} value in RC rat-only millimole regression: Log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621.

[4]IC_{50} values are the geometric mean IC_{50} values for each substance in each lab.

Appendix L

Outlier Information

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Appendix L1

Outlier Characterization for the 3T3 and NHK NRU Test Methods with the RC Millimole Regression

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L.1 Outlier Analysis for the 3T3 and NHK NRU Test Methods and RC Millimole Regression

The RC millimole regression and each *in vitro* NRU test method were used to identify outlier substances among the reference substances tested in the validation study (i.e., those for which the rodent LD_{50} was not accurately predicted by the *in vitro* NRU IC_{50}) (see **Section 6.2**). The outliers, identified for each test method in **Table 6-3**, were evaluated for common characteristics that may assist in determining the types of chemicals that are not suited for use in the 3T3 and NHK NRU test methods to determine starting doses for acute systemic toxicity test methods.

A number of physico-chemical characteristics were evaluated for their frequency of occurrence among the 28 outlier substances for the 3T3 NRU test method and 31 outlier substances for the NHK NRU test method versus the entire set of reference substances. The frequency of occurrence of outliers versus the total number of reference substances for each category of each characteristic examined is shown in **Table L1-1**.

Table L1-1 Outliers per Category and NRU Test Method

Category	3T3 NRU Test Method[1]		NHK NRU Test Method[2]	
	Number of Outliers	Total Substances in Category	Number of Outliers	Total Substances in Category
Boiling Point (BP) [in degrees C]				
No information	13	34	13	34
< 100	1	6	2	7
100-200	1	5	2	5
200-300	3	4	3	4
300-400	5	6	4	6
465	1	1	1	1
960	0	1	0	1
1500	0	1	0	1
decompose, sublime, or BPs were provided at less than atmospheric pressure	4	12	6	12
Molecular Weight (g/mol)				
< 100	3	14	4	15
100-200	6	18	9	18
200-300	12	20	12	20
300-400	3	11	3	11
400-500	2	4	3	4
500-600	1	1	0	1
600-700	0	1	0	1
700-800	1	1	0	1
IC_{50} (mM)				
≤ 0.0001	0	3	0	4
0.0001 – 0.001	1	1	1	2
0.001 – 0.01	1	4	3	7
0.01 – 0.1	8	14	5	8
0.1 – 1	13	21	12	19
1 – 10	3	13	7	19
10 – 100	1	9	2	7
> 100	1	5	1	5
pH				
< 7.1	0	0	0	6
7.1	0	0	0	0
7.2	0	0	1	1
7.3	0	0	0	0
7.4	0	0	1	4
7.5	0	0	4	7
< 7.6	0	9	0	0
7.6	0	0	4	7
7.7	1	1	8	22
7.8	0	1	11	17
7.9	2	6	0	3
8.0	5	11	0	1
8.1	10	18	0	0
8.2	3	6	1	1
8.3	3	8	0	0

Table L1-1 Outliers per Category and NRU Test Method

Category	3T3 NRU Test Method[1]		NHK NRU Test Method[2]	
	Number of Outliers	Total Substances in Category	Number of Outliers	Total Substances in Category
8.4	1	5	0	0
8.5	0	1	1	1
> 8.5	3	4	0	1
log K_{ow}				
< -4	0	1	1	1
> -4 to < -3	0	1	0	1
> -3 to < -2	0	0	0	0
-2 to -1	1	5	1	5
-1 to 0	3	6	5	7
0 to 1	4	7	3	7
1 to 2	5	13	5	13
2 to 3	1	4	1	4
3 to 4	5	8	5	8
4 to 5	2	2	2	2
5 to 6	1	2	1	2
6 to 7	0	1	0	1
No information	6	20	7	20
Chemical Class				
Organic Compounds				
Acyclic hydrocarbon	1	1	1	1
Alcohol	3	9	4	10
Alkalies	0	1	0	1
Amide	1	3	0	3
Amine	2	3	2	3
Carbohydrate	1	1	0	1
Carboxylic acid	4	14	6	14
Cyclic hydrocarbon	0	3	1	3
Ester	1	1	1	1
Ether	1	1	1	1
Halogenated hydrocarbon	1	3	0	3
Heterocyclic compound	7	14	10	14
Ketone	0	1	0	1
Lipids	0	1	0	1
Nitrile	1	2	1	2
Nitro compound	0	1	0	1
Sodium compound	0	1	1	1
Sulfur compound	5	5	5	5
Organometallic compound	0	1	0	1
Organophosphorous compound	3	3	3	5
Phenol	1	5	2	5
Polycyclic compound	1	5	0	5
Urea	1	1	1	1
Inorganic Compounds				
Arsenical	1	2	1	2
Boron compound	0	1	0	1
Cadmium compound	0	1	0	1
Chlorine compound	2	5	2	5

Table L1-1 Outliers per Category and NRU Test Method

Category	3T3 NRU Test Method[1]		NHK NRU Test Method[2]	
	Number of Outliers	Total Substances in Category	Number of Outliers	Total Substances in Category
Chromium compound	0	1	0	1
Fluorine compound	0	1	0	1
Inorganic acid	0	1	0	1
Inorganic carbon compound	0	1	0	1
Lithium compound	0	1	0	1
Mercury compound	1	1	1	1
Metal	1	2	0	2
Nitrogen compound	1	1	1	1
Oxygen compound	1	1	1	1
Potassium compound	1	2	1	2
Selenium compound	1	1	1	1
Sodium compound	2	6	2	6
Sulfur compound	1	2	0	2
Substance Physical Form				
Solid	21	54	22	54
Liquid	7	16	9	17

Abbreviations: NRU=Neutral red uptake; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; BP=Boiling point; K_{ow}= Octanol:water partition coefficient.

[1]28 discordant chemicals (i.e., outliers) are characterized for the 3T3 NRU test method by counting the number of outliers in each category and comparing to the total number of chemicals in the category. Analysis excludes carbon tetrachloride and methanol since no IC_{50} values were obtained. Total chemicals = 70.

[2]31 discordant chemicals (i.e., outliers) are characterized for the NHK NRU test method by counting the number of outliers in each category and comparing to the total number of chemicals in the category. Analysis excludes carbon tetrachloride since no IC_{50} values were obtained. Total chemicals = 71.

Appendix L2

Discordant Substances for GHS Acute Oral Toxicity Category Predictions Using the 3T3 and NHK NRU Test Methods and RC Rat-Only Regressions

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L.2 Discordant Substances for GHS Acute Toxicity Category Predictions Using the 3T3 and NHK NRU Test Methods and RC Rat-Only Regressions

This appendix provides a more detailed discussion of the discordant substances identified for the GHS acute oral toxicity category predictions using the NRU test methods and the RC rat-only regressions evaluated in **Section 6.4.**

L.2.1 Discordant Substances for Prediction of GHS Acute Oral Toxicity Category by the 3T3 and NHK NRU Test Methods and the RC Rat-Only Millimole Regression

Table L2-1 identifies the discordant substances for which the *in vitro* predicted GHS toxicity category (using the 3T3 and NHK NRU test methods with the RC rat-only millimole regression) did not match the GHS toxicity category assigned based on the reference rat oral LD_{50} data. For the 3T3 NRU test method, the toxicity category was underpredicted for 23 (34%) and overpredicted for 23 (34%) of the 46 discordant substances. Of the 23 substances for which toxicity was underpredicted,

- 15 (65%) were underpredicted by one toxicity category
- 2 (9%) were underpredicted by two toxicity categories
- 6 (26%) were underpredicted by three toxicity categories

For the 23 substances for which toxicity was overpredicted,

- 14 (61%) were overpredicted by one toxicity category
- 9 (39%) were overpredicted by two toxicity categories

For the NHK NRU test method, toxicity was underpredicted for 21 (54%) and overpredicted for 27 (46%) of the 48 discordant substances. Of the 21 substances for which toxicity was underpredicted,

- 12 (57%) were underpredicted by one toxicity category
- 5 (24%) were underpredicted by two toxicity categories
- 4 (19%) were underpredicted by three toxicity categories

For the 27 substances for which toxicity was overpredicted,

- 18 (67%) were overpredicted by one toxicity category
- 9 (33%) were overpredicted by two toxicity categories

Table L2-1 Discordant Substances[1] for the Prediction of GHS Acute Oral Toxicity Categories by the 3T3 and NHK NRU Test Methods and the RC Rat-Only Millimole Regression[2]

***In Vivo* GHS Toxicity Category[3] (mg/kg)**	**3T3 NRU Test Method**		**NHK NRU Test Method**	
	Toxicity Overpredicted	**Toxicity Underpredicted**	**Toxicity Overpredicted**	**Toxicity Underpredicted**
LD_{50} <5		Cycloheximide (1) Disulfoton (3) Phenylthiourea (3) Physostigmine (3) Sodium selenate (3) Triethylenemelamine (1)		Cycloheximide (1) Disulfoton (3) Phenylthiourea (3) Physostigmine (3) Sodium selenate (2) Triethylenemelamine (2)
5 < LD_{50} ≤50		Arsenic trioxide (1) Busulfan (2) Digoxin (3) Endosulfan (1) Mercury chloride (1) Parathion (2) Potassium cyanide (1) Sodium arsenite (1) Strychnine (3) Thallium sulfate (1)		Aminopterin (3) Arsenic trioxide (1) Busulfan (2) Endosulfan (1) Mercury chloride (1) Parathion (2) Potassium cyanide (1) Strychnine (2) Thallium sulfate (1)
50 < LD_{50} ≤300		Dichlorvos (1) Fenpropathrin (1) Lindane (1) Nicotine (1) Paraquat (1) Phenobarbital (1) Verapamil HCl (1)	Hexachlorophene (1)	Lindane (1) Nicotine (1) Paraquat (1) Phenobarbital (1) Verapamil HCl (1)
300 < LD_{50} ≤2000	Amitriptyline HCl (1) Haloperidol (1) Triphenyltin hydroxide (2)		Amitriptyline HCl (1) Haloperidol (1) Triphenyltin hydroxide (2)	Procainamide HCl (1)
2000 < LD_{50} ≤5000	Acetaminophen (1) Acetonitrile (1) 5-Aminosalicylic acid (1) Boric acid (1) Carbamazepine (1) Chloramphenicol (1)		Acetaminophen (1) Acetonitrile (1) 5-Aminosalicylic acid (1) Boric acid (1) Carbamazepine (1) Chloramphenicol (1)	

***In Vivo* GHS Toxicity Category[3] (mg/kg)**	**3T3 NRU Test Method**		**NHK NRU Test Method**	
	Toxicity Overpredicted	**Toxicity Underpredicted**	**Toxicity Overpredicted**	**Toxicity Underpredicted**
	Lactic acid (1) Potassium chloride (1) Sodium chloride (1) Xylene (1)		Lactic acid (1) Potassium chloride (1) Sodium chloride (1) Xylene (1)	
LD_{50} >5000	Citric acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Dimethylformamide (2) Ethanol (2) Ethylene glycol (1) Glycerol (1) 2-Propanol (2) Sodium hypochlorite (2) Trichloroacetic acid (2)		Citric acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Dimethylformamide (2) Ethanol (1) Gibberellic Acid (1) Glycerol (1) Methanol (2) 2-Propanol (2) Sodium hypochlorite (2) Trichloroacetic acid (2) 1,1,1-Trichloroethane (1)	

Abbreviations: GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; NRU=Neutral red uptake; RC=Registry of Cytotoxicity

[1]Substances for which the *in vitro* predicted GHS acute oral toxicity category was different from the category assigned to the substance based on reference rat oral LD_{50} data. Numbers in parentheses indicate the number of categories different. Three substances were excluded because no rat LD_{50} was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride was excluded from the 3T3 and NHK NRU analyses because no laboratory attained sufficient toxicity for the calculation of an IC_{50}. Methanol was excluded from the 3T3 analysis because no laboratory attained sufficient toxicity for the calculation of an IC_{50}.

[2]The RC rat-only millimole regression is log LD_{50} (mmol/kg) = 0.439 log IC_{50} (mM) + 0.621.

[3]Reference rat oral LD_{50} values from **Table 4-2**.

L.2.2 Discordant Substances for Prediction of Toxicity Category by the 3T3 and NHK NRU Test Methods and the RC Rat-Only Weight Regression

Table L2-2 shows the discordant substances for which the *in vitro* predicted GHS toxicity category (using the 3T3 and NHK NRU test methods with the RC rat-only weight regression) did not match that based on the reference rat oral LD_{50} data. The two *in vitro* NRU cytotoxicity test methods over- and under-predicted the GHS toxicity category for a similar number of substances. For the 3T3 NRU test method, the GHS toxicity category of 22 of 46 (48%) discordant substances was overpredicted, with:

- 16 (73%) overpredicted by one GHS toxicity category
- 6 (27%) overpredicted by two GHS toxicity categories

The toxicity of 24 substances (52%) was underpredicted by this test method, with:

- 13 (54%) underpredicted by one GHS toxicity category
- 7 (29%) underpredicted by two GHS toxicity categories
- 4 (17%) underpredicted by three GHS toxicity categories

For the NHK NRU test method, the GHS toxicity category of 25 (53%) of the 47 discordant substances was overpredicted. Of these,

- 18 (72%) were overpredicted by one GHS toxicity category
- 7 (28%) were overpredicted by two GHS toxicity categories

For this assay, the toxicity of 22 (47%) of the discordant substances was underpredicted, with

- 12 (55%) underpredicted by one GHS toxicity category
- 7 (32%) underpredicted by two GHS toxicity categories
- 3 (14%) underpredicted by three toxicity categories

Table L2-2 Discordant Substances[1] for the Prediction of GHS Acute Oral Toxicity Categories by the 3T3 and NHK NRU Test Methods and the RC Rat-Only Weight Regression[2]

In Vivo GHS Category[3] (mg/kg)	3T3 NRU Test Method		NHK NRU Test Method	
	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted
LD_{50} <5		Cycloheximide (2) Disulfoton (3) Phenylthiourea (3) Physostigmine (3) Sodium selenate (3) Triethylenemelamine (2)		Cycloheximide (1) Disulfoton (3) Phenylthiourea (3) Physostigmine (3) Sodium selenate (2) Triethylenemelamine (2)
5 < LD_{50} ≤50		Arsenic trioxide (1) Busulfan (2) Digoxin (2) Endosulfan (1) Mercury chloride (1) Parathion (2) Potassium cyanide (2) Sodium arsenite (1) Strychnine (2) Thallium sulfate (1)		Aminopterin (2) Arsenic trioxide (1) Busulfan (2) Endosulfan (1) Mercury chloride (1) Parathion (2) Potassium cyanide (2) Sodium arsenite (1) Strychnine (2) Thallium sulfate (1)
50 < LD_{50} ≤300		Dichlorvos (1) Fenpropathrin (1) Lindane (1) Nicotine (1) Paraquat (1) Phenobarbital (1) Sodium fluoride (1) Verapamil HCl (1)	Hexachlorophene (1)	Lindane (1) Nicotine (1) Paraquat (1) Phenobarbital (1) Sodium fluoride (1) Verapamil HCl (1)
300 < LD_{50} ≤2000	Amitriptyline HCl (1) Haloperidol (1) Propranolol HCl (1) Triphenyltin hydroxide (2)		Amitriptyline HCl (1) Haloperidol (1) Triphenyltin hydroxide (2)	
2000 < LD_{50} ≤5000	Acetaminophen (1) 5-Aminosalicylic acid (1) Boric acid (1) Carbamazepine (1) Chloramphenicol (1)		Acetaminophen (1) 5-Aminosalicylic acid (1) Boric acid (1) Carbamazepine (1) Chloramphenicol (1)	

***In Vivo* GHS Category[3] (mg/kg)**	**3T3 NRU Test Method**		**NHK NRU Test Method**	
	Toxicity Overpredicted	**Toxicity Underpredicted**	**Toxicity Overpredicted**	**Toxicity Underpredicted**
	Xylene (1)		Lactic acid (1) Potassium chloride (1) Sodium chloride (1) Xylene (1)	
LD_{50} >5000	Citric acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Dimethylformamide (1) Ethanol (1) Ethylene glycol (1) Gibberellic acid (1) Glycerol (1) 2-Propanol (1) Sodium hypochlorite (2) Trichloroacetic acid (2) 1,1,1-Trichloroethane (1)		Citric acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Dimethylformamide (1) Ethanol (1) Gibberellic acid (1) Glycerol (1) Methanol (2) 2-Propanol (1) Sodium hypochlorite (2) Trichloroacetic acid (2) 1,1,1-Trichloroethane (1)	

Abbreviations: GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; NRU=Neutral red uptake; RC=Registry of Cytotoxicity

[1]Substances for which the *in vitro* predicted GHS acute oral toxicity category was different from the category assigned to the substance based on reference rat oral LD_{50} data. Numbers in parentheses indicate the number of categories different. Three substances were excluded because no rat LD_{50} was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride was excluded from the 3T3 and NHK NRU analyses because no laboratory attained sufficient toxicity for the calculation of an IC_{50}. Methanol was excluded from the 3T3 analysis because no laboratory attained sufficient toxicity for the calculation of an IC_{50}.

[2]The RC rat-only weight regression is $\log LD_{50}$ (mg/kg) = 0.372 $\log IC_{50}$ (µg/mL) + 2.024.

[3]Reference rat oral LD_{50} values from **Table 4-2**.

Appendix L3

Analysis of Outliers by Halle (1998, 2003) for the RC Millimole Regression

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L.3 Analysis of Outliers for the RC Millimole Regression

The RC millimole regression was constructed from the *in vitro* IC_{50X} cytotoxicity data from multiple cell lines and the *in vivo* acute toxicity data from rats and mice (i.e., LD_{50} values) for 347 chemicals (Halle 1998, 2003). Halle (1998, 2003) investigated the 95 (27.4%) chemicals for which the observed log LD_{50} values were greater than 0.699 (i.e., 0.5 log) from predicted log LD_{50} values. Of the 95 outliers, 46 were positive outliers and 49 were negative outliers. The positive outliers have IC_{50X} values that predict a far higher *in vivo* toxicity (i.e., lower LD_{50}) than the actual animal experiment. The negative outliers are more important since the IC_{50X} values predict lower toxicity (i.e. higher LD_{50}) than the observed *in vivo* toxicity. It seems that Halle (1998, 2003) was not concerned about the positive outliers since the prediction erred in a health protective direction. Halle (1998, 2003) was much more concerned about trying to explain the reasons for the negative outliers since the error was in a nonconservative direction.

Halle (1998, 2003) investigated three factors that could have explained the negative outliers.

1. Variation in the oral LD_{50} values.
   They reported oral LD_{50} values for a particular chemical might vary by a factor of 4 to 14 even when experiments were highly standardized. LD_{50} values were found from other sources for 23 of the 95 outliers. They found that the variations in the LD_{50} values (difference between the RTECS® value and the "new" value found for the 23 chemicals) were larger for the negative outliers than for the positive outliers.
2. Species-specificity of the oral LD_{50} values.
   Halle (1998, 2003) compared an IC_{50x}–LD_{50} regression using mouse LD_{50} values (242 values) with a regression using rat LD_{50} values (285 values) and found no significant difference between the two regressions. The RC millimole regression with 347 chemicals has 285 rat values and 62 mouse values and is not statistically different from either the rat or mouse regressions.
3. The cell culture(s) used may have been unsuitable for the detection of cytotoxic potential or it may have been unable to simulate the complex

process of toxicity *in vivo.* Halle (1998, 2003) expected, *a priori*, that three classes of compounds, insecticides (**Table L3-1**), neurotoxins (**Table L3-2**), and those requiring metabolic activation for toxicity **(Table L3-3**), would not fit the RC millimole regression (i.e., cytotoxicity data would not predict *in vivo* toxicity). Sixty-two of the 347 chemicals belong to these three classes. Twenty-three (37.1%) of the 62 chemicals were negative outliers. Of the 23, 10 were insecticides, five were neurotoxins, and eight required metabolic activation. No positive outliers were identified in the three classes.

Of the 49 negative outliers, 23 (46.9%) belonged to the three classes of concern. Examination of these classes showed that the RC millimole prediction was accurate (i.e., predicted log LD_{50} [mmol/kg] was within 0.699 of observed log LD_{50} in [mmol/kg]) for 50% of the insecticides (**Table L3-1**) and chemicals that required metabolic activation (**Table L3-3**). For neurotoxins (**Table L3-2**), the results were even better, since 21 (80.8%) fell within the prediction interval. Halle (1998, 2003) felt that the ability to predict the acute LD_{50} for 50% of the insecticides and xenobiotics requiring metabolic activation and for 81% of the neurotoxic xenobiotics was sufficiently accurate for practical purposes.

Of the 49 negative outliers in the RC millimole regression, 23 (46.9%) of these belonged to the three classes of concern that may explain the false negative IC_{50X} values. Findings were contrary to Halle's assumption that *in vitro* cytotoxicity would not predict *in vivo* toxicity for these types of chemicals. The RC millimole prediction of LD_{50} was applicable to 50% of the insecticides and chemicals that required metabolic activation. For neurotoxic chemicals the results were even better, since 21 (80.8%) fell within the prediction interval. Halle felt that the ability to predict the acute LD_{50} for 50% of the insecticides and chemicals requiring metabolic activation and for 81% of the neurotoxic chemicals was sufficiently accurate for practical purposes.

In separate analyses, Halle (1998, 2003) considered the physicochemical properties of chemicals (i.e., molecular weight and the octanol/water partition coefficient) as independent

variables in a multiple regression analysis, but they did not improve the prediction of LD_{50} by IC_{50}.

L3-1 The Error of Prediction[a] of 20 of The Most Important Insecticides in the RC Ordered According to Their Chemical Characteristics[b]

Chemical Class	RC No	Name	LD_{50} Error of Prediction[a]
Chlorinated hydrocarbon			
	26	Kelthane	0.340
	40	Chlordan	-0.046
	43	Aldrin	**-1.074**[b]
	61	DDT	**-0.775**
	167	DDD	-0.378
	185	Heptachlor	**-1.050**
	195	DDA	0.133
	197	DDE	0.251
	207	Dieldrin	**-1.223**
	223	Lindane	**-1.043**
Organophosphorus compounds			
	49	Parathion	**-2.339**
	51	Disulfoton	**-2.346**
	67	Malathion	0.106
	75	Trichlorfon	-0.136
	96	Cygon	**-0.848**
Carbamate compounds			
	73	Carbaryl	-0.279
	186	Zineb	**1.185**
Other compounds			
	134	Rotenone	0.583
	173	Pentachlorophenol	**-0.720**
	235	Paraquat	**-1.019**

Abbreviations: RC=Registry of Cytotoxicity; No=RC number; DDA=*p*,*p*'-DDA [2,2-bis(4-chlorophenyl)acetic acid]; DDD=*p*,*p*'-DDD [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane]; DDE= *p*,*p*'-DDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene]; DDT=*p*,*p*'-DDT [1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane]

[a] Defined as observed log LD_{50} (mmol/kg) - predicted log LD_{50} (mmol/kg).

[b] Modified from Table 10 of Halle (1998, 2003).

Bold numbers: outliers (i.e., observed log LD_{50} [mmol/kg] - predicted log LD_{50} [mmol/kg] > 0.699).

Table L3-2 The Error of Prediction [a] of 26 Neurotoxic Xenobiotics in the RC Ordered According to Their *In Vivo* Potency[b]

Chemical Class	RC No	Name	LD_{50} Error of Prediction[a]
Sedative, hypnotic, CNS depressants			
	69	Secobarbital sod.	-0.651
	83	Thiopental	-0.119
	84	Amobarbital	-0.335
	87	Pentobarbital sodium	-0.654
	101	Gluthetimide	-0.270
	118	Phenobarbital	**-1.035**[b]
	247	(+)-Thalidomide	-0.397
	264	Chloral hydrate	-0.349
	317	Barbital sodium	-0.591
Antidepressant			
	38	Imipramine HCl	-0.093
	90	Iproniazid	-0.273
	183	Amitriptyline	0.021
Antipsychotic, anxiolytic			
	27	Chlorpromazine	-0.176
	44	Hydroxyzine HCl	0.248
	63	Diazepam	0.116
	170	Thioridazine HCl	-0.013
Stimulants			
	112	Caffeine	**-0.815**
	262	Amphetamine sulfate	**-1.579**
Anticonvulsants			
	82	Diphenylhydantoin	-0.551
Analgetic (general anesthesia)			
	229	Dextropropoxyphene HCl	**-1.150**
Anticholinergic			
	251	Scopolamine * HBr	-0.123
	296	Homatropine methylbromide	-0.532
Other Neurotoxins (not insecticide)			
	102	Acrylamide	-0.338
	137	Triethyltin chloride	**-0.852**
	142	Methylmercury chloride	0.105
	316	Toluene	0.571

Abbreviations: RC=Registry of Cytotoxicity; No=RC number; CNS=Central nervous system.

[a] Defined as observed log LD_{50} (mmol/kg) - predicted log LD_{50} (mmol/kg).

[b] Modified from Table 11 of Halle (1998, 2003).

Bold numbers: outliers (i.e., observed log LD_{50} [mmol/kg] - predicted log LD_{50} [mmol/kg] >0.699).

Table L3-3 The Error of Prediction[a] of the 16 Xenobiotics in the RC that Require Metabolic Activation[b]

RC No	Name	LD_{50} Error of Prediction[a]
13	Cycloheximide	**-1.370**[b]
33	p-Chloromercuribenzoic acid	**-1.077**
37	Aflatoxin B_1	**-1.783**
68	2.4-Dinitrophenol	**-1.128**
97	Phenacetin	0.292
109	Frusemide	0.109
113	Acetaminophen	0.386
116	Cyclophosphamide * H_2O	**-1.310**
123	Isoniazid	-0.332
125	Carbon tetrachloride	0.229
192	1.3-Bis(2-chloroethyl)-1-nitrosourea	**-1.176**
260	Coumarin	-0.427
273	Bromobenzene	0.374
279	Thioacetamide	-0.294
281	1.2-Dibromomethane	**-1.106**
292	Allylalcohol	**-0.952**

Abbreviations: RC=Registry of Cytotoxicity; No=RC number.

[a] Defined as observed log LD_{50} (mmol/kg) - predicted log LD_{50} (mmol/kg).

[b] Modified from Table 12 of Halle (1998, 2003).

Bold numbers: outliers (i.e., observed log LD_{50} [mmol/kg] - predicted log LD_{50} [mmol/kg] >0.699.

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Appendix M

Acute Oral Toxicity Test Guidelines

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Appendix M1

OECD UDP Test Guideline

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OECD/OCDE **425**

Adopted:
17th December 2001

OECD GUIDELINE FOR TESTING OF CHEMICALS

Acute Oral Toxicity – Up-and-Down Procedure

INTRODUCTION

1. OECD guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The concept of the up-and-down testing approach was first described by Dixon and Mood (1)(2)(3)(4). In 1985, Bruce proposed to use an up-and-down procedure (UDP) for the determination of acute toxicity of chemicals (5). There exist several variations of the up-and-down experimental design for estimating an LD50. This guideline is based on the procedure of Bruce as adopted by ASTM in 1987 (6) and revised in 1990. A study comparing the results obtained with the UDP, the conventional LD50 test and the Fixed Dose Procedure (FDP, Guideline 420) was published in 1995 (7). Since the early papers of Dixon and Mood, papers have continued to appear in the biometrical and applied literature, examining the best conditions for use of the approach (8)(9)(10)(11). Based on the recommendations of several expert meetings in 1999, an additional revision was considered timely because: i) international agreement had been reached on harmonised LD50 cut-off values for the classification of chemical substances, ii) testing in one sex (usually females) is generally considered sufficient, and iii) in order for a point estimate to be meaningful, there is a need to estimate confidence intervals (CI).

2. The test procedure described in this Guideline is of value in minimizing the number of animals required to estimate the acute oral toxicity of a chemical. In addition to the estimation of LD50 and confidence intervals, the test allows the observation of signs of toxicity. Revision of Test Guideline 425 was undertaken concurrently with revisions to the Test Guidelines 420 and 423.

3. Guidance on the selection of the most appropriate test method for a given purpose can be found in the Guidance Document on Oral Toxicity Testing (12). This Guidance Document also contains additional information on the conduct and interpretation of Guideline 425.

4. Definitions used in the context of this Guideline are set out in Annex 1.

INITIAL CONSIDERATIONS

5. The testing laboratory should consider all available information on the test substance prior to conducting the study. Such information will include the identity and chemical structure of the test substance; its physical chemical properties; the results of any other *in vitro* or *in vivo* toxicity tests on the substance; toxicological data on structurally related substances or similar mixtures; and the anticipated use(s) of the substance. This information is useful to determine the relevance of the test for the protection of human health and the environment, and will help in the selection of an appropriate starting dose.

6. The method permits estimation of an LD50 with a confidence interval and the results allow a substance to be ranked and classified according to the Globally Harmonised System for the classification of chemicals which cause acute toxicity (16).

7. When no information is available to make a preliminary estimate of the LD50 and the slope of the dose-response curve, results of computer simulations have suggested that starting near 175 mg/kg and using half-log units (corresponding to a dose progression of factor 3.2) between doses will produce the best results. This starting dose should be modified if the substance is likely to be highly toxic. The half-log spacing provides for a more efficient use of animals, and increases accuracy in the prediction of the LD50 value. Because the method has a bias toward the starting dose, it is essential that initial dosing occur below the estimated LD50. (See paragraphs 32 and Annex 2 for discussion of dose sequences and starting values). However, for chemicals with large variability (i.e., shallow dose-response slopes), bias can still be introduced in the lethality estimates and the LD50 will have a large statistical error, similar to other acute toxicity methods. To correct for this, the main test includes a stopping rule keyed to properties of the estimate rather than a fixed number of test observations (see paragraph 33).

8. The method is easiest to apply to materials that produce death within one or two days. The method would not be practical to use when considerably delayed death (five days or more) can be expected.

9. Computers are used to facilitate animal-by-animal calculations that establish testing sequences and provide final estimates.

10. Test substances, at doses that are known to cause marked pain and distress due to corrosive or severely irritant actions, need not be administered. Moribund animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death are the subject of a separate OECD Guidance Document (13).

11. A limit test can be used efficiently to identify chemicals that are likely to have low toxicity.

PRINCIPLE OF THE LIMIT TEST

12. The Limit Test is a sequential test that uses a maximum of 5 animals. A test dose of 2000, or exceptionally 5000 mg/kg, may be used. The procedures for testing at 2000 and 5000 mg/kg are slightly different (see paragraphs 23-25 for limit test at 2000 mg/kg and paragraphs 26-30 for limit test at 5000 mg/kg). The selection of a sequential test plan increases the statistical power and also has been made to intentionally bias the procedure towards rejection of the limit test for compounds with LD50s near the limit dose; i.e., to err on the side of safety. As with any limit test protocol, the probability of correctly classifying a compound will decrease as the actual LD50 more nearly resembles the limit dose.

PRINCIPLE OF THE MAIN TEST

13. The main test consists of a single ordered dose progression in which animals are dosed, one at a time, at a minimum of 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD50. If the animal survives, the dose for the next animal is increased by [a factor of] 3.2 times the original dose; if it dies, the dose for the next animal is decreased by a similar dose progression. (Note: 3.2 is the default factor corresponding to a dose progression of one half log unit). Paragraph 32 provides further guidance for choice of dose spacing factor.) Each animal should be observed carefully for up to 48 hours before making a decision on whether and how much to dose the next animal. That decision is based on the 48-hour survival pattern of all the animals up to that time. (See paragraphs 31 and 35 on choice of dosing interval). A combination of stopping criteria is used to keep the

number of animals low while adjusting the dosing pattern to reduce the effect of a poor starting value or low slope (see paragraph 34). Dosing is stopped when one of these criteria is satisfied (see paragraphs 33 and 41), at which time an estimate of the LD50 and a confidence interval are calculated for the test based on the status of all the animals at termination. For most applications, testing will be completed with only 4 animals after initial reversal in animal outcome. The LD50 is calculated using the method of maximum likelihood (14)(15). (See paragraphs 41 and 43.)

14. The results of the main test procedure serve as the starting point for a computational procedure to provide a confidence interval estimate where feasible. A description of the basis for this CI is outlined in paragraph 45.

DESCRIPTION OF THE METHOD

Selection of animals species

15. The preferred rodent species is the rat although other rodent species may be used. Normally female rats are used (12). This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally slightly more sensitive (7). However, if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive then this sex should be used. When the test is conducted in males, adequate justification should be provided.

16. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. At the commencement of its dosing, each animal should be between 8 and 12 weeks old and its weight should fall in an interval within ± 20 % of the mean initial weight of any previously dosed animals.

Housing and feeding conditions

17. The temperature in the experimental animal room should be 22°C (± 3°C). Although the relative humidity should be at least 30 % and preferably not exceed 70 % other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. The animals are housed individually. For feeding, conventional rodent laboratory diets may be used with an unlimited supply of drinking water.

Preparation of animals

18. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. As with other sequential test designs, care must be taken to ensure that animals are available in the appropriate size and age range for the entire study.

Preparation of doses

19. In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested, however, the use of the undiluted test substance, i.e., at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory

authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1 mL/100g of body weight; however in the case of aqueous solutions, 2 mL/100g body weight can be considered. With respect to the formulation of the dosing preparations, the use of an aqueous solution/suspension/emulsion is recommended wherever possible, followed in order of preference by a solution/suspension/emulsion in oil (e.g. corn oil) and then possibly solution in other vehicles. For vehicles other than water the toxicological characteristics of the vehicle should be known. Doses must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known and shown to be acceptable.

PROCEDURE

Administration of doses

20. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

21. Animals should be fasted prior to dosing (e.g., with the rat, food but not water should be withheld overnight; with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period of time, it may be necessary to provide the animals with food and water depending on the length of the period.

Limit test and main test

22. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations where there is little or no information about its toxicity, or in which the the test material is expected to be toxic, the main test should be performed.

Limit test

Limit test at 2000 mg/kg

23. Dose one animal at the test dose. If the animal dies, conduct the main test to determine the LD50. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD50 is greater than 2000 mg/kg if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period (see paragraph 31 for initial observation period). Late deaths should be counted the same as other deaths. The results are evaluated as follows (O=survival, X=death).

24. The LD50 is less than the test dose (2000 mg/kg) when three or more animals die.

O XO XX
O OX XX
O XX OX
O XX X

If a third animal dies, conduct the main test.

25. Test five animals. The LD50 is greater than the test dose (2000 mg/kg) when three or more animals survive.

O OO OO
O OO XO
O OO OX
O OO XX
O XO XO
O XO OO/X
O OX XO
O OX OO/X
O XX OO

Limit Test at 5000 mg/kg

26. Exceptionally, and only when justified by specific regulatory needs, the use of a dose at 5000 mg/kg may be considered (see Annex 4). For reasons of animal welfare concern, testing of animals in GHS Category 5 ranges (2000-5000mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.

27. Dose one animal at the test dose. If the animal dies, conduct the main test to determine the LD50. If the animal survives, dose two additional animals. If both animals survive, the LD50 is greater than the limit dose and the test is terminated (i.e. carried to full 14-day observation without dosing of further animals).

28. If one or both animals die, then dose an additional two animals, one at a time. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period (see paragraph 10 for initial observation period). Late deaths should be counted the same as other deaths. The results are evaluated as follows (O=survival, X=death, and U=Unnecessary).

29. The LD50 is less than the test dose (5000 mg/kg) when three or more animals die.

O XO XX
O OX XX
O XX OX
O XX X

30. The LD50 is greater than the test dose (5000 mg/kg) when three or more animals survive.

O OO
O XO XO

O XO O
O OX XO
O OX O
O XX OO

Main test

31. Single animals are dosed in sequence usually at 48 h intervals. However, the time intervals between dosing is determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose should be delayed until one is confident of survival of the previously dosed animal. The time interval may be adjusted as appropriate, e.g., in case of inconclusive response. The test is simpler to implement when a single time interval is used for making sequential dosing decisions. Nevertheless, it is not necessary to recalculate dosing or likelihood-ratios if the time interval changes midtest. For selecting the starting dose, all available information, including information on structurally related substances and results of any other toxicity tests on the test material, should be used to approximate the LD50 as well as the slope of the dose-response curve.

32. The first animal is dosed a step below the best preliminary estimate of the LD50. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. The dose progression factor should be chosen to be the antilog of 1/(the estimated slope of the dose-response curve) (a progression of 3.2 corresponds to a slope of 2) and should remain constant throughout testing. When there is no information on the slope of the substance to be tested, a dose progression factor of 3.2 is used. Using the default progression factor, doses would be selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550, 2000 (or 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000 for specific regulatory needs). If no estimate of the substance's lethality is available, dosing should be initiated at 175 mg/kg. In most cases, this dose is sublethal and therefore serves to reduce the level of pain and suffering. If animal tolerances to the chemical are expected to be highly variable (i.e., slopes are expected to be less than 2.0), consideration should be given to increasing the dose progression factor beyond the default 0.5 on a log dose scale (i.e., 3.2 progression factor) prior to starting the test. Similarly, for test substances known to have very steep slopes, dose progression factors smaller than the default should be chosen. (Annex 2 includes a table of dose progressions for whole number slopes ranging from 1 to 8 with starting dose 175 mg/kg).

33. Dosing continues depending on the fixed-time interval (e.g., 48-hour) outcomes of all the animals up to that time. The testing stops when one of the following stopping criteria first is met:

(a) 3 consecutive animals survive at the upper bound;

(b) 5 reversals occur in any 6 consecutive animals tested;

(c) at least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value. (See paragraph 44 and Annex 3. Calculations are made at each dosing, following the fourth animal after the first reversal).

For a wide variety of combinations of LD50 and slopes, stopping rule (c) will be satisfied with 4 to 6 animals after the test reversal. In some cases for chemicals with shallow slope dose-response curves, additional animals (up to a total of fifteen tested) may be needed.

34. When the stopping criteria have been attained, the estimated LD50 should be calculated from the animal outcomes at test termination using the method described in paragraphs 40 and 41.

35. Moribund animals killed for humane reasons are considered in the same way as animals that died on test. If an animal unexpectedly dies late in the study and there are other survivors at that dose or above, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. If subsequent survivors also die, *and* it appears that all dose levels exceed the LD50 it would be most appropriate to start the study again beginning at least two steps below the lowest dose with deaths (and increasing the observation period) since the technique is most accurate when the starting dose is below the LD50. If subseqent animals survive at or above the dose of the animal that dies, it is not necessary to change the dose progression since the information from the animal that has now died will be included into the calculations as a death at a lower dose than subsequent survivors, pulling the LD50 down.

OBSERVATIONS

36. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions and time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed (17). All observations are systematically recorded with individual records being maintained for each animal.

37. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document (13) should be taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

Body weight

38. Individual weights of animals should be determined shortly before the test substance is administered and at least weekly thereafter. Weight changes should be calculated and recorded. At the end of the test surviving animals are weighed and then humanely killed.

Pathology

39. All animals (including those which die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours after the initial dosing may also be considered because it may yield useful information.

DATA AND REPORTING

Data

40. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test dose the number of animals used, the number of animals displaying signs of toxicity (17), the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings. A rationale for the starting dose and the dose progression and any data used to support this choice should be provided.

Calculation of LD50 for the main test

41. The LD50 is calculated using the maximum likelihood method (14)(15), except in the exceptional cases described in paragraph 42. The following statistical details may be helpful in implementing the maximum likelihood calculations suggested (with an assumed *sigma*). All deaths, whether immediate or delayed or humane kills, are incorporated for the purpose of the maximum likelihood analysis. Following Dixon (4), the likelihood function is written as follows:

$L = L_1 L_2 \ldots L_n$,

where

L is the likelihood of the experimental outcome, given *mu* and *sigma*, and n the total number of animals tested.

$L_i = 1 - F(Z_i)$ if the i^{th} animal survived, or
$L_i = F(Z_i)$ if the i^{th} animal died,

where

F = cumulative standard normal distribution,
$Z_i = [\log(d_i) - mu\ ] / sigma$
d_i = dose given to the i^{th} animal, and
sigma = standard deviation in log units of dose (which is not the log standard deviation).

An estimate of the true LD50 is given by the value of *mu* that maximizes the likelihood L (see paragraph 43).

An estimate of *sigma* of 0.5 is used unless a better generic or case-specific value is available.

42. Under some circumstances, statistical computation will not be possible or will likely give erroneous results. Special means to determine/report an estimated LD50 are available for these circumstances as follows:

(a) If testing stopped based on criterion (a) in paragraph 33 (i.e., a boundary dose was tested repeatedly), or if the upper bound dose ended testing, then the LD50 is reported to be above the upper bound. Classification is completed on this basis.

(b) If all the dead animals have higher doses than all the live animals (or if all live animals have higher doses than all the dead animals, although this is practically unlikely), then the LD50 is between the doses for the live and the dead animals. These observations give no further information on the exact value of the LD50. Still, a maximum likelihood LD50 estimate can be

made provided there is a value for *sigma*. Stopping criterion (b) in paragraph 33 describes one such circumstance.

(c) If the live and dead animals have only one dose in common and all the other dead animals have higher doses and all the other live animals lower doses, or vice versa, then the LD50 equals their common dose. If a closely related substance is tested, testing should proceed with a smaller dose progression.

If none of the above situations occurs, then the LD50 is calculated using the maximum likelihood method.

43. Maximum likelihood calculation can be performed using either SAS (14) (e.g., PROC NLIN) or BMDP (15) (e.g., program AR) computer program packages as described in Appendix 1D in Reference 3. Other computer programs may also be used. Typical instructions for these packages are given in appendices to the ASTM Standard E 1163-87 (6). [The *sigma* used in the BASIC program in (6) will need to be edited to reflect the parameters of this OECD 425 Guideline.] The program's output is an estimate of log(LD50) and its standard error.

44. The likelihood-ratio stopping rule (c) in paragraph 33 is based on three measures of test progress, that are of the form of the likelihood in paragraph 41 with different values for *mu*. Comparisons are made after each animal tested after the sixth that does not already satisfy criterion (a) or (b) of paragraph 33. The equations for the likelihood-ratio criteria are provided in Annex 3. These comparisons are most readily performed in an automated manner and can be executed repeatedly, for instance, by a spreadsheet routine such as that also provided in Annex 3. If the criterion is met, testing stops and the LD50 can be calculated by the maximum likelihood method.

Computation of confidence interval

45. Following the main test and estimated LD50 calculation, it may be possible to compute interval estimates for the LD50. Any of these confidence intervals provides valuable information on the reliability and utility of the main test that was conducted. A wide confidence interval indicates that there is more uncertainty associated with the estimated LD50. The reliability of the estimated LD50 is low and the usefulness of the estimated LD50 may be marginal. A narrow interval indicates that there is relatively little uncertainty associated with the estimated LD50. The reliability of the estimated LD50 is high and the usefulness of the estimated LD50 is good. This means that if the main test were to be repeated, the new estimated LD50 should be close to the original estimated LD50 and both of these estimates should be close to the true LD50.

46. Depending on the outcome of the main test, one of two different types of interval estimates of the true LD50 is calculated.

- When at least three different doses have been tested and the middle dose has at least one animal that survived and one animal that died, a profile-likelihood-based computational procedure is used to obtain a confidence interval that is expected to contain the true LD50 95% of the time. However, because small numbers of animals are expected to be used, the actual level of confidence is generally not exact (18). The random stopping rule improves the ability of the test overall to respond to varying underlying conditions, but also causes the reported level of confidence and the actual level of confidence to differ somewhat (19).

- If all animals survive at or below a given dose level and all animals die when dosed at the next higher dose level, an interval is calculated that has as its lower limit the highest dose

tested where all the animals survive and has as its upper limit the dose level where all the animals died. This interval is labeled as "approximate." The exact confidence level associated with this interval cannot be specifically determined. However, because this type of response would only occur when the dose response is steep, in most cases, the true LD50 is expected to be contained within the calculated interval or be very close to it. This interval will be relatively narrow and sufficiently accurate for most practical use.

47. In some instances, confidence intervals are reported as infinite, through including either zero as its lower end or infinity as its upper end, or both. Such intervals, for example, may occur when all animals die or all animals live. Implementing this set of procedures requires specialized computation which is either by use of a dedicated program to be available from the USEPA or OECD or developed following technical details available from the USEPA or OECD (20). Achieved coverage of these intervals and properties of the dedicated program are described in reports (21) also available through the USEPA.

Test report

48. The test report must include the following information:

Test substance:

- physical nature, purity and,where relevant, physico-chemical properties (including isomerisation);
- identification data, including CAS number.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the animals, when known;
- number, age and sex of animals (including, where appropriate, a rationale for use of males instead of females);
- source, housing conditions, diet, etc.;

Test conditions:

- rationale for initial dose level selection, dose progression factor and for follow-up dose levels
- details of test substance formulation including details of the physical form of the material administered.;
- details of the administration of the test substance including dosing volumes and time of dosing;
- details of food and water quality (including diet type/source, water source).

Results:

- body weight/body weight changes;
- tabulation of response data and dose level for each animal (i.e., animals showing signs of toxicity including nature, severity, duration of effects, and mortality);

- individual weights of animals at the day of dosing, in weekly intervals thereafter, and at the time of death or sacrifice ;
- time course of onset of signs of toxicity and whether these were reversible for each animal;
- necropsy findings and any histopathological findings for each animal, if available;
- LD50 data;
- statistical treatment of results (description of computer routine used and spreadsheet tabulation of calculations).

Discussion and interpretation of results.

Conclusions.

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ANNEX 1

DEFINITIONS

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.

Delayed death means that an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period.

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg) or as weight of test substance per unit weight of test animal (e.g. mg/kg).

Dose progression factor, sometimes termed a dose spacing factor, refers to the multiple by which a dose is increased (i.e., the dose progression) when an animal survives or the divisor by which it is decreased when an animal dies. The dose progression factor is recommended to be the antilog of 1/ (the estimated slope of the dose response curve). The default dose progression factor is recommended to be 3.2 = antilog 0.5 = antilog ½.

GHS: Globally Harmonised Classification System for Chemical Substances and Mixtures. A joint activity of OECD (human health and the environment), UN Committee of Experts on Transport of Dangerous Goods (physical–chemical properties) and ILO (hazard communication) and co-ordinated by the Interorganisation Programme for the Sound Management of Chemicals (IOMC).

Impending death: when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, lateral position, recumbence, and tremor. (See the Humane Endpoint Guidance Document (13) for more details).

LD50 (median lethal oral dose), is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Limit dose refers to a dose at an upper limitation on testing (2000 or 5000 mg/kg).

Moribund status : being in a state of dying or inability to survive, even if treated. (See the Humane Endpoint Guidance Document (13) for more details).

Nominal sample size refers to the total number of tested animals, reduced by one less than the number of like responses at the beginning of the series, or by the number of tested animals up to but not including the pair that creates the first reversal. For example, for a series where X and O indicate opposite animal outcomes (for instance, X could be: "dies within 48 hours" and O: " survives") in a pattern as follows: OOOXXOXO, we have the total number of tested animals (or sample size in the conventional sense) as 8 and the nominal sample size as 6. This particular example shows 4 animals following a reversal. It is important to note whether a count in a particular part of the guideline refers to the nominal sample size or to the total number tested. For example, the maximum actual number tested is 15. When testing is stopped based on that maximum number, the nominal sample size will be less than or equal to 15. Members of the nominal sample start with the (r-1)st animal (the animal before the second in the reversal pair) (see reversal below).

Predictable death: presence of clinical signs indicative of death at a known time in the future before the planned end of the experiment, for example: inability to reach water or food. (See the Humane Endpoint Guidance Document (13) for more details).

Probit is an abbreviation for the term "probability integral transformation" and a probit dose-response model permits a standard normal distribution of expected responses (i.e., one centered to its mean and scaled to its standard deviation, *sigma*) to doses (typically in a logarithmic scale) to be analyzed as if it were a straight line with slope the reciprocal of *sigma*. A standard normal lethality distribution is symmetric; hence, its mean is also its true LD50 or median response.

Reversal is a situation where nonresponse is observed at some dose, and a response is observed at the next dose tested, or vice versa (i.e., response followed by nonresponse). Thus, a reversal is created by a pair of responses. The first such pair occurs at animals numbered r-1 and r.

Sigma is the standard deviation of a log normal curve describing the range of tolerances of test subjects to the chemical (where a subject is expected capable of responding if the chemical dose exceeds the subject's tolerance). The estimated *sigma* provides an estimate of the variation among test animals in response to a full range of doses.
See slope and probit.

Slope (of the dose-response curve) is a value related to the angle at which the dose response curve rises from the dose axis. In the case of probit analysis, when responses are analyzed on a probit scale against dose on a log scale this curve will be a straight line and the slope is the reciprocal of *sigma,* the standard deviation of the underlying test subject tolerances, which are assumed to be normally distributed. See probit and *sigma.*

Stopping rule is used in this guideline synonymously with 1) a specific stopping criterion and 2) the collection of all criteria determining when a testing sequence terminates. In particular, for the main test, stopping rule is used in paragraph 7 as a shorthand for the criterion that relies on comparison of ratios to a critical value.

ANNEX 2

DOSING PROCEDURE

Dose Sequence for Main Test

1. Up-and-Down Dosing Procedure. For each run, animals are dosed, one at a time, usually at 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD50. This selection reflects an adjustment for a tendency to bias **away from the LD50 in the direction of** the initial starting dose in the final estimate (see paragraph 7 of the Guideline). The overall pattern of outcomes is expected to stabilize as dosing is adjusted for each subsequent animal. Paragraph 3 below provides further guidance for choice of dose spacing factor.

2. Default Dose Progression. Once the starting dose and dose spacing are decided, the toxicologist should list all possible doses including the upper bound (usually 2000 or 5000 mg/kg). Doses that are close to the upper bound should be removed from the progression. The stepped nature of the TG 425 design provides for the first few doses to function as a self-adjusting sequence. Because of the tendency for positive bias, in the event that nothing is known about the substance, a starting dose of 175 mg/kg is recommended. If the default procedure is to be used for the main test, dosing will be initiated at 175 mg/kg and doses will be spaced by a factor of 0.5 on a log dose scale. The doses to be used include 1.75, 5.5, 17.5, 55, 175, 550, 2000 or, for specific regulatory needs, 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000. For certain highly toxic substances, the dosing sequence may need to be extended to lower values.

3. In the event a dose progression factor other than the default is deemed suitable, Table 1 provides dose progressions for whole number multiples of slope, from 1 to 8.

Table 1 Dose Progressions for OECD Guideline 425
Choose a Slope and Read Down the Column
All doses in mg/kg bw

Slope =	1	2	3	4	5	6	7	8
	0.175*	0.175*	0.175*	0.175*	0.175*	0.175*	0.175*	0.175*
							0.24	0.23
					0.275	0.26		
				0.31			0.34	0.31
			0.375			0.375		
								0.41
					0.44		0.47	
		0.55		0.55		0.55		0.55
					0.69		0.65	
								0.73
			0.81			0.82		
				0.99			0.91	0.97
					1.09	1.2		
							1.26	1.29
	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
							2.4	2.3
					2.75	2.6		
				3.1			3.4	3.1
			3.75			3.75		
					4.4			4.1
							4.7	
		5.5		5.5		5.5		5.5
					6.9		6.5	
								7.3
			8.1			8.2		
				9.9			9.1	9.7
					10.9	12		
							12.6	12.9
	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5
							24	23
					27.5	26		
				31			34	31

Table 1 continued

		37.5			37.5		
				44			41
						47	
	55		55		55		55
						65	
				69			73
		81			82		
			99			91	97
				109	120		
						126	129
175	175	175	175	175	175	175	175
						240	230
				275	260		
			310			340	310
		375			375		
				440			410
						470	
	550		550		550		550
						650	
				690			730
		810			820		
			990			910	970
				1090	1200		
						1260	1290
1750	1750	1750	1750	1750	1750	1750	1750
						2400	2300
				2750	2600		
			3100				3100
					3750	3400	
							4100
5000	5000	5000	5000	5000	5000	5000	5000

* If lower doses are needed, continue progressions to a lower dose

ANNEX 3

COMPUTATIONS FOR THE LIKELIHOOD-RATIO STOPPING RULE

1. As described in Guideline paragraph 33, the main test may be completed on the basis of the first of three stopping criteria to occur. In any case, even if none of the stopping criteria is satisfied, dosing would stop when 15 animals are dosed. Tables 2-5 illustrate examples where testing has started with no information, so the recommended default starting value, 175 mg/kg, and the recommended default dose progression factor, 3.2 or one half log, have been used. Please note the formatting of these tables is only illustrative.

2. Table 2 shows how the main test would stop if 3 animals have survived at the limit dose of 2000 mg/kg; Table 3 shows a similar situation when the limit dose of 5000 mg/kg is used. (These illustrate situations where a Limit Test was not thought appropriate *a priori*.) Table 4 shows how a particular sequence of 5 reversals in 6 tested animals could occur and allow test completion. Finally, Table 5 illustrates a situation where neither criterion (a) nor criterion (b) has been met, a reversal of response has occurred followed by 4 tested animals, and, consequently, criterion (c) must be evaluated as well.

3. Criterion (c) calls for a likelihood-ratio stopping rule to be evaluated after testing each animal, starting with the fourth tested following the reversal. Three "measures of test progress" are calculated. Technically, these measures of progress are likelihoods, as recommended for the maximum-likelihood estimation of the LD50. The procedure is closely related to calculation of a confidence interval by a likelihood-based procedure.

4. The basis of the procedure is that when enough data have been collected, a point estimate of the LD50 should be more strongly supported than values above and below the point estimate, where statistical support is quantified using likelihood. Therefore three likelihood values are calculated: a likelihood at an LD50 point estimate (called the rough estimate or dose-averaging estimate in the example), a likelihood at a value below the point estimate, and a likelihood at a value above the point estimate. Specifically, the low value is taken to be the point estimate divided by 2.5 and the high value is taken to be the point estimate multiplied by 2.5.

5. The likelihood values are compared by calculating ratios of likelihoods, and then determining whether these likelihood-ratios (LR) exceed a critical value. Testing stops when the ratio of the likelihood for the point estimate exceeds each of the other likelihoods by a factor of 2.5, which is taken to indicate relatively strong statistical support for the point estimate. Therefore two likelihood-ratios (LRs) are calculated, a ratio of likelihoods for the point estimate and the point estimate divided by 2.5, and a ratio for the point estimate and the estimate times 2.5.

6. The calculations are easily performed in any spreadsheet with normal probability functions. The calculations are illustrated in Table 5, which is structured to promote spreadsheet implementation. The computation steps are illustrated using an example where the upper limit dose is 5000 mg/kg, but the computational steps are carried out in the same fashion when the upper boundary dose is 2000 mg/kg. Empty spreadsheets preprogrammed with the necessary formulas are available for direct downloading on the OECD and EPA web sites.

Hypothetical example using an upper limit dose of 5000 mg/kg (Table 5)

7. In the hypothetical example utilizing an upper boundary dose of 5000 mg/kg, the LR stopping criterion was met after nine animals had been tested. The first "reversal" occurred with the 3rd animal tested. The LR stopping criterion is checked when four animals have been tested following the reversal. In this example, the fourth animal tested following the reversal is the seventh animal actually tested. Therefore, for this example, the spreadsheet calculations are only needed after the seventh animal had been tested and the data could be entered at that time. Subsequently, the LR stopping criterion would have been checked after testing the seventh animal, the eighth animal, and the ninth. The LR stopping criterion is first satisfied after the ninth animal is tested in this example.

A. Enter the dose-response information animal by animal.

Column 1. Steps are numbered 1-15. No more than 15 animals may be tested.
Column 2. Place an I in this column as each animal is tested.
Column 3. Enter the dose received by the i^{th} animal.
Column 4. Indicate whether the animal responded (shown by an X) or did not respond (shown by an O).

B. The nominal and actual sample sizes.

8. The nominal sample consists of the two animals that represent the first reversal (here the second and third animals), plus all animals tested subsequently. Here, Column 5 indicates whether or not a given animal is included in the nominal sample.

The nominal sample size (nominal n) appears in Row 16. This is the number of animals in the nominal sample. In the example, nominal n is 8.
The actual number tested appears in Row 17.

C. Rough estimate of the LD50.

9. The geometric mean of doses for the animals in the current nominal sample is used as a rough estimate of the LD50 from which to gauge progress. In the table, this is called the "dose-averaging estimator." It is updated with each animal tested. This average is restricted to the nominal sample in order to allow for a poor choice of initial test dose, which could generate either an initial string of responses or an initial string of nonresponses. (However, the results for all animals are used in the likelihood calculations for final LD50 calculation below.) Recall that the geometric mean of n numbers is the product of the n numbers, raised to a power of $1/n$.

The dose-averaging estimate appears in Row 18 (e.g., $(175 * 550 * ... * 1750)^{1/8} = 1292.78$).
Row 19 shows the logarithm (base 10) of the value in Row 18 (e.g., $\log_{10} 1292.8 = 3.112$).

D. Likelihood for the rough LD50 estimate.

10. Likelihood is a statistical measure of how strongly the data support an estimate of the LD50 or other parameter. Ratios of likelihood values can be used to compare how well the data support different estimates of the LD50.

11. In column 8 calculate the likelihood for Step C's rough LD50 estimate. The likelihood (Row 21) is the product of likelihood contributions for individual animals (see Guideline paragraph 41). The likelihood contribution for the i^{th} animal is denoted L_i.

12. In column 7 enter the estimate of the probability of response at dose d_i, denoted P_i. P_i is calculated from a dose-response curve. Note that the parameters of a probit dose-response curve are the

slope and the LD50, so values are needed for each of those parameters. For the LD50 the dose-averaging estimate from Row 18 is used. For the slope in this example the default value of 2 is used. The following steps may be used to calculate the response probability P_i.

1. Calculate the base-10 log of dose d_i (Column 6).

2. For each animal calculate the z-score, denoted Z_i (not shown in the table), using the formulae
   sigma = 1 / *slope*,
   $Z_i = (\log_{10}(d_i) - \log_{10}(\text{LD50})) / \textit{sigma}$

   For example, for the first animal (Row 1),
   sigma = 1 / 2
   $Z_1 = (2.243 - 3.112) / 0.500 = -1.738$

3. For the i^{th} dose the estimated response probability is

   $P_i = F(Z_i)$

where F denotes the cumulative distribution function for the standard normal distribution (i.e., the normal distribution with mean 0 and variance 1).

For example (Row 1),

$$P_1 = F(-1.738) = 0.0412$$

The function F (or something very close) is ordinarily what is given for the normal distribution in statistical tables, but the function is also widely available as a spreadsheet function. It is available under different names, for example the @NORMAL function of Lotus 1-2-3 (1) and the @NORMDIST function in Excel (2). To confirm that you have used correctly the function available in your software, you may wish to verify familiar values such as $F(1.96) \approx 0.975$ or $F(1.64) \approx 0.95$.

13. Column 8. Calculate the natural log of the likelihood contribution ($\ln(L_i)$). L_i is simply the probability of the response that actually was observed for the i^{th} animal:

responding animals: $\ln(L_i) = \ln (P_i)$
non-responding animals: $\ln(L_i) = \ln(1 - P_i)$

Note that here the natural logarithm (ln) is used, whereas elsewhere the base-10 (common) logarithm was used. These choices are what are ordinarily expected in a given context.

The steps above are performed for each animal. Finally:

Row 20: Sum the log-likelihood contributions in Column 8.
Row 21: Calculate the likelihood by applying the exp function applied to the log-likelihood value in Row 20 (e.g., $\exp(-3.389) = e^{-3.389} = 0.0337$).

E. Calculate likelihoods for two dose values above and below the rough estimate.

14. If the data permit a precise estimate, then one expects the likelihood should be high if the estimate is a reasonable estimate of the LD50, relative to likelihoods for values distant from this estimate. Compare the likelihood for the dose-averaging estimate (1292.8, Row 18) to values differing by a factor of

2.5 from that value (i.e., to 1292.8*2.5 and 1292.8/2.5). The calculations (displayed in Columns 9-12) are carried out in a fashion similar to those described above, except that the values 517.1 (=1292.8/2.5) and 3232.0 (=1292.8*2.5) have been used for the LD50, instead of 1292.8. The likelihoods and log-likelihoods are displayed in Rows 20-21.

F. Calculate likelihood-ratios.

15. The three likelihood values (Row 21) are used to calculate two likelihood-ratios (Row 22). A likelihood-ratio is used to compare the statistical support for the estimate of 1292.8 to the support for each of the other values, 517.1 and 3232.0. The two likelihood-ratios are therefore:

LR1 = [likelihood of 1292.8] / [likelihood of 517.1]
= 0.0337 / 0.0080
= 4.21

and

LR2 = [likelihood of 1292.8] / [likelihood of 3232.0]
= 0.0337 / 0.0098
= 3.44

G. Determine if the likelihood-ratios exceed the critical value.

16. High likelihood-ratios are taken to indicate relatively high support for the point estimate of the LD50. Both of the likelihood-ratios calculated in Step F (4.21 and 3.44) exceed the critical likelihood-ratio, which is 2.5. Therefore the LR stopping criterion is satisfied and testing stops. This is indicated by a TRUE in Row 24 and a note at the top of the example spreadsheet that the LR criterion is met.

LITERATURE

(1) Lotus Development Corporation (1999). Lotus® 1-2-3. Version 9.5, Millenium Edition. Cambridge, MA, USA.

(2) Microsoft Corporation (1985-1997). Microsoft® Excel Version 5.0 or later. Seattle, WA, USA.

Table 2. Example of stopping criterion (a) using 2000 mg/kg.

Stop after animal #5 because 3 animals survive at limit of 2000 mg/kg (#3-#5).

1	2	3	4	5	6	7	8	9	10	11	12
Step	(I)nclude; (E)xclude	Dose	(X)response (O)non-resp. OK	Included in nominal *n*	log10 Dose	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)
1	I	175	O	no	2.2430	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
2	I	550	O	no	2.7404	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3	I	2000	O	no	3.3010	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4	I	2000	O	no	3.3010	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5	I	2000	O	no	3.3010	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6	E				-						
7	E				-						
8	E				-	-	-	-	-	-	-
9	E				-	-	-	-	-	-	-
10	E				-	-	-	-	-	-	-
11	E				-	-	-	-	-	-	-
12	E				-	-	-	-	-	-	-
13	E				-			-	-	-	-
14	E				-			-	-	-	-
15	E				-			-	-	-	-
Nominal Sample size =				0							
Actual number tested =				5							

Ignore all calculation cells. No reversal in direction of response.

Maximum Likelihood Calculations cannot be completed. LD50 is greater than 2000 mg/kg.

Calculated maximum likelihood estimate of LD50 = none

Table 3. Example of stopping criterion (a) using 5000 mg/kg.

Stop after animal #6 because 3 animals survive at limit of 5000 mg/kg (#4-#6).

1	2	3	4	5	6	7	8	9	10	11	12
Step	(I)nclude; (E)xclude	Dose	(X)response (O)non-resp. OK	Included in nominal *n*	log10 Dose	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)
1	I	175	O	no	2.2430	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
2	I	550	O	no	2.7404	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3	I	1750	O	no	3.2430	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4	I	5000	O	no	3.6990	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5	I	5000	O	no	3.6990	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6	I	5000	O	no	3.6990	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
7	E				-	Ignore all calculation cells. No reversal in direction of response.					
8	E				-						
9	E				-	-	-	-	-	-	-
10	E				-	-	-	-	-	-	-
11	E				-	-	-	-	-	-	-
12	E				-	Maximum Likelihood Calculations cannot be completed. LD50 is greater than 5000 mg/kg.			-	-	-
13	E				-				-	-	-
14	E				-				-	-	-
15	E				-	-	-	-	-	-	-
Nominal Sample size =				0							
Actual number tested =				6							

Calculated maximum likelihood estimate of LD50 = none

Table 4. Example of stopping criterion (b)

Stop after animal #7 because 5 reversals in 6 consecutive animals tested (#2-#7).

1	2	3	4	5	6	7	8	9	10	11	12
Step	(I)nclude; (E)xclude	Dose	(X)response (O)non-resp. OK	Included in nominal *n*	log10 Dose	LD50 = Prob. of response	31.0 likelihood contribn. (ln *Li*)	LD50 = Prob. of response	12.4 likelihood contribn. (ln *Li*)	LD50 = Prob. of response	77.6 likelihood contribn. (ln *Li*)
1	I	175	X	no	2.2430	0.9335	-0.0688	0.9892	-0.0108	0.7602	-0.2742
2	I	55	X	yes	1.7404	0.6905	-0.3703	0.9020	-0.1031	0.3826	-0.9607
3	I	17.5	O	yes	1.2430	0.3095	-0.3703	0.6174	-0.9607	0.0980	-0.1031
4	I	55	X	yes	1.7404	0.6905	-0.3703	0.9020	-0.1031	0.3826	-0.9607
5	I	17.5	O	yes	1.2430	0.3095	-0.3703	0.6174	-0.9607	0.0980	-0.1031
6	I	55	X	yes	1.7404	0.6905	-0.3703	0.9020	-0.1031	0.3826	-0.9607
7	I	17.5	O	yes	1.2430	0.3095	-0.3703	0.6174	-0.9607	0.0980	-0.1031
8	E				-	-	-	-	-	-	-
9	E				-	-	-	-	-	-	-
10	E				-	-	-	-	-	-	-
11	E				-	-	-	-	-	-	-
12	E				-	-	-	-	-	-	-
13	E				-	-	-	-	-	-	-
14	E				-	-	-	-	-	-	-
15	E				-	-	-	-	-	-	-
Nominal Sample size =				6							
Actual number tested =				7							
Dose-averaging estimator log10 =				31.02 1.492							
log-likelihood sums:							-2.2906		-3.2021		-3.4655
likelihoods:							0.1012		0.0407		0.0313
likelihood ratios:									2.4880		3.2378
Individual ratios exceed critical value?				critical=	2.5		Automated calculation; not relevant to this case.		FALSE		TRUE
Both ratios exceed critical value?									FALSE		
Calculated maximum likelihood estimate of LD50 =					29.6	Final estimate obtained from Maximum Likelihood Calculations					

Table 5. Example of stopping criterion (c)

Stop when LR criterion is first met, here at animal #9.
Check LR criterion starting at animal #6.

Assumed slope	2	sigma =	0.5

Parameters of convergence criterion	
critical LR	2.5
factor of LD50	2.5

Result:	The LR criterion is met

1	2	3	4	5	6		7	8	9	10	11	12
Step	(I)nclude; (E)xclude	Dose	(X)response (O)non-resp.	Included in nominal *n*	log10 Dose	Contrib.to DAE	LD50 =	1292.8	LD50 =	517.1	LD50 =	3232.0
			OK				Prob. of response	likelihood contribn. (ln *Li*)	Prob. of response	likelihood contribn. (ln *Li*)	Prob. of response	likelihood contribn. (ln *Li*)
1	I	175	O	no	2.2430	0.0000	0.0412	-0.0421	0.1733	-0.1903	0.0057	-0.0057
2	I	550	O	yes	2.7404	2.7404	0.2289	-0.2600	0.5214	-0.7368	0.0620	-0.0640
3	I	1750	X	yes	3.2430	3.2430	0.6037	-0.5046	0.8552	-0.1564	0.2971	-1.2138
4	I	550	O	yes	2.7404	2.7404	0.2289	-0.2600	0.5214	-0.7368	0.0620	-0.0640
5	I	1750	X	yes	3.2430	3.2430	0.6037	-0.5046	0.8552	-0.1564	0.2971	-1.2138
6	I	550	O	yes	2.7404	2.7404	0.2289	-0.2600	0.5214	-0.7368	0.0620	-0.0640
7	I	1750	O	yes	3.2430	3.2430	0.6037	-0.9257	0.8552	-1.9323	0.2971	-0.3525
8	I	5000	X	yes	3.6990	3.6990	0.8800	-0.1279	0.9756	-0.0247	0.6477	-0.4344
9	I	1750	X	yes	3.2430	3.2430	0.6037	-0.5046	0.8552	-0.1564	0.2971	-1.2138
10	E				-	0.0000	-	-	-	-	-	-
11	E				-	0.0000	-	-	-	-	-	-
12	E				-	0.0000	-	-	-	-	-	-
13	E				-	0.0000	-	-	-	-	-	-
14	E				-	0.0000	-	-	-	-	-	-
15	E				-	0.0000	-	-	-	-	-	-
Nominal Sample size =				8								
Actual number tested =				9								
Dose-averaging estimator				1292.78								
log10 =				3.112								
log-likelihood sums:								-3.3894		-4.8270		-4.6260
likelihoods:								0.0337		0.0080		0.0098
likelihood ratios:										4.2104		3.4436
Individual ratios exceed critical value?				critical=	2.5					TRUE		TRUE
Both ratios exceed critical value?										TRUE		

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ANNEX 4

CRITERIA FOR CLASSIFICATION OF TEST SUBSTANCES WITH EXPECTED LD50 VALUES EXCEEDING 2000 MG/KG WITHOUT THE NEED FOR TESTING

1. Criteria for hazard Category 5 are intended to enable the identification of test substances which are of relatively low acute toxicity hazard but which, under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD50 in the range of 2000-5000 mg/kg or equivalent doses for other routes. Test substances could be classified in the hazard category defined by: 2000 mg/kg<LD50<5000 mg/kg (Category 5 in the GHS) in the following cases:

a) if reliable evidence is already available that indicates the LD50 to be in the range of Category 5 values; or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature.

b) through extrapolation, estimation or measurement of data if assignment to a more hazardous category is not warranted, and
   - reliable information is available indicating significant toxic effects in humans, or
   - any mortality is observed when tested up to Category 4 values by the oral route, or
   - where expert judgement confirms significant clinical signs of toxicity, when tested up to Category 4 values, except for diarrhoea, piloerection or an ungroomed appearance, or
   - where expert judgement confirms reliable information indicating the potential for significant acute effect from the other animal studies.

TESTING AT DOSES ABOVE 2000 MG/KG

2. Recognising the need to protect animal welfare, testing in animals in Category 5 ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test would have a direct relevance for protecting human health.

Appendix M2

EPA UDP Test Guideline

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United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–02–190
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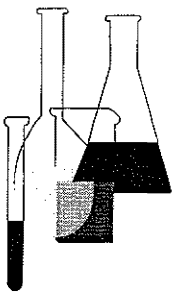
EPA

Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's Internet Web site at http://www.epa.gov/opptsfrs/home/guidelin.htm. Also, the Agency has developed, and strongly recommends users to solely use, the software program for performing the Up-and-Down Procedure and calculating the LD50 and confidence interval. The software program (AOT425StatPgm) is available on EPA's Internet Web site at http://www.epa.gov/oppfead1/harmonized.

OPPTS 870.1100 Acute oral toxicity.

(a) **Scope—Applicability**. This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticida Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background**. The source material for this revised harmonized test guideline is OPPTS 870.1100 Acute Oral Toxicity, dated August 1998 and OECD test Guideline 425 Acute Oral Toxicity–Up-and-Down Procedure.

(b) **Purpose**. In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health and environmental hazards likely to arise from short-term exposure by the oral route. Data from an acute study may serve as a basis for classification and labeling. It is traditionally a step in establishing a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a substance. An evaluation of acute toxicity data should include the relationship, if any, between the exposure of animals to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects.

(c) **Definitions**. The definitions in Section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours.

Confidence interval (CI) is an interval estimate, a range of values, intended to include the true LD_{50} with a specified degree of confidence.

Delayed death means that an animal does not die or appear moribund within 48 hours, but dies later during the 14-day observation period.

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg (grams, milligrams)) or as weight of test substance per unit weight of test animal (e.g., mg/kg (milligrams/kilograms)).

Dose progression factor, sometimes termed a dose spacing factor, refers to the multiple by which a dose is increased (i.e., the dose progression) when an animal survives or the divisor by which it is decreased when an animal dies. The dose progression factor is recommended to be the antilog of 1/(the estimated slope of the dose-response curve). The default

dose progression factor is recommended to be 3.2 = antilog 0.5 = antilog (1/2).

LD_{50} (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Limit dose refers to a dose at an upper limitation on testing (2000–5000 mg/kg).

Moribund status of an animal refers to being in a state of dying or inability to survive, even if treated.

Nominal sample size refers to the total number of tested animals, reduced by one less than the number of like responses at the beginning of the series, or by the number of tested animals up to but not including the pair that creates the first reversal. For example, for a series where X and O indicate opposite animal outcomes (for instance, X could be dies within 48 hours and O survives) in a pattern as follows: OOOXXOXO, we have the total number of tested animals (or sample size in the conventional sense) as 8 and the nominal sample size as 6. This particular example shows 4 animals following a reversal. It is important to note whether a count in a particular part of the guideline refers to the nominal sample size or to the total number tested. For example, the maximum actual number tested is 15. When testing is stopped based on that basis, the nominal sample size will be less than or equal to 15. Members of the nominal sample start with the (r-1)st animal (the animal before the second in the reversal pair) (see reversal below).

Probit is an abbreviation for the term "*prob*ability *i*ntegral *t*ransformation" and a probit dose-response model permits a standard normal distribution of expected responses (i.e., one centered to its mean and scaled to its standard deviation, *sigma*) to doses (typically in a logarithmic scale) to be analyzed as if it were a straight line with slope the reciprocal of *sigma*. A standard normal lethality distribution is symmetric; hence, its mean is also its true LD_{50} or median response.

Reversal is a situation where nonresponse is observed at some dose, and a response is observed at the next dose tested, or vice versa (i.e., response followed by nonresponse). Thus, a reversal is created by a pair of responses. The first such pair occurs at animals numbered r-1 and r.

Sigma is the standard deviation of a log normal curve describing the range of tolerances of test subjects to the chemical (where a subject is expected capable of responding if the chemical dose exceeds the subject's tolerance). The estimated *sigma* provides an estimate of the variation

among test animals in response to a full range of doses. See slope and probit.

Slope (of the dose-response curve) is a value related to the angle at which the dose response curve rises from the dose axis. In the case of probit analysis, when responses are analyzed on a probit scale against dose on a log scale this curve will be a straight line and the slope is the reciprocal of *sigma*, the standard deviation of the underlying test subject tolerances, which are assumed to be normally distributed. See probit and *sigma*.

Stopping rule is used in this guideline synonymously with (1) a specific stopping criterion and (2) the collection of all criteria determining when a testing sequence terminates. In particular, for the main test, stopping rule is used in paragraph (e)(2)(ii) of this guideline as a shorthand for the criterion that relies on comparison of ratios to a critical value.

(d) **Approaches to the determination of acute toxicity**. EPA recommends the Up-and-Down Procedure (UDP) as detailed in this guideline and adopted by the Organization for Economic Cooperation and Development (OECD) as test Guideline 425 (see paragraph (n)(1) of this guideline), to assess acute oral toxicity. This method provides a point estimate of lethality and confidence interval around the LD50. Acute oral toxicity testing may also be performed using the Fixed Dose Method of OECD Guideline 420 (see paragraph (n)(2) of this guideline) or the Acute Toxic Class Method of OECD Guideline 423 (see paragraph (n)(3) of this guideline). These methods assess lethality within a dose range.

(e) **Introduction to the UDP**—(1) **Background**. (i) The concept of the up-and-down testing approach was first described by Dixon and Mood (see paragraphs (n)(4) through (n)(7) of this guideline). In 1985, Bruce proposed to use an UDP for the determination of acute toxicity of chemicals (see paragraph (n)(8) of this guideline). There exist several variations of the up-and-down experimental design for estimating an LD_{50}. This guideline is derived from the UDP of Bruce as adopted by the American Society for Testing and Materials (ASTM) in 1987 (see paragraph (n)(9) of this guideline) and revised in 1990. A study comparing the results obtained with the UDP, the conventional LD_{50} test and the Fixed Dose Procedure (FDP, OECD Guideline 420) was published in 1995 (see paragraph (n)(10) of this guideline).

(ii) The UDP described in this guideline is of value in minimizing the number of animals required to estimate the acute oral toxicity of a chemical. In addition to the estimation of LD_{50} and CI, the test procedure allows the observation of signs of toxicity. The UDP does not provide information about the slope of the dose-response curve.

(iii) The guideline significantly reduces the number of animals used in comparison to the traditional LD_{50} test, which often required at least 30 animals in a test: (A) The stopping rule limits the number of animals

in a test; (B) sequential dosing introduces further efficiencies in animal use; (C) initial dosing is now set to be below the LD_{50} increasing the percentage of animals in which dosing levels will be sublethal and thereby providing some reduction in pain and distress; and (D) the use of a single sex (usually females) reduces the number of animals needed and minimizes the variability in the test population. In addition, the OECD Guidance Document on Humane Endpoints (see paragraph (n)(11) of this guideline) should be followed in order to reduce the overall suffering of test animals used in this type of toxicity test.

(2) **Initial considerations**—(i) **Choice of starting dose and dose progression factor.** All available information on the test substance should be considered by the testing laboratory prior to conducting the study in order to determine if a preliminary estimate of the LD_{50} and the slope of the dose-response curve can be made. Because the method has a bias toward the starting dose, it is essential that initial dosing occur below the LD_{50}. In addition, the UDP performs best when the spacing between doses or dose progression factor is based on an accurate estimate of the slope of the dose-response curve. (See paragraphs (i)(3)(ii) and (m)(1) of this guideline for discussion of dose sequences and starting values.) Initial information may include the identity and chemical structure of the substance; its physical chemical properties; the results of any other *in vitro* or *in vivo* toxicity tests on the substance or mixtures; toxicological data on structurally related substances or similar mixtures; and the anticipated use(s) of the substance. For example, data from an *in vitro* cytotoxicity assay can also be useful as one of the tools in setting a starting dose for the *in vivo* assessment of acute oral toxicity (see paragraphs (n)(10) through (n)(12) of this guideline). (A Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity is available (see paragraph (n)(11) of this guideline), and preliminary information suggests that the use of this approach may further reduce the number of animals used for *in vivo* testing (see paragraph (n)(11) of this guideline). Preliminary estimates of the LD_{50} and the dose-response slope will help in selecting a dose progression factor and a starting dose for testing.

(ii) **Default starting dose and dose progression factor.** If no information is available to make a preliminary estimate of the LD_{50} and the slope of the dose-response curve, results of computer simulations have suggested that starting near 175 mg/kg and using half-log units (corresponding to a dose progression of 3.2) between doses will produce the best results. This starting dose should be modified if the substance is likely to be highly toxic. The half-log spacing provides for a more efficient use of animals, and increases accuracy in the prediction of the LD_{50} value. However, for chemicals with large variability (i.e., shallow dose- response slopes), bias can still be introduced in the lethality estimates and the LD_{50} estimate will have a large statistical error, similar to other acute toxicity methods. To correct for this, the main test includes a stopping rule keyed

to properties of the estimate rather than a fixed number of test observations. (See paragraph (i)(3)(iii) of this guideline.)

(iii) **Delayed toxicity**. The method is easiest to apply to materials that produce death within one or two days. The method would not be practical to use when considerably delayed death (five days or more) can be expected.

(iv) **Computation**. Computers are used to facilitate animal-by-animal calculations that establish testing sequences and provide final estimates. The users of this protocol are strongly urged to solely use the Agency-developed software package (AOT425StatPgm) for performing the test and the calculation of the LD 50. The software is available on EPA's Internet Web site at http://www.epa.gov/oppfead1/harmonized.

(v) **Humane practices**. Moribund animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death are the subject of an OECD guidance document (see paragraph (n)(11) of this guideline).

(vi) **Limit test**. A limit test can be used efficiently to identify chemicals that are likely to have low acute toxicity.

(f) **Principle of the limit test**. The limit test is a sequential test that uses a maximum of 5 animals (see paragraphs (i)(2)(i) through (i)(2)(iv) of this guideline). A test dose of 5000 mg/kg is used. The selection of a sequential test plan increases the statistical power and also has been made to intentionally bias the procedure towards rejection of the limit test for compounds with LD_{50}s near the limit dose; i.e., to err on the side of safety. As with any limit test protocol, the probability of correctly classifying a compound will decrease as the actual LD_{50} more nearly resembles the limit dose.

(g) **Principle of the Main Test**. (1) The main test consists of a single ordered dose progression in which animals are dosed, one at a time, at 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD_{50}. If the animal survives, the dose for the next animal is increased to a factor of one half log times the original dose; if it dies, the dose for the next animal is decreased by a similar dose progression. (Note: 3.2 is the default factor corresponding to a dose progression of one half log unit in the Agency developed software program (AOT425StatPgm). However, this value may be changed. Paragraphs (i)(3)(ii) and (m)(12) of this guideline provide further guidance for choice of dose spacing factor.) Each animal should be observed carefully for up to 48 hours before making a decision on whether and how much to dose the next animal. That decision is based on the 48-hour survival pattern

of all the animals up to that time. (See paragraphs (i)(3)(i) and (i)(3)(v) of this guideline on choice of survival interval.) A combination of stopping criteria is used to keep the number of animals low while adjusting the dosing pattern to reduce the effect of a poor starting value or low slope (see paragraph (i)(3)(iv) of this guideline). Dosing is stopped when one of these criteria is satisfied (see paragraphs (i)(3)(iii) and (k)(2) of this guideline), at which time an estimate of the LD_{50} and a CI are calculated for the test based on the status of all the animals at termination. For most applications, testing will be completed with only 4 animals after initial reversal in animal outcome. The LD_{50} is calculated using the method of maximum likelihood (see paragraphs (k)(2) and (k)(2)(iii) of this guideline.)

(2) The results of the main test procedure serve as the starting point for a computational procedure to provide a CI estimate where feasible. A description of the basis for this CI is outlined in paragraph (k)(3) of this guideline.

(h) **Preparation for testing**—(1) **Selection of animals species.** The preferred rodent species is the rat although other rodent species may be used.

(2) **Single sex selection.** The test is conducted using a single sex in order to reduce variability and as a means of minimizing the number of animals used. Either sex may be used, however, if there is information available indicating differences in sensitivity, the most sensitive sex (usually females) should be tested (see paragraph (n)(11) of this guideline).

(i) Literature surveys of conventional LD_{50} tests show that usually there is little difference in sensitivity between the sexes but, in those cases where differences were observed, females were often slightly more sensitive (see paragraph (n)(10) of this guideline). For chemicals that are direct acting in their toxic mechanism, female rats may have a lower detoxification capacity than males, as measured by specific activity of phase I and II enzymes. However, all available information should be evaluated, for example on chemical analogues and the results of testing for other toxicological endpoints on the chemical itself, as this may indicate that males may be more sensitive than females. Knowledge that metabolic activation is required for a chemical's toxicity can also indicate that males may be the more sensitive sex.

(ii) Occasionally, the results of subsequent testing, for example a subchronic test, may raise concerns that the more sensitive sex had not been used. In such cases, and only when considerable differences between the sexes are suspected, it may be necessary to conduct another full acute oral toxicity study in the second sex. This is preferable to conducting confirmatory testing in a small group of animals of the second sex as a late satellite to the original test because there is a strong possibility that this

would produce results that are difficult to interpret. The impact of conducting a second full test on the overall number of animals used in acute toxicity testing should be small because re-testing is anticipated to be infrequent and the results of the test in one sex, together with data from any subsequent studies, will greatly assist in the selection of starting doses closer to the LD_{50} in the second test.

(3) **Age and weight ranges**. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. At the commencement of its dosing, each animal should be between 8 weeks and 12 weeks old. In order to minimize the contribution of developmental variability to study outcome, 10 weeks, with a range of ± 1 week is recommended if practical. The weight of each animal should fall in an interval ± 20% of the mean initial weight of all previously dosed animals.

(4) **Housing and feeding conditions**. The temperature in the experimental animal room should be 22°C (± 3°C). The relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. The animals are housed individually. For feeding, conventional rodent laboratory diets may be used with an unlimited supply of drinking water.

(5) **Preparation of animals**. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. As with other sequential test designs, care must be taken to ensure that animals are available in the appropriate size and age range for the entire study.

(6) **Preparation of doses**. (i) When necessary, the test substance is dissolved or suspended in a suitable vehicle. The use of an aqueous solution/suspension/emulsion is recommended wherever possible, followed in order of preference by a solution/suspension/emulsion in oil (e.g. corn oil) and then possibly solution in other vehicles. For vehicles other than water the toxicological characteristics of the vehicle should be known. Dosing preparations must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known. Where preparation shortly before administration is not practicable and the stability of the preparation is not known, this will need to be demonstrated analytically.

(ii) Constant concentration should be used in dosing unless there is clear scientific or regulatory justification for not doing so. The maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed

1 ml/100g of body weight; however, in the case of aqueous solutions, 2 ml/100g body weight can be considered.

(7) **Administration of doses**. (i) The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

(ii) Animals should be fasted prior to dosing (e.g., with the rat, food but not water should be withheld overnight; with the mouse, food but not water should be withheld for 3–4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food may be withheld for a further 3–4 hours in rats or 1–2 hours in mice. Where a dose is administered in fractions over a period of time, it may be necessary to provide the animals with food and water depending on the length of the period.

(i) **The up-and-down testing procedure**—(1) **Choice of limit test and main test**. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed.

(2) **Implementation of the limit test**. (i) The Agency has developed dedicated software for performing the test and calculation of test results (see paragraph (e) (2)(iv) of this guideline).

(ii) Dose one animal at 5000 mg/kg. If the animal dies, conduct the main test starting at 175 mg/kg to determine the LD_{50}. If the animal survives, dose two additional animals. If both animals survive, the LD_{50} is greater than the limit dose and the test is terminated (i.e. carried to full 14-day observation without dosing of further animals). If one or both animals die, then dose an additional two animals, one at a time. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period (see paragraph (g)(1) of this guideline for initial observation period). Late deaths should be counted the same as other deaths. The results are evaluated as follows (O=survival and X=death).

(iii) The LD_{50} is less than the test dose (5000 mg/kg) when three or more animals die. If a third animal dies, conduct the main test.

O XO XX

O OX XX

O XX OX

O XX X

(iv) The LD_{50} is greater than the test dose (5000 mg/kg) when three or more animals survive.

O OO

O XO XO

O XO O

O OX XO

O OX O

O XX OO

(v) If a limit test is performed at 2000 mg/kg, animals should be dosed sequentially and testing should be performed on all five animals.

(3) **Implementation of the main test.** (i) The Agency has developed dedicated software for performing the test and calculation of test results (see paragraph (e) (2)(iv) of this guideline).

(ii) Performing the UDP. Single animals are dosed in sequence usually at 48-hour intervals. However, the time interval between dosing is determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose should be delayed until one is confident of survival of the previously dosed animal. The time interval may be adjusted as appropriate, e.g., in case of inconclusive response. The test is simpler to implement when a single time interval is used for making sequential dosing decisions. Nevertheless, it is not necessary to recalculate dosing or likelihood-ratios if the time interval changes midtest. For selecting the starting dose, all available information, including information on structurally related substances and results of any other toxicity tests on the test material, should be used to approximate the LD_{50} as well as the slope of the dose-response curve.

(iii) Choice of starting dose and dose progression. The first animal is dosed a step below the toxicologist's best estimate of the LD_{50}. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. The same dosing decision pattern is followed for each subsequent animal.

The dose progression factor should be chosen to be the antilog of 1/(the estimated slope of the dose-response curve) (a progression of 3.2 corresponds to a slope of 2) and should remain constant throughout testing. Thus, when there is no information on the slope of the substance to be tested, a default dose progression factor of 3.2 is used. Using the default progression factor, doses would be selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000. If no estimate of the substance's lethality is available, dosing should be initiated at 175 mg/kg. In most cases, this dose is sublethal and therefore serves to reduce the level of pain and suffering. If animal tolerances to the chemical are expected to be highly variable (i.e., slopes are expected to be less than 2.0), consideration should be given to increasing the dose progression factor beyond the default 0.5 on a log dose scale (i.e., 3.2 progression factor) prior to starting the test. Similarly, for test substances known to have very steep slopes, dose progression factors smaller than the default should be chosen. (Paragraph (m)(3) of this guideline relates choice of dose progression to assumed slope and *sigma* and discusses test performance. Paragraph (m)(1) of this guideline includes a table of dose progressions for whole number slopes ranging from 1 to 8 with starting dose 175 mg/kg.)

(iv) Stopping rules. Dosing continues depending on the fixed-time interval (e.g., 48-hours) outcomes of all the animals up to that time. The testing stops when one of the following stopping criteria first is met:

(A) 3 consecutive animals survive at the upper bound;

(B) 5 reversals occur in any 6 consecutive animals tested;

(C) At least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value. (See paragraphs (k)(2)(iv) and (m)(2) of this guideline). Calculations are made at each dosing, following the fourth animal after the first reversal.).

(v) Total number of doses. For a wide variety of combinations of LD_{50} and slopes, stopping rule in paragraph (i)(3)(iii)(C) of this guideline will be satisfied with 4 to 6 animals after the test reversal. In some cases for chemicals with shallow slope dose-response curves, additional animals (up to a total of fifteen tested) may be needed.

(vi) Calculation. When the stopping criteria have been attained, the estimated LD_{50} should be calculated from the animal outcomes at test termination using the method described in paragraphs (k)(1)(i) and (k)(2)(i) of this guideline.

(vii) Humane practices. Moribund animals killed for humane reasons are considered in the same way as animals that died on test. If an animal unexpectedly dies late in the study and there are other survivors at that dose or above, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period.

If subsequent survivors also die, and it appears that all dose levels exceed the LD_{50} it would be most appropriate to start the study again beginning at least two steps below the lowest dose with deaths (and increasing the observation period) since the technique is most accurate when the starting dose is below the LD_{50}. If subsequent animals survive at or above the dose of the animal that dies, it is not necessary to change the dose progression since the information from the animal that has now died will be included into the calculations as a death at a lower dose than subsequent survivors, pulling the LD_{50} down.

(j) **Observations**. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions and time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed (see paragraph (n)(15) of this guideline). All observations of toxic signs are systematically recorded with individual records being maintained for each animal. Additional observations will be necessary if the animals continue to display signs of toxicity.

(1) **Toxic signs**. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document (see paragraph (n)(11) of this guideline) should be taken into consideration. Animals found in a moribund condition and animals showing severe pain and enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

(2) **Body weight**. Individual weights of animals should be determined shortly before the test substance is administered and at least weekly thereafter. Weight changes should be calculated and recorded. At the end of the test surviving animals are weighed and then humanely killed.

(3) **Pathology**. All animals (including those which die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours after the

initial dosing may also be considered because it may yield useful information.

(k) **Data and reporting**—(1) **Data**. Individual animal data should be provided. Additionally, all data should be summarized in tabular form, showing for each test dose the number of animals used, the number of animals displaying signs of toxicity (see paragraph (n)(15) of this guideline), the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings. A rationale for the starting dose and the dose progression and any data used to support this choice should be provided.

(2) **Calculation of LD_{50} for the main test**—(i) **Maximum likelihood**. The LD_{50} is calculated using the maximum likelihood method, except in the exceptional cases described in paragraphs (k)(2)(ii) and (m)(3) of this guideline. The Agency-developed software program (AOT425StatPgm) available on EPA's Internet Web site at http://www.epa.gov/oppfead1/harmonized should be used to perform this calculation. The following statistical details may be helpful in implementing the maximum likelihood calculations suggested (with an assumed *sigma*). All deaths, whether immediate or delayed or humane kills, are incorporated for the purpose of the maximum likelihood analysis. Following Dixon (see paragraph (n)(5) of this guideline), the likelihood function is written as follows:

$L = L_1 L_2 \ldots L_n$,

where

L is the likelihood of the experimental outcome, given μ and *sigma*, and n the total number of animals tested.

$L_i = 1 - F(Z_i)$ if the i^{th} animal survived, or

$L_i = F(Z_i)$ if the i^{th} animal died,

where

F = cumulative standard normal distribution,

$Z_i = [\log(d_i) - \mu\ ] / sigma$

d_i = dose given to the i^{th} animal, and

sigma = standard deviation in log units of dose (which is not the log standard deviation).

An estimate of the log of the true LD_{50} is given by the value of μ that maximizes the likelihood L (see paragraph (k)(2)(iii) of this guideline).

An estimate of *sigma* of 0.5 is used unless a better generic or case-specific value is available.

(ii) **Special circumstances**. Under some circumstances, statistical computation will not be possible or will likely give erroneous results. Special means to determine/report an estimated LD_{50} are available for these circumstances as described in the following paragraphs (k)(2)(ii)(A), (k)(2)(ii)(B), and (k)(2)(ii)(C). If none of these situations occurs, then the LD_{50} is calculated using the maximum likelihood method.

(A) If testing stopped based on the criterion in paragraph (i)(3)(iii)(C) of this guideline (i.e., a boundary dose was tested repeatedly), or if the upper bound dose ended testing, then the LD_{50} is reported to be above the upper bound.

(B) If all the dead animals have higher doses than all the live animals (or if all live animals have higher doses than all the dead animals, although this is practically unlikely), then the LD_{50} is between the doses for the live and the dead animals. These observations give no further information on the exact value of the LD_{50}. Still, a maximum likelihood LD_{50} estimate can be made provided there is a prior value for *sigma*. The LD_{50} estimate is only as good as the validity of the assumed signa. However, Case 3 as described in paragraph (m)(3)(iii) of this guideline and here is most likely to occur because the dose progression (based on the assumed signma) is too wide. The stopping criterion in paragraph (i)(3)(iii)(C) describes one such circumstance.

(C) If the live and dead animals have only one dose in common and all the other dead animals have higher doses and all the other live animals lower doses, or vice versa, then the LD_{50} equals their common dose. If a closely related substance is tested, testing should proceed with a smaller dose progression.

(iii) **Maximum likelihood calculation**. Maximum likelihood calculation should be performed using a dedicated program developed by and available from EPA (see paragraph (n)(16) of this guideline). If other computer programs are used, the laboratory should take care in handling special cases described in this guideline and the documentation of test performance available on EPA's Internet Web site at http://www.epa.gov/oppfead1/harmonized. Typical instructions for these packages are given in appendices to the ASTM Standard E 1163-87 (see paragraph (n)(9) of this guideline). (The *sigma* used in the BASIC program in (see paragraph (n)(9) of this guideline) will need to be edited to reflect the parameters of the UDP.) The program's output is an estimate of log (LD_{50}) and its standard error.

(iv) **Stopping rule**. The likelihood-ratio stopping rule in paragraph (i)(3)(iii)(C) of this guideline is based on three measures of test progress, that are of the form of the likelihood in paragraph (k)(2) of this guideline,

with different values for μ. Comparisons are made after each animal tested after the sixth that does not already satisfy the criteria in paragraph (i)(3)(iii)(A) or paragraph (i)(3)(iii)(B) guideline. The equations for the likelihood-ratio criteria are provided by following the steps in paragraph (m)(2)(vii) of this guideline. These comparisons are most readily performed in an automated manner and can be executed repeatedly, for instance, by a spreadsheet routine such as that also provided in paragraph (m)(2)(vii) of this guideline. If the criterion is met, testing stops and the LD_{50} can be calculated by the maximum likelihood method.

(3) **Computation of CI**. (i) Following the main test and estimated LD_{50} calculation, it may be possible to compute interval estimates for the LD_{50}. The Agency-developed software program AOT425StatPgm will perform the calculations. Any of these CIs provides valuable information on the reliability and utility of the main test that was conducted. A wide CI indicates that there is more uncertainty associated with the estimated LD_{50}. In this case, the reliability of the estimated LD_{50} is low and the usefulness of the estimated LD_{50} may be marginal. A narrow interval indicates that there is relatively little uncertainty associated with the estimated LD_{50}. In this case, the reliability of the estimated LD_{50} is high and the usefulness of the estimated LD_{50} is good. This means that if the main test were to be repeated, the new estimated LD_{50} is expected to be close to the original estimated LD_{50} and both of these estimates are expected to be close to the true LD_{50}.

(ii) Depending on the outcome of the main test, one of two different types of interval estimates of the true LD_{50} is calculated:

(A) When at least three different doses have been tested and the middle dose has at least one animal that survived and one animal that died, a profile-likelihood-based computational procedure is used to obtain a CI that is expected to contain the true LD_{50} 95% of the time. However, because small numbers of animals are expected to be used, the actual level of confidence is generally not exact (see paragraph (n)(19) of this guideline). The random stopping rule improves the ability of the test overall to respond to varying underlying conditions, but also causes the reported level of confidence and the actual level of confidence to differ somewhat (see paragraph (n)(18) of this guideline).

(B) If all animals survive at or below a given dose level and all animals die when dosed at the next higher dose level, an interval is calculated that has as its lower limit the highest dose tested where all the animals survive and has as its upper limit the dose level where all the animals died. This interval is labeled as ''approximate.'' The exact confidence level associated with this interval cannot be specifically determined. However, because this type of response would only occur when the dose-response is steep, in most cases, the true LD_{50} is expected to be contained

within the calculated interval or be very close to it. This interval will be relatively narrow and sufficiently accurate for most practical use.

(iii) In some instances, CIs are reported as infinite, through including either zero at the lower end or infinity at the upper end, or both. Such intervals may occur, for example, when the response profile is relatively flat or relatively uncertain.

(iv) Implementing this set of procedures requires specialized computation which is either by use of a dedicated program to be available through the Environmental Protection Agency (EPA) or OECD or developed following technical details available from the EPA or OECD. Achieved coverage of these intervals and properties of the dedicated program are described in a report (see paragraph (n)(16) of this guideline) also available through the EPA. Paragraph (m)(3) of this guideline provides information on choice of dose progression and initial dose level for the UDP and describes test performance under a variety of circumstances.

(l) **Test reporting**. The test report must include the following information:

(1) Test substance:

(i) Physical nature, purity and physicochemical properties (including isomerization);

(ii) Identification data.

(2) Vehicle (if appropriate): Justification for choice of vehicle, if other than water.

(3) Test animals:

(i) Species/strain used;

(ii) Microbiological status of the animals, when known;

(iii) Number, age and sex of animals;

(iv) Rationale for use of males instead of females;

(v) Source, housing conditions, diet, etc.;

(vi) Individual weights of animals at the start of the test, at day 7, and at day 14.

(4) Test conditions:

(i) Rationale for initial dose level selection, dose progression factor and for follow-up dose levels;

(ii) Details of test substance formulation;

(iii) Details of the administration of the test substance;

(iv) Details of food and water quality (including diet type/source, water source).

(5) Results:

(i) Body weight/body weight changes;

(ii) Tabulation of response data by sex (if both sexes are used) and dose level for each animal (i.e., animals showing signs of toxicity including nature, severity, duration of effects, and mortality);

(iii) Time course of onset of signs of toxicity and whether these were reversible for each animal;

(iv) Necropsy findings and any histopathological findings for each animal, if available;

(v) LD_{50} and CI (which the AOT425StatPgm software package uses);

(vi) Statistical treatment of results (description of computer routine used and spreadsheet tabulation of calculations). If other than Agency-supplied software is used, give explanation of now the program was verified against Agency software.

(6) Discussion and interpretation of results.

(7) Conclusions.

(m) **Additional guidance for toxicologists**—(1) **Dosing procedure—dose sequence for main test**. (i) Up-and-down dosing procedure. For each run, animals are dosed, one at a time, usually at 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD_{50}. This selection reflects an adjustment for a tendency to bias away from the LD_{50} in the direction of the initial starting dose in the final estimate (see paragraph (e)(2)(ii) of the guideline). The overall pattern of outcomes is expected to stabilize as dosing is adjusted for each subsequent animal. Paragraph (m)(1)(iii) of this guideline provides further guidance for choice of dose spacing factor.

(ii) Default dose progression. Once the starting dose and dose spacing are decided, the toxicologist should list all possible doses including the upper bound (usually 2000 or 5000 mg/kg). Doses that are close to the upper bound should be removed from the progression. The stepped nature of the UDP design provides for the first few doses to function as a self-adjusting sequence. Because of the tendency for positive bias, in the event that nothing is known about the substance, a starting dose of 175 mg/kg is recommended. If the default procedure is to be used for the main test, dosing will be initiated at 175 mg/kg and doses will be spaced by a factor of 0.5 on a log dose scale. The doses to be used include 1.75,

5.5, 17.5, 55, 175, 550, 2000 or, for specific regulatory needs, 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000. For certain highly toxic substances, the dosing sequence may need to be extended to lower values.

(iii) In the event a dose progression factor other than the default is deemed suitable, the following Table 1 provides dose progressions for whole number multiples of slope, from 1 to 8. (See paragraph (m)(3) of this guideline for discussion of influence of dose progression on test performance.)

Table 1.—Dose Progressions for UDP

(Choose a Slope and Read Down the Column. All doses in mg/kg body weight)

Slope =	1	2	3	4	5	6	7	8
	0.175*	0.175*	0.175*	0.175*	0.175*	0.175*	0.175*	0.175*
							0.243*	0.233*
					0.28	0.26		
				0.31			0.34	0.31
			0.38			0.38		
								0.41
					0.44		0.47	
		0.55		.55		0.55		0.55
				0.70		0.65		
							0.74	
			.81			.81		
				0.98			0.91	0.98
					110	1.19		
							1.26	1.31
	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
							2.43	2.33
					2.8	2.6		
				3.1			3.4	3.1
			3.8			3.8		
					4.4			4.1
							4.7	
		5.5		5.5	5.5		5.5	
					7.0		6.5	
								7.4
			8.1			8.1		
				9.8			9.1	9.8
					11.0	11.9		
							12.6	13.1
	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5
							24.3	23.3
					28	26		
				31			34	31
			,38			38		
					44			41
							47	
		55		55		55		55
							65	
					70			74
			81			81		
				98			91	98
					110	119		
							126	131
	175	175	175	175	175	175	175	175
							243	233
					280	260		
				310			340	310
			380			380		
					440			410
							470	
		550		550		550		550
							650	
					700			740
			810			810		
				980			910	980
					1100	1190		
							1260	1310
	1750	1750	1750	1750	1750	1750	1750	1750
							2430	2330
					2800	2600		
				3100				3100
						3800	3400	
								4100
	5000	5000	5000	5000	5000	5000	5000	5000

* If lower doses are needed, continue progressions to a lower dose

(2) **Computations for the likelihood-ratio stopping rules.** (i) As described in paragraph (i)(3)(iii) of this guideline, the main test may be completed on the basis of the first of three stopping criteria to occur. In any case, even if none of the stopping criteria is satisfied, dosing would stop when 15 animals are dosed. Tables 2, 4, and 6 in paragraphs (m)(2)(ii), (m)(2)(iii), and (m)(2)(iv), respectively, of this guideline illustrate examples where testing has started with no information, so the rec-

ommended default starting value, 175 mg/kg, and the recommended default dose progression factor, 3.2 or one half log, have been used. Tables 3, 5, and 7 in paragraphs (m)(2)(ii), (m)(2)(iii), and (m)(2)(iv), respectively, illustrate how Tables 2, 4, and 6, respectively, would appear in the dedicated program referenced in paragraph (k)(3)(iv) (see also paragraph (n)(16)).

(ii) The following Tables 2 and 3 show how the main test would stop if 3 animals have survived at the limit dose of 5000 mg/kg. (This example illustrates situations where a limit test was not thought appropriate *a priori*).

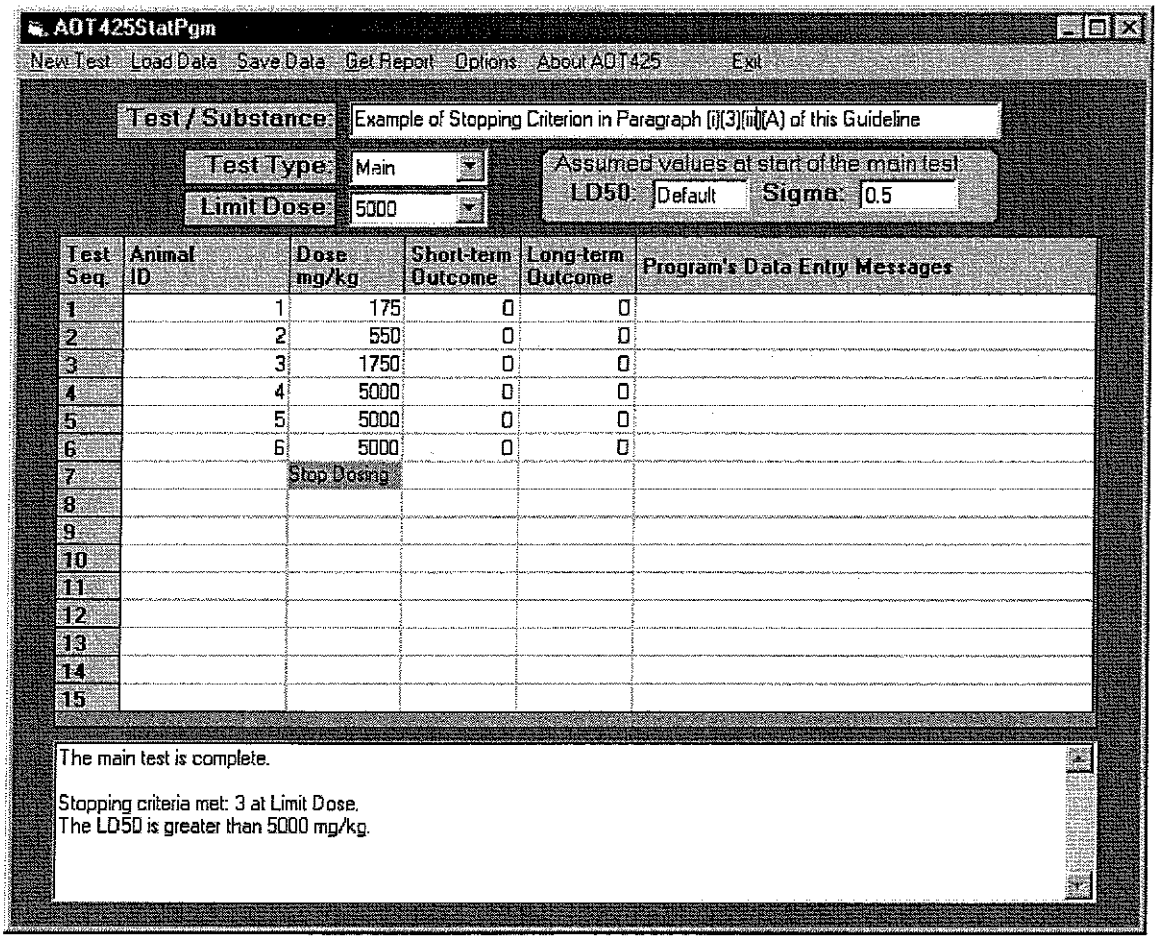
Table 2. Example of Stopping Criterion in Paragraph (i)(3)(iii)(A) using 5000 mg/kg.

Stop after animal #6 because 3 animals survive at limit of 5000 mg/kg (#4-#6).

1	2	3	4	5	6	7	8	9	10	11	12
Step	(I)nclude; (E)xclude	Dose	(X)response (O)non-resp. OK	Included in nominal *n*	log10 Dose	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)	LD50 = Prob. of response	#DIV/0! likelihood contribn. (ln *Li*)
1	I	175	O	no	2.2430	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
2	I	550	O	no	2.7404	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3	I	1750	O	no	3.2430	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4	I	5000	O	no	3.6990	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5	I	5000	O	no	3.6990	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6	I	5000	O	no	3.6990	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
7	E				-	Ignore all calculation cells. No reversal in direction of response.					
8	E				-						
9	E				-	-	-	-	-	-	-
10	E				-	-	-	-	-	-	-
11	E				-	-	-	-	-	-	-
12	E				-	Maximum Likelihood Calculations cannot be completed. LD50 is greater than 5000 mg/kg.			-	-	-
13	E				-				-	-	-
14	E				-				-	-	-
15	E				-	-	-	-	-	-	-
Nominal Sample size =				0							
Actual number tested =				6							

Calculated maximum likelihood estimate of LD50 = none

Table 3. Example of Stopping Criterion in Paragraph (i)(3)(iii)(A) of this Guideline Using 5000 mg/kg



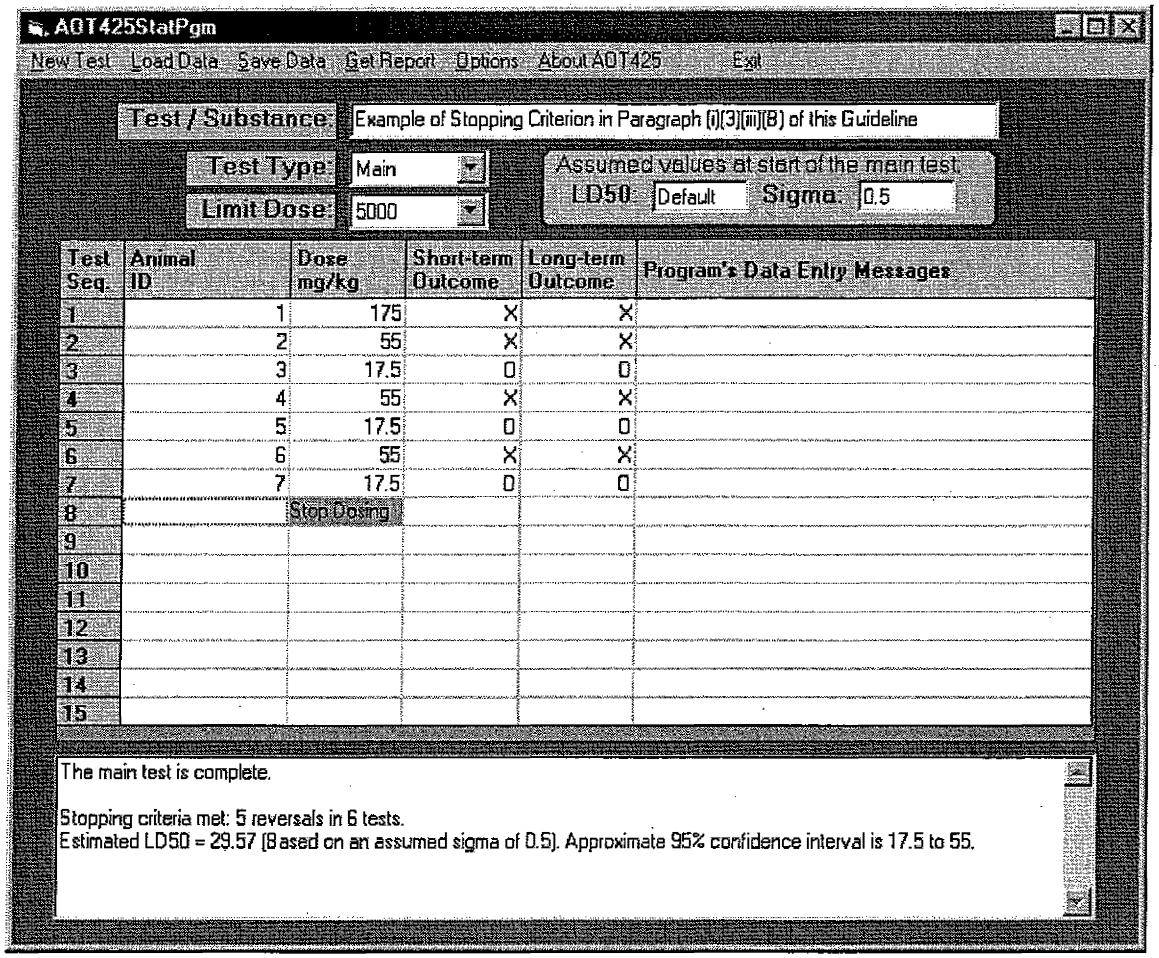
(iii) The following Tables 4 and 5 show how a particular sequence of 5 reversals in 6 tested animals could occur and allow test completion.

Table 4. Example of Stopping Criterion in Paragraph (i)(3)(iii)(B).

Stop after animal #7 because 5 reversals in 6 consecutive animals tested (#2-#7).

1	2	3	4	5	6	7	8	9	10	11	12
Step	(I)nclude; (E)xclude	Dose	(X)response (O)non-resp. OK	Included in nominal *n*	log10 Dose	LD50 = Prob. of response	31.0 likelihood contribn. (ln *Li*)	LD50 = Prob. of response	12.4 likelihood contribn. (ln *Li*)	LD50 = Prob. of response	77.6 likelihood contribn. (ln *Li*)
1	I	175	X	no	2.2430	0.9335	-0.0688	0.9892	-0.0108	0.7602	-0.2742
2	I	55	X	yes	1.7404	0.6905	-0.3703	0.9020	-0.1031	0.3826	-0.9607
3	I	17.5	O	yes	1.2430	0.3095	-0.3703	0.6174	-0.9607	0.0980	-0.1031
4	I	55	X	yes	1.7404	0.6905	-0.3703	0.9020	-0.1031	0.3826	-0.9607
5	I	17.5	O	yes	1.2430	0.3095	-0.3703	0.6174	-0.9607	0.0980	-0.1031
6	I	55	X	yes	1.7404	0.6905	-0.3703	0.9020	-0.1031	0.3826	-0.9607
7	I	17.5	O	yes	1.2430	0.3095	-0.3703	0.6174	-0.9607	0.0980	-0.1031
8	E				-	-	-	-	-	-	-
9	E				-	-	-	-	-	-	-
10	E				-	-	-	-	-	-	-
11	E				-	-	-	-	-	-	-
12	E				-	-	-	-	-	-	-
13	E				-	-	-	-	-	-	-
14	E				-	-	-	-	-	-	-
15	E				-	-	-	-	-	-	-
Nominal Sample size =				6							
Actual number tested =				7							
Dose-averaging estimator log10 =				31.02 1.492							
log-likelihood sums:							-2.2906		-3.2021		-3.4655
likelihoods:							0.1012		0.0407		0.0313
likelihood ratios:									2.4880		3.2378
Individual ratios exceed critical value?				critical=	2.5		Automated calculation; not relevant to this case.		FALSE		TRUE
Both ratios exceed critical value?									FALSE		
Calculated maximum likelihood estimate of LD50 =					29.6	Final estimate obtained from Maximum Likelihood Calculations					

Table 5. Example of Stopping Criterion in Paragraph (i)(3)(iii)(B) of this Guideline.



(iv) Finally, the following Tables 6 and 7 illustrate a situation several animals into a test, where neither the criterion in paragraph (i)(3)(iii)(A) nor the criterion in paragraph (i)(3)(iii)(B) of this guideline has been met, a reversal of response has occurred followed by 4 tested animals, and, consequently, the criterion in paragraph (i)(3)(iii)(C) of this guideline must be evaluated as well.

Table 6. Example of Stopping Criterion in Paragraph (i)(3)(iii)(C).

Stop when LR criterion is first met, here at animal #9.
Check LR criterion starting at animal #6.

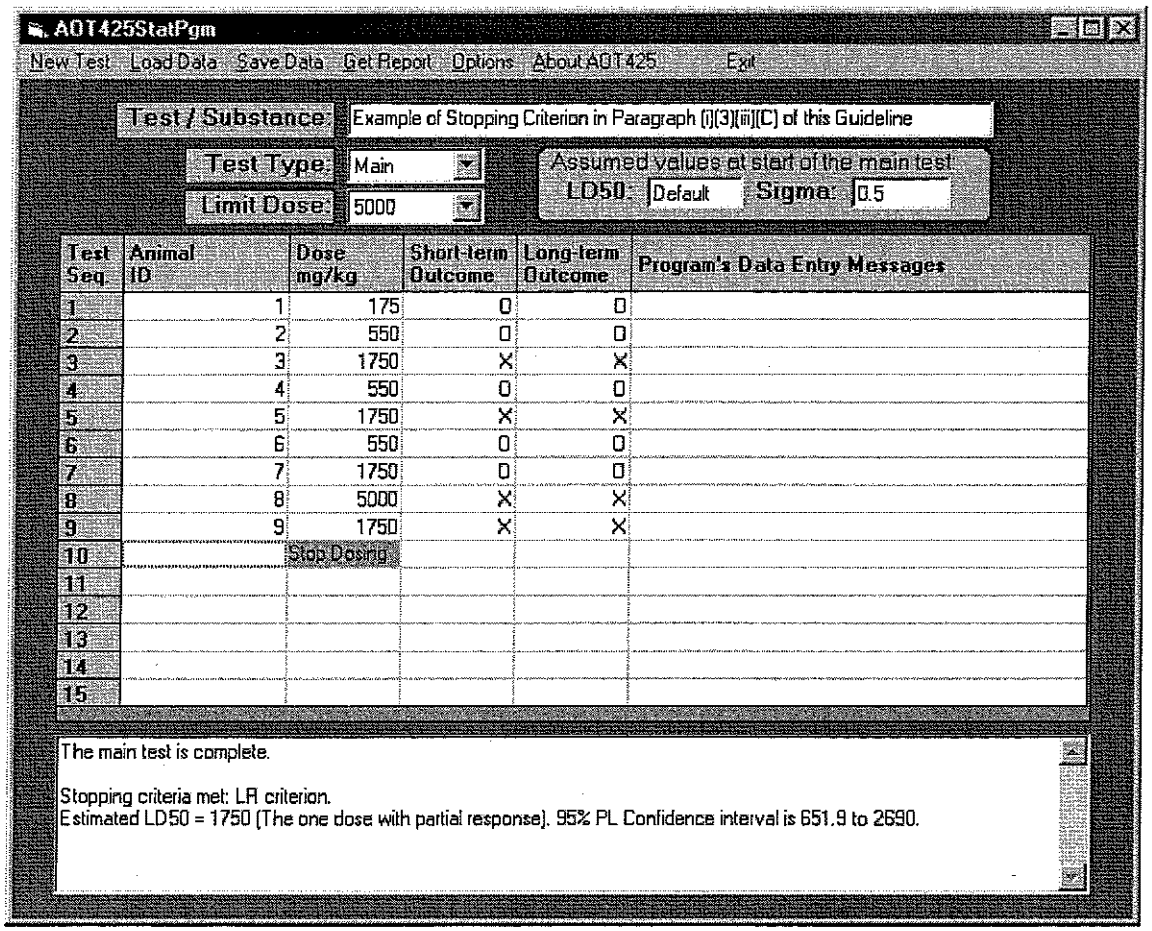
Assumed slope	2	sigma =	0.5

Parameters of convergence criterion	
critical LR	2.5
factor of LD50	2.5

Result: The LR criterion is met

1	2	3	4	5	6		7	8	9	10	11	12
Step	(I)nclude; (E)xclude	Dose	(X)response (O)non-resp. OK	Included in nominal *n*	log10 Dose	Contrib.to DAE	LD50 = Prob. of response	1292.8 likelihood contribn. (ln *Li*)	LD50 = Prob. of response	517.1 likelihood contribn. (ln *Li*)	LD50 = Prob. of response	3232.0 likelihood contribn. (ln *Li*)
1	I	175	O	no	2.2430	0.0000	0.0412	-0.0421	0.1733	-0.1903	0.0057	-0.0057
2	I	550	O	yes	2.7404	2.7404	0.2289	-0.2600	0.5214	-0.7368	0.0620	-0.0640
3	I	1750	X	yes	3.2430	3.2430	0.6037	-0.5046	0.8552	-0.1564	0.2971	-1.2138
4	I	550	O	yes	2.7404	2.7404	0.2289	-0.2600	0.5214	-0.7368	0.0620	-0.0640
5	I	1750	X	yes	3.2430	3.2430	0.6037	-0.5046	0.8552	-0.1564	0.2971	-1.2138
6	I	550	O	yes	2.7404	2.7404	0.2289	-0.2600	0.5214	-0.7368	0.0620	-0.0640
7	I	1750	O	yes	3.2430	3.2430	0.6037	-0.9257	0.8552	-1.9323	0.2971	-0.3525
8	I	5000	X	yes	3.6990	3.6990	0.8800	-0.1279	0.9756	-0.0247	0.6477	-0.4344
9	I	1750	X	yes	3.2430	3.2430	0.6037	-0.5046	0.8552	-0.1564	0.2971	-1.2138
10	E				-	0.0000	-	-	-	-	-	-
11	E				-	0.0000	-	-	-	-	-	-
12	E				-	0.0000	-	-	-	-	-	-
13	E				-	0.0000	-	-	-	-	-	-
14	E				-	0.0000	-	-	-	-	-	-
15	E				-	0.0000	-	-	-	-	-	-
Nominal Sample size =				8								
Actual number tested =				9								
Dose-averaging estimator log10 =				1292.78 3.112								
log-likelihood sums:								-3.3894		-4.8270		-4.6260
likelihoods:								0.0337		0.0080		0.0098
likelihood ratios:										4.2104		3.4436
Individual ratios exceed critical value?				critical=	2.5					TRUE		TRUE
Both ratios exceed critical value?										TRUE		
Calculated maximum likelihood estimate of LD50 =						1329.6	Final estimate obtained from Maximum Likelihood Calculations					

Table 7. Example of Stopping Criterion in Paragraph (i)(3)(iii)(C) of this Guideline.



(v) Criterion in paragraph (i)(3)(iii)(C) of this guideline calls for a likelihood-ratio stopping rule to be evaluated after testing each animal, starting with the fourth tested following the reversal. Three ''measures of test progress'' are calculated. Technically, these measures of progress are likelihoods, as recommended for the maximum-likelihood estimation of the LD_{50}. The procedure is closely related to calculation of a CI by a likelihood-based procedure.

(vi) The basis of the procedure is that when enough data have been collected, a point estimate of the LD_{50} should be more strongly supported than values above and below the point estimate, where statistical support is quantified using likelihood. Therefore three likelihood values are calculated: A likelihood at an LD_{50} point estimate (called the rough estimate or dose-averaging estimate in the example), a likelihood at a value below the point estimate, and a likelihood at a value above the point estimate. Specifically, the low value is taken to be the point estimate divided by 2.5 and the high value is taken to be the point estimate multiplied by 2.5.

(vii) The likelihood values are compared by calculating ratios of likelihoods, and then determining whether these likelihood-ratios (LR) exceed a critical value. Testing stops when the ratio of the likelihood for the point estimate exceeds each of the other likelihoods by a factor of 2.5, which is taken to indicate relatively strong statistical support for the point estimate. Therefore two likelihood-ratios (LRs) are calculated, a ratio of likelihoods for the point estimate and the point estimate divided by 2.5, and a ratio for the point estimate and the estimate times 2.5.

(viii) The calculations are easily performed in any spreadsheet with normal probability functions. The calculations are illustrated in Tables 6 and 7 in paragraph (m)(2)(iv) of this guideline, which is structured to promote spreadsheet implementation. The computation steps are illustrated using an example where the upper limit dose is 5000 mg/kg.

(A) Hypothetical example (Tables 6 and 7 in paragraph (m)(2)(iv) of this guideline). In the hypothetical example utilizing an upper boundary dose of 5000 mg/kg, the LR stopping criterion was met after nine animals had been tested. The first ''reversal'' occurred with the 3rd animal tested. The LR stopping criterion is checked when four animals have been tested following the reversal. In this example, the fourth animal tested following the reversal is the seventh animal actually tested. Therefore, for this example, the spreadsheet calculations are only needed after the seventh animal had been tested and the data could be entered at that time. Subsequently, the LR stopping criterion would have been checked after testing the seventh animal, the eighth animal, and the ninth. The LR stopping criterion is first satisfied after the ninth animal is tested in this example.

(*1*) Enter the dose-response information animal by animal.

(*i*) Column 1. Steps are numbered 1–15. No more than 15 animals may be tested.

(*ii*) Column 2. Place an I in this column as each animal is tested.

(*iii*) Column 3. Enter the dose received by the i^{th} animal.

(*iv*) Column 4. Indicate whether the animal responded (shown by an X) or did not respond (shown by an O).

(*2*) The nominal and actual sample sizes. The nominal sample consists of the two animals that represent the first reversal (here the second and third animals), plus all animals tested subsequently. Here, Column 5 indicates whether or not a given animal is included in the nominal sample.

(*i*) The nominal sample size (nominal *n*) appears in Row 16. This is the number of animals in the nominal sample. In the example, nominal *n* is 8.

(*ii*) The actual number tested appears in Row 17.

(*3*) Rough estimate of the LD_{50}. The geometric mean of doses for the animals in the current nominal sample is used as a rough estimate of the LD_{50} from which to gauge progress. In the table, this is called the ''dose-averaging estimator.'' It is updated with each animal tested. This average is restricted to the nominal sample in order to allow for a poor choice of initial test dose, which could generate either an initial string of responses or an initial string of nonresponses. (However, the results for all animals are used in the likelihood calculations for final LD_{50} calculation below.) Recall that the geometric mean of *n* numbers is the product of the *n* numbers, raised to a power of $1/n$.

(*i*) The dose-averaging estimate appears in Row 18 (e.g., $(175 * 550 * ... * 1750)^{1/8} = 1292.78$).

(*ii*) Row 19 shows the logarithm (base 10) of the value in Row 18 (e.g., $\log_{10} 1292.8 = 3.112$).

(*4*) Likelihood for the rough LD_{50} estimate.

(*i*) ''Likelihood'' is a statistical measure of how strongly the data support an estimate of the LD_{50} or other parameter. Ratios of likelihood values can be used to compare how well the data support different estimates of the LD_{50}.

(*ii*) In Column 8 calculate the likelihood for Step C's rough LD_{50} estimate. The likelihood (Row 21) is the product of likelihood contributions for individual animals (see paragraph (k)(2) of this guideline). The likelihood contribution for the i^{th} animal is denoted L_i.

(*iii*) Column 7. Enter the estimate of the probability of response at dose d_i, denoted P_i. P_i is calculated from a dose-response curve. Note that the parameters of a probit dose-response curve are the slope and the LD_{50}, so values are needed for each of those parameters. For the LD_{50} the dose-averaging estimate from Row 18 is used. For the slope in this example the default value of 2 is used. The following steps may be used to calculate the response probability P_i.

1. Calculate the base-10 log of dose d_i (Column 6).

2. For each animal calculate the z-score, denoted Z_i (not shown in the table), using the formulae

$sigma = 1 / slope$,

$Z_i = (\log_{10}(d_i) - \log_{10}(LD_{50})) / sigma$

For example, for the first animal (Row 1),

$sigma = 1 / 2$

$Z_1 = (2.243 - 3.112) / 0.500 = -1.738$

3. For the i^{th} dose the estimated response probability is

$P_i = F(Z_i)$

where F denotes the cumulative distribution function for the standard normal distribution (i.e., the normal distribution with mean 0 and variance 1).

For example (Row 1),

$P_1 = F(-1.738) = 0.0412$

The function F (or something very close) is ordinarily what is given for the normal distribution in statistical tables, but the function is also widely available as a spreadsheet function. It is available under different names, for example the @NORMAL function of Lotus 1-2-3 (see paragraph (n)(19) of this guideline) and the @NORMDIST function in Excel (see paragraph (n)(20) of this guideline). To confirm that you have used correctly the function available in your software, you may wish to verify familiar values such as $F(1.96) \approx 0.975$ or $F(1.64) \approx 0.95$.

(*iv*) Column 8. Calculate the natural log of the likelihood contribution ($\ln(L_i)$). L_i is simply the probability of the response that actually was observed for the i^{th} animal:

Responding animals: $\ln(L_i) = \ln(P_i)$

Non-responding animals: $\ln(L_i) = \ln(1 - P_i)$

Note that here the natural logarithm (ln) is used, whereas elsewhere the base-10 (common) logarithm was used. These choices are what are ordinarily expected in a given context.

The steps above are performed for each animal. Finally:

Row 20: Sum the log-likelihood contributions in Column 8.

Row 21: Calculate the likelihood by applying the exp function applied to the log-likelihood value in Row 20 (e.g., exp(-3.389) = $e^{-3.389}$ = 0.0337).

(*5*) Calculate likelihoods for two dose values above and below the rough estimate. If the data permit a precise estimate, then one expects the likelihood should be high if the estimate is a reasonable estimate of the LD_{50}, relative to likelihoods for values distant from this estimate. Compare the likelihood for the dose-averaging estimate (1292.8, Row 18) to values differing by a factor of 2.5 from that value (i.e., to 1292.8*2.5 and 1292.8/2.5). The calculations (displayed in Columns 9–12) are carried out in a fashion similar to those described above, except that the values 517.1 (=1292.8/2.5) and 3232.0 (=1292.8*2.5) have been used for the LD_{50}, instead of 1292.8. The likelihoods and log-likelihoods are displayed in Rows 20–21.

(*6*) Calculate likelihood-ratios. The three likelihood values (Row 21) are used to calculate two likelihood-ratios (Row 22). A likelihood-ratio is used to compare the statistical support for the estimate of 1292.8 to the support for each of the other values, 517.1 and 3232.0. The two likelihood-ratios are therefore:

LR1 = [likelihood of 1292.8] / [likelihood of 517.1]

= 0.0337 / 0.0080

= 4.21

and

LR2 = [likelihood of 1292.8] / [likelihood of 3232.0]

= 0.0337 / 0.0098

= 3.44

(*7*) Determine if the likelihood-ratios exceed the critical value. High likelihood-ratios are taken to indicate relatively high support for the point estimate of the LD_{50}. Both of the likelihood-ratios calculated in paragraph (m)(2)(viii)(A)(*6*) of this guideline (4.21 and 3.44) exceed the critical likelihood-ratio, which is 2.5. Therefore the LR stopping criterion is satisfied and testing stops. This is indicated by a TRUE in Row 24 and a note at the top of the example spreadsheet that the LR criterion is met. Determination of the point estimate and CI is carried out separately.

(B) [Reserved]

(3) **Performance of the UDP**. This section addresses choice of dose progression and initial dose level for the UDP and describes the performance of the test under a variety of circumstances. A companion document titled ''Toxicology Summary: Performance of the Up-and-Down Procedure'' provides assistance to the user in interpretation of the test results and is available on the ICCVAM web site at http://iccvam.niehs.nih.gov/methods/udpdocs/udprpt/udp_ciprop.htm. The statistical methods applied will depend upon the case into which the test response patterns fall (see Table 8 in paragraph (m)(3)(iii) of this guideline.

(i) Adjusting the dose progression and initial dose. For optimum performance of the UDP, the dose progression used should be based on an accurate prior estimate of *sigma*. The following two cases describe the outcome when an accurate estimate of *sigma* is not available. In addition, to account conservatively for any bias in the LD_{50} estimate, it is essential that dosing be initiated below the actual LD_{50}.

(A) Assumed *sigma* << true *sigma*: When the assumed *sigma* (i.e., the *sigma* on which the dose progression is based) is much smaller than the true *sigma* of the actual test population, the estimated LD_{50} may be ''biased'' in the direction of starting dose. For example, if the starting dose is less than the true LD_{50} of the test population, the estimated LD_{50} will generally be below the true LD_{50}. Also, if the starting dose is greater than the true LD_{50} of the test population, the estimated LD_{50} will tend to be greater than the true LD_{50}. To minimize the chance of overestimating the LD_{50} due to this bias, the UDP guideline recommends a choice of starting dose just below the assumed LD_{50}.

(B) Assumed *sigma* >> true *sigma*: If the assumed sigma on which the dose progression is based is much larger than the true *sigma* of the test population, the median estimated LD_{50} can be much larger or much smaller than the true LD_{50} depending on the starting dose. In this case, the LD_{50} can be estimated only within a range. (This is Case 3 described below.)

(ii) CI. Coverage of the CI is the probability that a calculated CI encloses the true LD_{50} for an experimental sample. Because the profile likelihood method is approximate, coverage of the CI does not always correspond to its nominal value. For example, coverage falls below 95% for populations with shallow slopes and is better than 95% for populations with steep slopes. In addition, the width of the CI is limited by the dose progression chosen. Generally, no type of CI would be more narrow than the dose progression.

(iii) Response Patterns. Data gathered under the UDP fall into one of five animal response patterns. The five types of animal response patterns, referred to as Case 1 through Case 5 in the following Table 8, can

be distinguished for the purpose of describing the performance of the UDP. These cases can be distinguished by looking at the experimental outcome (survival or death) as reflected in the AOT425StatPgm Data Grid or Report windows (see paragraph (n)(18) of this guideline). In considering these cases, note that doses can be repeated more than once in the course of sequential dosing.

Table 8.—Outcomes of the UDP: Cases and Confidence Intervals

Case #	Definition of Case	Approach Proposed	Possible Findings
1	No positive dose-response association. (1a) All animals tested in the study responded, or (1b) none responded, or (1c) the geometric mean dose is lower for animals that responded than for animals that did not respond.	LD_{50} cannot be calculated. CI not applicable.	Possible inferences: (1a) LD_{50} < lowest dose; (1b) LD_{50} > highest dose; (1c) reverse dose-response curve; unlikely test outcome. In case 1b, the highest dose tested is equivalent to a limit dose.
2	Multiple partial responses. One or more animals responded at a dose below some other dose where one or more did not respond. The conditions defining Case 1 do not hold. (The definition of Case 2 holds if there are 2 doses with partial responses, but holds in some other cases as well.)	Maximum likelihood estimate and profile likelihood computations of CI are straightforward.	The LD_{50} can be estimated and its CI calculated.
3	No intermediate response fractions. One or more test doses is associated with 0% response and one or more is associated with 100% response (all of the latter being greater than all of the former), and no test doses are associated with a partial response.	Lower bound = highest test dose with 0% response. Upper bound = lowest test dose with 100% response.	High confidence that the true LD_{50} falls between the two bounding doses. Any value of LD_{50} between highest dose with 0% response and lowest dose with 100% response is equally plausible.
4	One partial response fraction, first subcase. An intermediate partial response is observed at a single test dose. That dose is greater than doses associated with 0% response and lower than doses associated with 100% response.	The LD_{50} is set at the single dose showing partial response and its CI is calculated using profile likelihood method.	The LD_{50} can be estimated and its CI calculated.
5	One partial response fraction, second subcase. There is a single dose associated with partial response, which is either the highest test dose (with no responses at all other test doses) or the lowest test dose (with 100% response at all other test doses).	The LD_{50} is set at the dose with the partial response. A profile likelihood CI is calculated and may be finite or infinite.	The true LD_{50} could be at the boundary of the testing range with more or less confidence.

(n) References. The following references should be consulted for additional background material on this test guideline.

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Appendix M3

OECD ATC Method Test Guideline

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OECD/OCDE **423**

Adopted:
17th December 2001

OECD GUIDELINE FOR TESTING OF CHEMICALS

Acute Oral Toxicity – Acute Toxic Class Method

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 423 was adopted in March 1996 as the second alternative to the conventional acute toxicity test, described in Test Guideline 401. Based on the recommendations of several expert meetings, revision was considered timely because: i) international agreement has been reached on harmonised LD50 cut-off values for the classification of chemical substances, which differ from the cut-offs recommended in the 1996 version of the Guideline, and ii) testing in one sex (usually females) is now considered sufficient.

2. The acute toxic class method (1) set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods (Test Guidelines 420 and 425). The acute toxic class method is based on biometric evaluations (2)(3)(4)(5) with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated *in vivo* against LD50 data obtained from the literature, both nationally (6) and internationally (7).

3. Guidance on the selection of the most appropriate test method for a given purpose can be found in the Guidance Document on Acute Oral Toxicity Testing (8). This Guidance Document also contains additional information on the conduct and interpretation of Test Guideline 423.

4. Definitions used in the context of this Guideline are set out in Annex 1.

INITIAL CONSIDERATIONS

5. Test substances, at doses that are known to cause marked pain and distress due to corrosive or severely irritant actions, need not be administered. Moribund animals, or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document (9).

6. The method uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonised System for the classification of chemicals which cause acute toxicity (10).

7. In principle, the method is not intended to allow the calculation of a precise LD_{50}, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%. The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

8. The testing laboratory should consider all available information on the test substance prior to conducting the study. Such information will include the identity and chemical structure of the substance; its physico-chemical properties; the result of any other *in vivo* or *in vitro* toxicity tests on the substance; toxicological data on the structurally related substances; and the anticipated use(s) of the substance. This information is necessary to satisfy all concerned that the test is relevant for the protection of human health and will help in the selection of the most appropriate starting dose.

PRINCIPLE OF THE TEST

9. It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level.

10. Details of the test procedure are described in Annex 2. The method will enable a judgement with respect to classifying the test substance to one of a series of toxicity classes defined by fixed LD50 cut-off values.

DESCRIPTION OF THE METHOD

Selection of animal species

11. The preferred rodent species is the rat, although other rodent species may be used. Normally females are used (9). This is because literature surveys of conventional LD50 tests show that, although there is little difference in sensitivity between the sexes, in those cases where differences are observed females are generally slightly more sensitive (11). However if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive, then this sex should be used. When the test is conducted in males adequate justification should be provided.

12. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within $\pm$ 20 % of the mean weight of any previously dosed animals.

Housing and feeding conditions

13. The temperature in the experimental animal room should be 22°C (± 3°C). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

14. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions.

Preparation of doses

15. In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, ie at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1mL/100g of body weight: however in the case of aqueous solutions 2 mL/100g body weight can be considered. With respect to the formulation of the dosing preparation, the use of an aqueous solution/suspension/emulsion is recommended wherever possible, followed in order of preference by a solution/suspension/emulsion in oil (e.g. corn oil) and then possibly solution in other vehicles. For vehicles other than water the toxicological characteristics of the vehicle should be known. Doses must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known and shown to be acceptable.

PROCEDURE

Administration of doses.

16. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

17. Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period it may be necessary to provide the animals with food and water depending on the length of the period.

Number of animals and dose levels

18. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses.

19. When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight.

20 The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals.

21. Exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 5000 mg/kg body weight may be considered (see Annex 3). For reasons of animal welfare concern, testing of animals in GHS Category 5 ranges (2000-5000mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.

Limit test

22. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations where there is little or no information about its toxicity, or in which the the test material is expected to be toxic, the main test should be performed.

23. A limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals (three animals per step). Exceptionally a limit test at one dose level of 5000 mg/kg may be carried out with three animals (see Annex 3). If test substance-related mortality is produced, further testing at the next lower level may need to be carried out.

OBSERVATIONS

24. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed (12). All observations are systematically recorded with individual records being maintained for each animal.

25. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep

and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document (9) should be taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

Body weight

26. Individual weights of animals should be determined shortly before the test substance is administered, and at least weekly thereafter. Weight changes should be calculated and recorded. At the end of the test surviving animals are weighed and humanely killed.

Pathology

27. All test animals (including those that die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours may also be considered because it may yield useful information.

DATA AND REPORTING

Data

28. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings.

Test report

29. The test report must include the following information, as appropriate:

Test substance:

- physical nature, purity, and, where relevant, physico-chemical properties (including isomerisation);
- identification data, including CAS number.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the animals, when known;
- number, age, and sex of animals (including, where appropriate, a rationale for the use of males instead of females);
- source, housing conditions, diet etc.

Test conditions:

- details of test substance formulation including details of the physical form of the

material administered;
- details of the administration of the test substance including dosing volumes and time of dosing;
- details of food and water quality (including diet type/source, water source);
- the rationale for the selection of the starting dose.

Results:

- tabulation of response data and dose level for each animal (i.e. animals showing signs of toxicity including mortality; nature, severity, and duration of effects);
- tabulation of body weight and body weight changes;
- individual weights of animals at the day of dosing, in weekly intervals thereafter, and at the time of death or sacrifice;
- date and time of death if prior to scheduled sacrifice;
- time course of onset of signs of toxicity, and whether these were reversible for each animal;
- necropsy findings and histopathological findings for each animal, if available.

Discussion and interpretation of results.

Conclusions.

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ANNEX 1

DEFINITIONS

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.

Delayed death means that an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period.

Dose is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of test animal (e.g. mg/kg).

GHS: Globally Harmonised Classification System for Chemical Substances and Mixtures. A joint activity of OECD (human health and the environment), UN Committee of Experts on Transport of Dangerous Goods (physical–chemical properties) and ILO (hazard communication) and co-ordinated by the Interorganisation Programme for the Sound Management of Chemicals (IOMC).

Impending death: when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, lateral position, recumbence, and tremor (See the Humane Endpoint Guidance Document (9) for more details).

LD50 (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Limit dose refers to a dose at an upper limitation on testing (2000 or 5000 mg/kg).

Moribund status: being in a state of dying or inability to survive, even if treated (See the Humane Endpoint Guidance Document (9) for more details).

Predictable death: presence of clinical signs indicative of death at a known time in the future before the planned end of the experimen ; for example: inability to reach water or food. (See the Humane Endpoint Guidance Document (9) for more details).

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ANNEX 2

PROCEDURE TO BE FOLLOWED FOR EACH OF THE STARTING DOSES

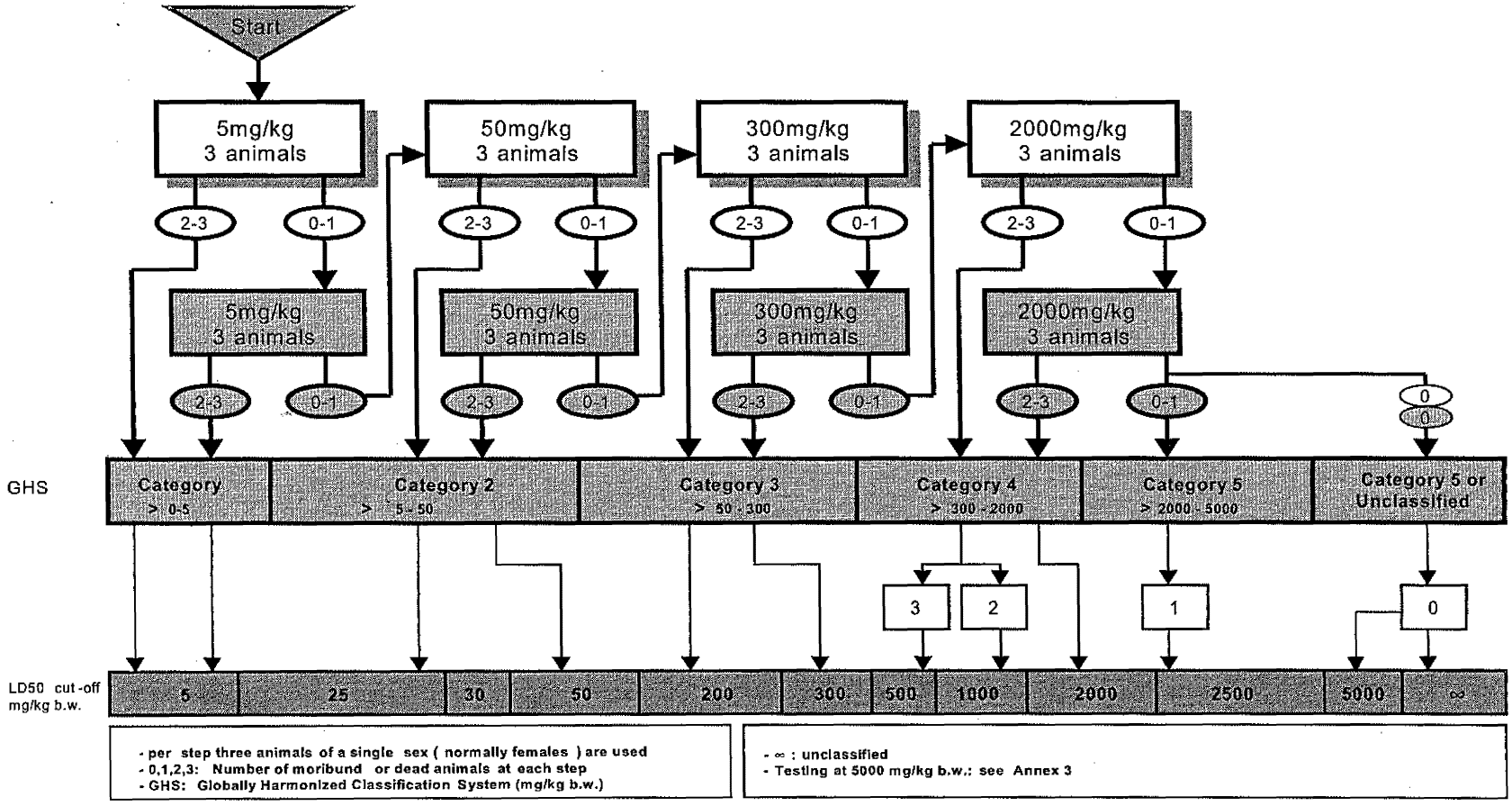
GENERAL REMARKS

1. For each starting dose, the respective testing schemes as included in this Annex outline the procedure to be followed.

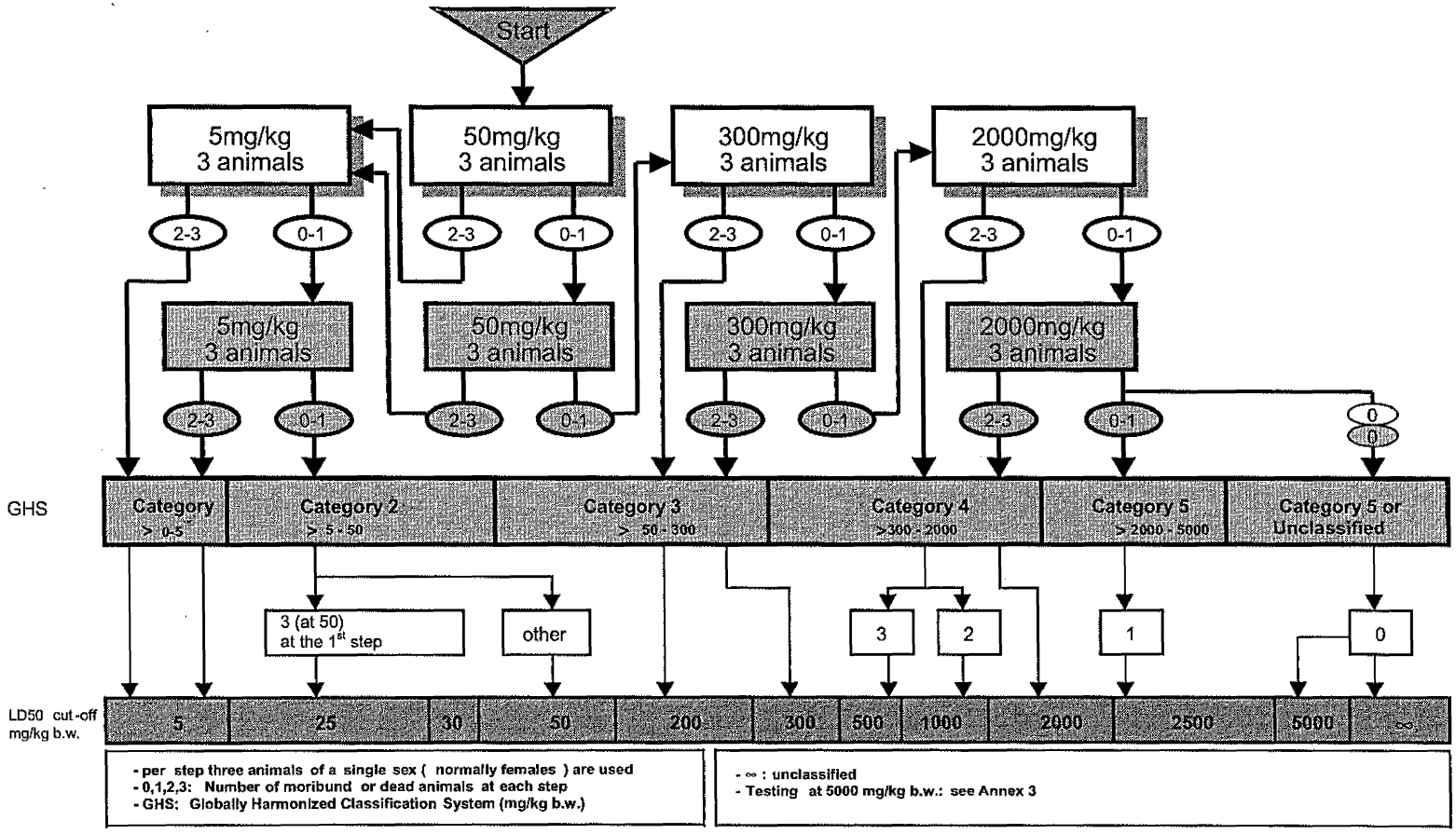
- Annex 2 a: Starting dose is 5 mg/kg bw
- Annex 2 b: Starting dose is 50 mg/kg bw
- Annex 2 c: Starting dose is: 300 mg/kg bw
- Annex 2 d: Starting dose is: 2000 mg/kg bw

Depending on the number of humanely killed or dead animals, the test procedure follows the indicated arrows.

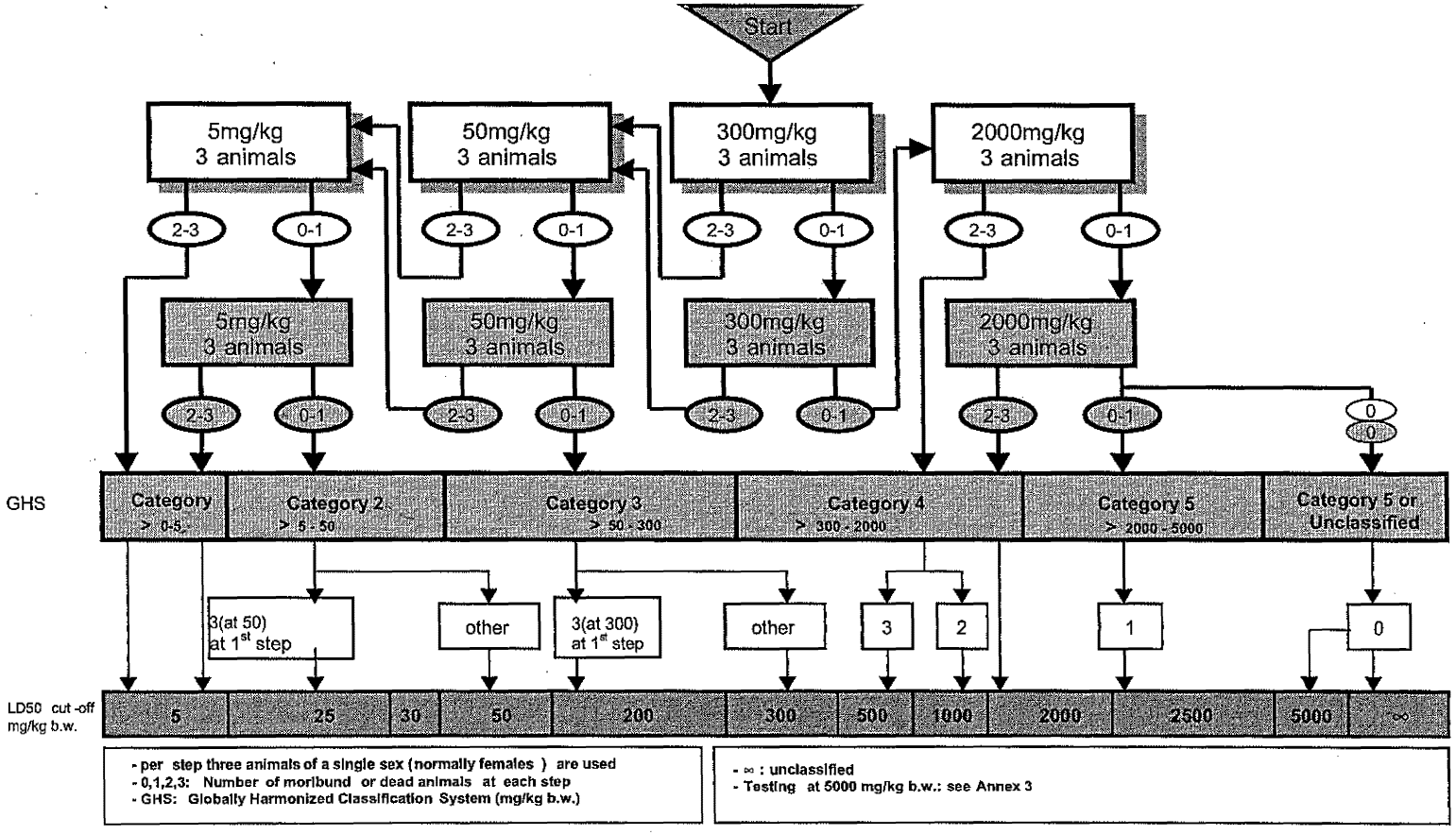
ANNEX 2a: TEST PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY WEIGHT



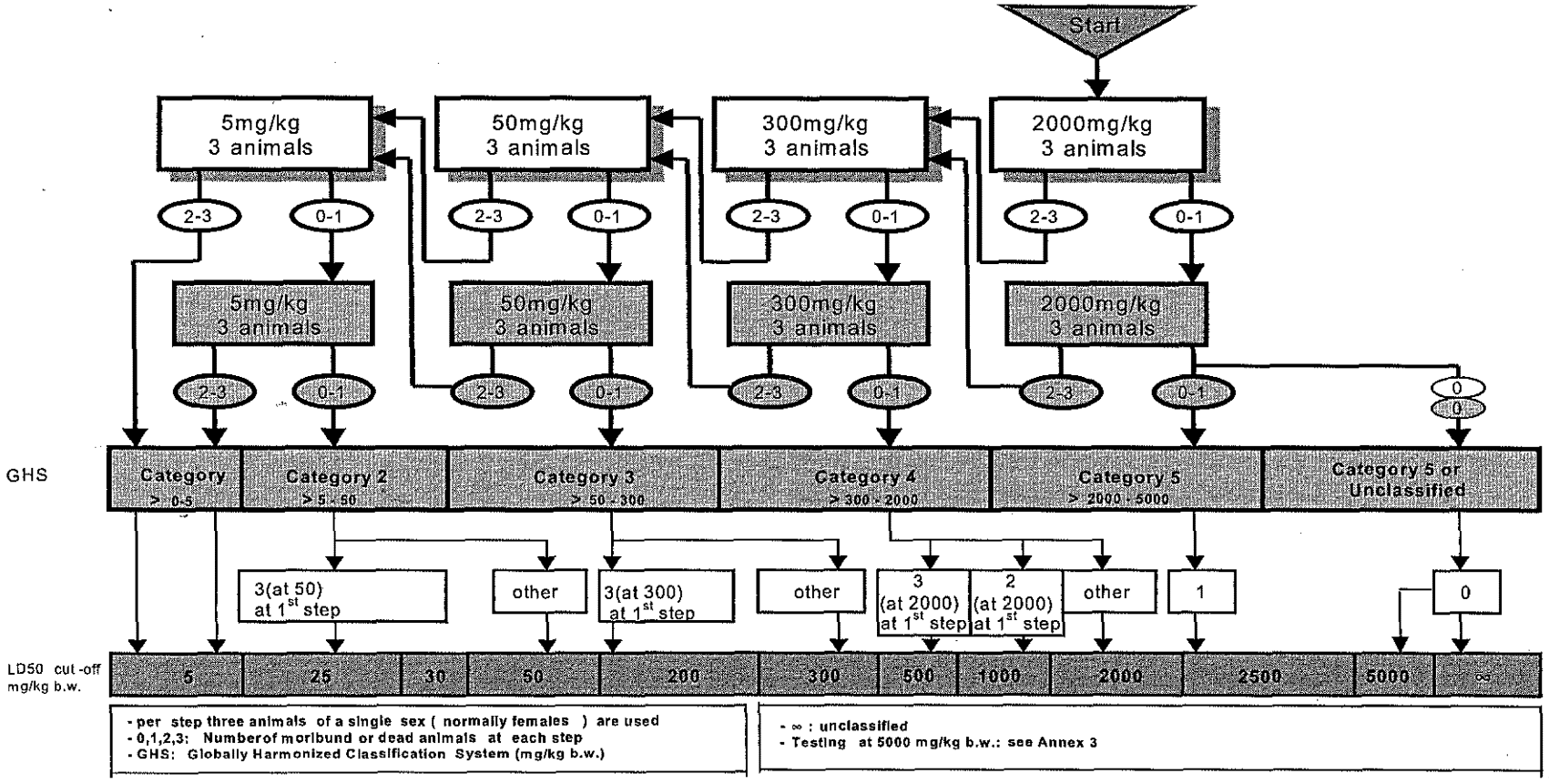
ANNEX 2b: TEST PROCEDURE WITH A STARTING DOSE OF 50 MG/KG BODY WEIGHT



ANNEX 2c: TEST PROCEDURE WITH A STARTING DOSE OF 300 MG/KG BODY WEIGHT



ANNEX 2d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



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ANNEX 3

CRITERIA FOR CLASSIFICATION OF TEST SUBSTANCES WITH EXPECTED LD50 VALUES EXCEEDING 2000 MG/KG WITHOUT THE NEED FOR TESTING

1. Criteria for hazard Category 5 are intended to enable the identification of test substances which are of relatively low acute toxicity hazard but which, under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD50 in the range of 2000-5000 mg/kg or equivalent doses for other routes. The test substance should be classified in the hazard category defined by: 2000mg/kg<LD50<5000mg/kg (Category 5 in the GHS) in the following cases:

a) If directed to this category by any of the testing schemes of Annex 2a-2d, based on mortality incidences;

b) if reliable evidence is already available that indicates the LD50 to be in the range of Category 5 values, or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature.

c) Through extrapolation, estimation or measurement of data if assignment to a more hazardous category is not warranted, and

- reliable information is available indicating significant toxic effects in humans, or
- any mortality is observed when tested up to Category 4 values by the oral route, or
- where expert judgement confirms significant clinical signs of toxicity, when tested up to Category 4 values, except for diarrhoea, piloerection or an ungroomed appearance, or
- where expert judgement confirms reliable information indicating the potential for significant acute effects from the other animal studies.

TESTING AT DOSES ABOVE 2000 MG/KG

2. Recognising the need to protect animal welfare, testing of animals in Category 5 (5000 mg/kg) ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health (10). No further testing should be conducted at higher dose levels.

3. When testing is required a dose of 5000mg/kg, only one step (i.e. three animals) is required. If the first animal dosed dies , then dosing procedes at 2000mg/kg in accordance with the flow charts in Annex 2. If the first animal survives, two further animals are dosed. If only one of the three animal dies , the LD50 value is expected to exceed 5000mg/kg. If both animals die, then dosing proceeds at 2000mg/kg.

Appendix M4

OECD FDP Test Guideline

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OECD/OCDE **420**

Adopted:
17th December 2001

OECD GUIDELINE FOR TESTING OF CHEMICALS

Acute Oral Toxicity – Fixed Dose Procedure

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 420 was adopted in July 1992 as the first alternative to the conventional acute toxicity test, described in Test Guideline 401. Based on the recommendations of several expert meetings, revision was considered timely because: i) international agreement had been reached on harmonised LD50 cut-off values for the classification of chemical substances, which differ from the cut-offs recommended in the 1992 version of the Guideline, and ii) testing in one sex (usually females) is now considered sufficient.

2. Traditional methods for assessing acute toxicity use death of animals as an endpoint. In 1984, a new approach to acute toxicity testing was suggested by the British Toxicology Society based on the administration at a series of fixed dose levels (1). The approach avoided using death of animals as an endpoint, and relied instead on the observation of clear signs of toxicity at one of a series of fixed dose levels. Following UK (2) and international (3) *in vivo* validation studies the procedure was adopted by the Council as a Test Guideline in 1992. Subsequently, the statistical properties of the Fixed Dose Procedure have been evaluated using mathematical models in a series of studies (4)(5)(6). Together, the *in vivo* and modelling studies have demonstrated that the procedure is reproducible, uses fewer animals and causes less suffering than the traditional methods and is able to rank substances in a similar manner to the other acute toxicity testing methods (Test Guidelines 423 and 425).

3. Guidance on the selection of the most appropriate test method for a given purpose can be found in the Guidance Document on Acute Oral Toxicity Testing (7). This Guidance Document also contains additional information on the conduct and interpretation of Guideline 420.

4. Definitions used in the context of this Guideline are set out in Annex 1.

INITIAL CONSIDERATIONS

5. It is a principle of the method that in the main study only moderately toxic doses are used, and that administration of doses that are expected to be lethal should be avoided. Also, doses that are known to cause marked pain and distress, due to corrosive or severely irritant actions, need not be administered. Moribund animals, or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document (8).

6. The method provides information on the hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity (9).

7. The testing laboratory should consider all available information on the test substance prior to conducting the study. Such information will include the identity and chemical structure of the substance; its physico-chemical properties; the results of any other *in vitro* or *in vivo* toxicity tests on the substance; toxicological data on structurally related substances; and the anticipated use(s) of the substance. This information is necessary to satisfy all concerned that the test is relevant for the protection of human health, and will help in the selection of an appropriate starting dose.

PRINCIPLE OF THE TEST

8. Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg (exceptionally an additional fixed dose of 5000 mg/kg may be considered, see paragraph 19). The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death, are described in detail in a separate OECD Guidance Document (8). Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose.

DESCRIPTION OF THE METHOD

Selection of animal species

9. The preferred rodent species is the rat, although other rodent species may be used. Normally females are used (7). This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between the sexes, but in those cases where differences are observed, females are generally slightly more sensitive (10). However, if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive then this sex should be used. When the test is conducted in males, adequate justification should be provided.

10. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within $\pm$ 20 % of the mean weight of any previously dosed animals.

Housing and feeding conditions

11. The temperature of the experimental animal room should be 22°C ($\pm$ 3°C). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

12. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to the start of dosing to allow for acclimatisation to the laboratory conditions.

Preparation of doses

13. In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, ie at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1mL/100g of body weight: however in the case of aqueous solutions 2 mL/100g body weight can be considered. With respect to the formulation of the dosing preparation, the use of an aqueous solution/suspension/emulsion is recommended wherever possible, followed in order of preference by a solution/suspension/emulsion in oil (e.g. corn oil) and then possibly solution in other vehicles. For vehicles other than water the toxicological characteristics of the vehicle should be known. Doses must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known and shown to be acceptable.

PROCEDURE

Administration of doses

14. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

15. Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night; with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period of time, it may be necessary to provide the animals with food and water depending on the length of the period.

Sighting study

16. The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study. The test substance is administered to single animals in a sequential manner following the flow charts in Annex 2. The sighting study is completed when a decision on the starting dose for the main study can be made (or if a death is seen at the lowest fixed dose).

17. The starting dose for the sighting study is selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg as a dose expected to produce evident toxicity based, when possible, on evidence from *in vivo* and *in vitro* data from the same chemical and from structurally related chemicals. In the absence of such information, the starting dose will be 300 mg/kg.

18. A period of at least 24 hours will be allowed between the dosing of each animal. All animals should be observed for at least 14 days.

19. Exceptionally, and only when justified by specific regulatory needs, the use of an additional upper fixed dose level of 5000 mg/kg may be considered (see Annex 4). For reasons of animal welfare concern, testing of animals in GHS Category 5 ranges (2000-5000mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.

20. In cases where an animal tested at the lowest fixed dose level (5mg/kg) in the sighting study dies, the normal procedure is to terminate the study and assign the substance to GHS Category 1 (as shown in Annex 2). However, if further confirmation of the classification is required, an optional supplementary procedure may be conducted, as follows. A second animal is dosed at 5mg/kg. If this second animal dies, then GHS Category 1 will be confirmed and the study will be immediately terminated. If the second animal survives, then a maximum of three additional animals will be dosed at 5mg/kg. Because there will be a high risk of mortality, these animals should be dosed in a sequential manner to protect animal welfare. The time interval between dosing each animal should be sufficient to establish that the previous animal is likely to survive. If a second death occurs, the dosing sequence will be immediately terminated and no further animals will be dosed. Because the occurence of a second death (irrespective of the number of animals tested at the time of termination) falls into outcome A (2 or more deaths), the classification rule of Annex 3 at the 5mg/kg fixed dose is followed (Category 1 if there are 2 or more deaths or Category 2 if there is no more than 1 death).

Main study

Numbers of animals and dose levels

21. The action to be taken following testing at the starting dose level is indicated by the flow charts in Annex 3. One of three actions will be required; either stop testing and assign the appropriate hazard classification class, test at a higher fixed dose or test at a lower fixed dose. However, to protect animals, a dose level that caused death in the sighting study will not be revisited in the main study (see Annex 3). Experience has shown that the most likely outcome at the starting dose level will be that the substance can be classified and no further testing will be necessary.

22. A total of five animals of one sex will normally be used for each dose level investigated. The five animals will be made up of one animal from the sighting study dosed at the selected dose level together with an additional four animals (except, unusually, if a dose level used on the main study was not included in the sighting study).

23. The time interval between dosing at each level is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. A period of 3 or 4 days between dosing at each dose level is recommended, if needed, to allow for the observation of delayed toxicity. The time interval may be adjusted as appropriate, e.g., in case of inconclusive response.

24. When the use of an upper fixed dose of 5000 mg/kg is considered, the procedure outlined in Annex 4 should be followed (see also paragraph 19).

Limit test

25. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested

compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations where there is little or no information about its toxicity, or in which the the test material is expected to be toxic, the main test should be performed.

26. Using the normal procedure, a sighting study starting dose of 2000mg/kg (or exceptionally 5000mg/kg) followed by dosing of a further four animals at this level serves as a limit test for this guideline.

OBSERVATIONS

27. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed (11). All observations are systematically recorded, with individual records being maintained for each animal.

28. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document should be taken into consideration (8). Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

Body weight

29. Individual weights of animals should be determined shortly before the test substance is administered and at least weekly thereafter. Weight changes should be calculated and recorded. At the end of the test surviving animals are weighed and then humanely killed.

Pathology

30. All test animals (including those that die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours after the initial dosing may also be considered because it may yield useful information.

DATA AND REPORTING

Data

31. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings.

Test report

32. The test report must include the following information, as appropriate:

Test substance:

- physical nature, purity, and, where relevant, physico-chemical properties (including isomerisation);
- identification data, including CAS number.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the animals, when known;
- number, age and sex of animals (including, where appropriate, a rationale for use of males instead of females);
- source, housing conditions, diet etc.

Test conditions:

- details of test substance formulation, including details of the physical form of the material administered;
- details of the administration of the test substance including dosing volumes and time of dosing;
- details of food and water quality (including diet type/source, water source);
- the rationale for the selection of the starting dose.

Results:

- tabulation of response data and dose level for each animal (i.e. animals showing signs of toxicity including mortality, nature, severity and duration of effects);
- tabulation of body weight and body weight changes;
- individual weights of animals at the day of dosing, in weekly intervals thereafter, and at time of death or sacrifice;
- date and time of death if prior to scheduled sacrifice;
- time course of onset of signs of toxicity and whether these were reversible for each animal;
- necropsy findings and histopathological findings for each animal, if available.

Discussion and interpretation of results.

Conclusions.

LITERATURE

(1) British Toxicology Society Working Party on Toxicity (1984). Special report: a new approach to the classification of substances and preparations on the basis of their acute toxicity. Human Toxicol., 3, 85-92.

(2) Van den Heuvel, M.J., Dayan, A.D. and Shillaker, R.O. (1987). Evaluation of the BTS approach to the testing of substances and preparations for their acute toxicity. Human Toxicol., 6, 279-291.

(3) Van den Heuvel, M.J., Clark, D.G., Fielder, R.J., Koundakjian, P.P., Oliver, G.J.A., Pelling, D., Tomlinson, N.J. and Walker, A.P. (1990). The international validation of a fixed-dose procedure as an alternative to the classical LD_{50} test. Fd. Chem. Toxicol. 28, 469-482.

(4) Whitehead, A. and Curnow, R.N. (1992). Statistical evaluation of the fixed-dose procedure. Fd. Chem. Toxicol., 30, 313-324.

(5) Stallard, N. and Whitehead, A. (1995). Reducing numbers in the fixed-dose procedure. Human Exptl. Toxicol. 14, 315-323.

(6) Stallard, N., Whitehead, A. and Ridgeway, P. (2000). Statistical evaluation of modifications to the fixed dose procedure (manuscript in preparation).

(7) OECD (2000). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No.24.

(8) OECD (2000). Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Environmental Health and Safety Monograph Series on Testing and Assesment No 19.

(9) OECD (1998). Harmonised Integrated Hazard Classification for Human Health and Environmental Effects of Chemical Substances as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals in November 1998, Part 2, p.11 [http://webnet1.oecd.org/oecd/pages/home/displaygeneral/0,3380,EN-documents-521-14-no-24-no-0,FF.html].

(10) Lipnick, R.L., Cotruvo, J.A., Hill, R.N., Bruce, R.D., Stitzel, K.A., Walker, A.P., Chu, I., Goddard, M., Segal, L., Springer, J.A. and Myers, R.C. (1995). Comparison of the Up-and-Down, Conventional LD_{50}, and Fixed-Dose Acute Toxicity Procedures. Fd. Chem. Toxicol. 33, 223-231.

(11) Chan P.K and A.W. Hayes (1994) Chapter 16 Acute Toxicity and Eye Irritation In: Principles and Methods of Toxicology. 3rd Edition. A.W. Hayes , Editor. Raven Press, Ltd. New York, USA.

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ANNEX 1

DEFINITIONS

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.

Delayed death means that an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period.

Dose is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of test animal (e.g. mg/kg).

Evident toxicity is a general term describing clear signs of toxicity following the administration of test substance, (see Van den Heuvel, M.J., Clark, D.G., Fielder, R.J., Koundakjian, P.P., Oliver, G.J.A., Pelling, D., Tomlinson, N.J. and Walker, A.P. (1990). The international validation of a fixed-dose procedure as an alternative to the classical LD_{50} test. Fd. Chem. Toxicol. 28, 469-482. (3) for examples) such that at the next highest fixed dose either severe pain and enduring signs of severe distress, moribund status (criteria are presented in the Humane Endpoints Guidance Document (8), or probable mortality in most animals can be expected.

GHS: Globally Harmonised Classification System for Chemical Substances and Mixtures. A joint activity of OECD (human health and the environment), UN Committee of Experts on Transport of Dangerous Goods (physical–chemical properties) and ILO (hazard communication) and co-ordinated by the Interorganisation Programme for the Sound Management of Chemicals (IOMC).

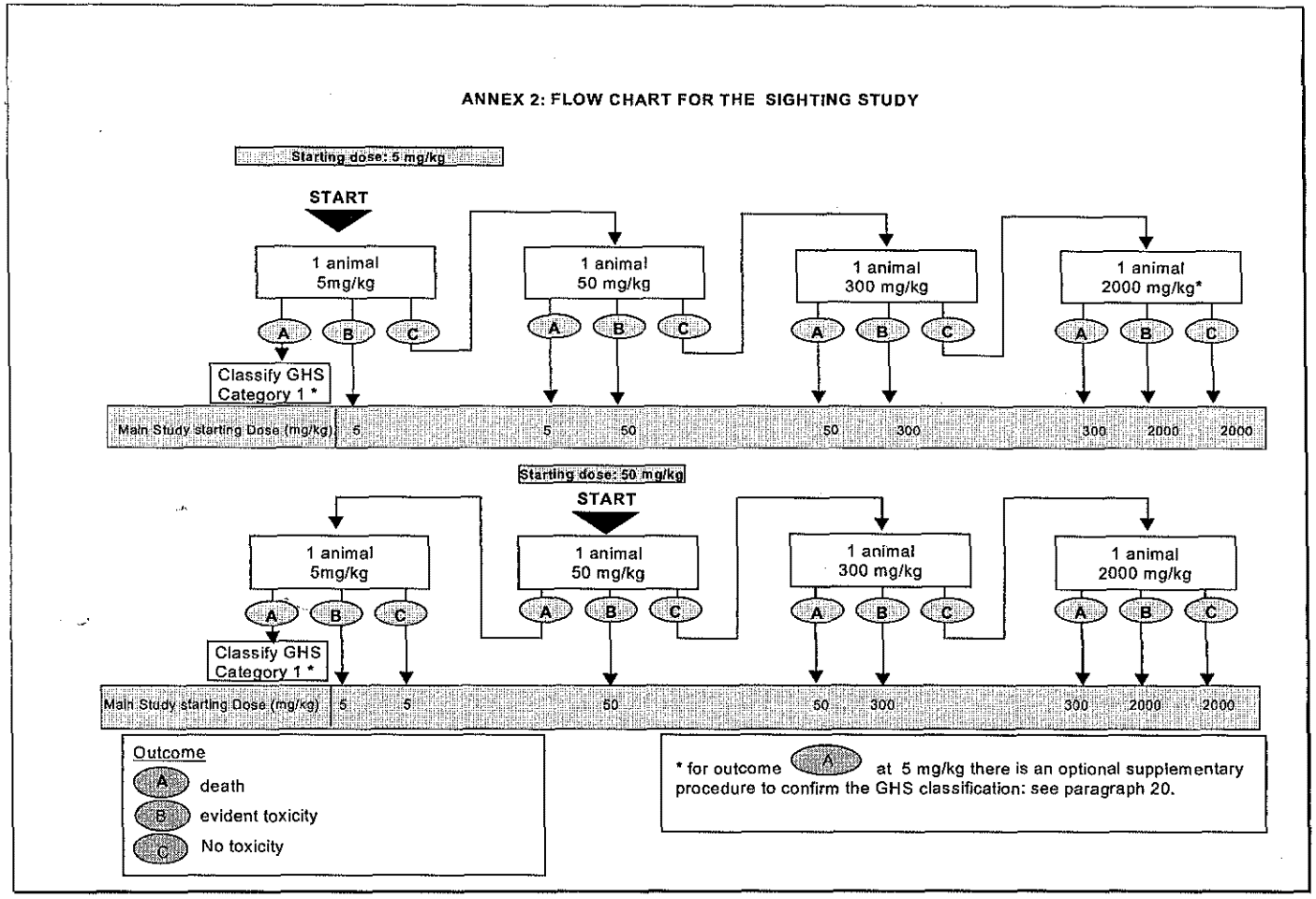
Impending death: when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, lateral position, recumbence, and tremor. (See the Humane Endpoint Guidance Document (8) for more details).

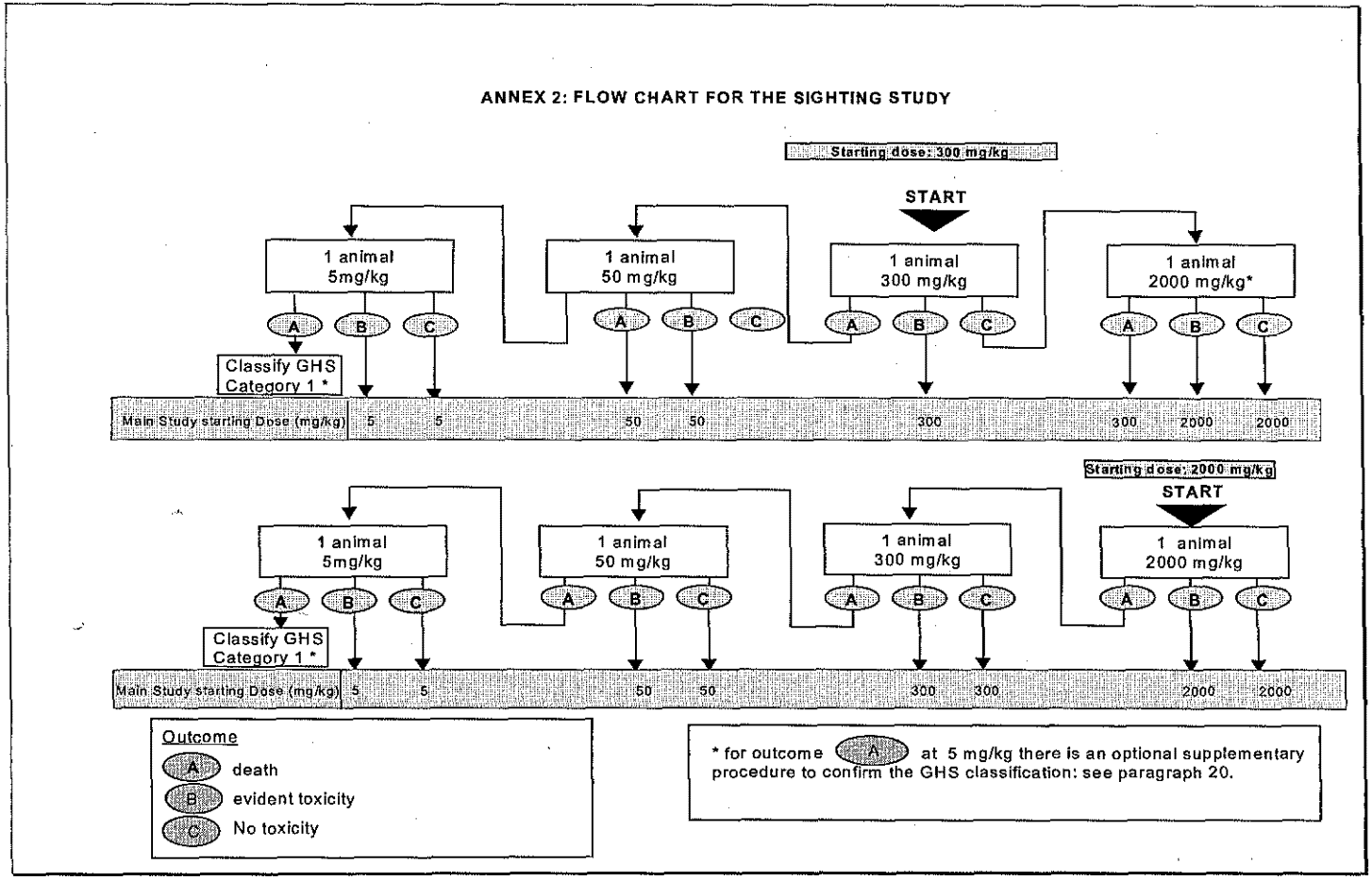
LD50 (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

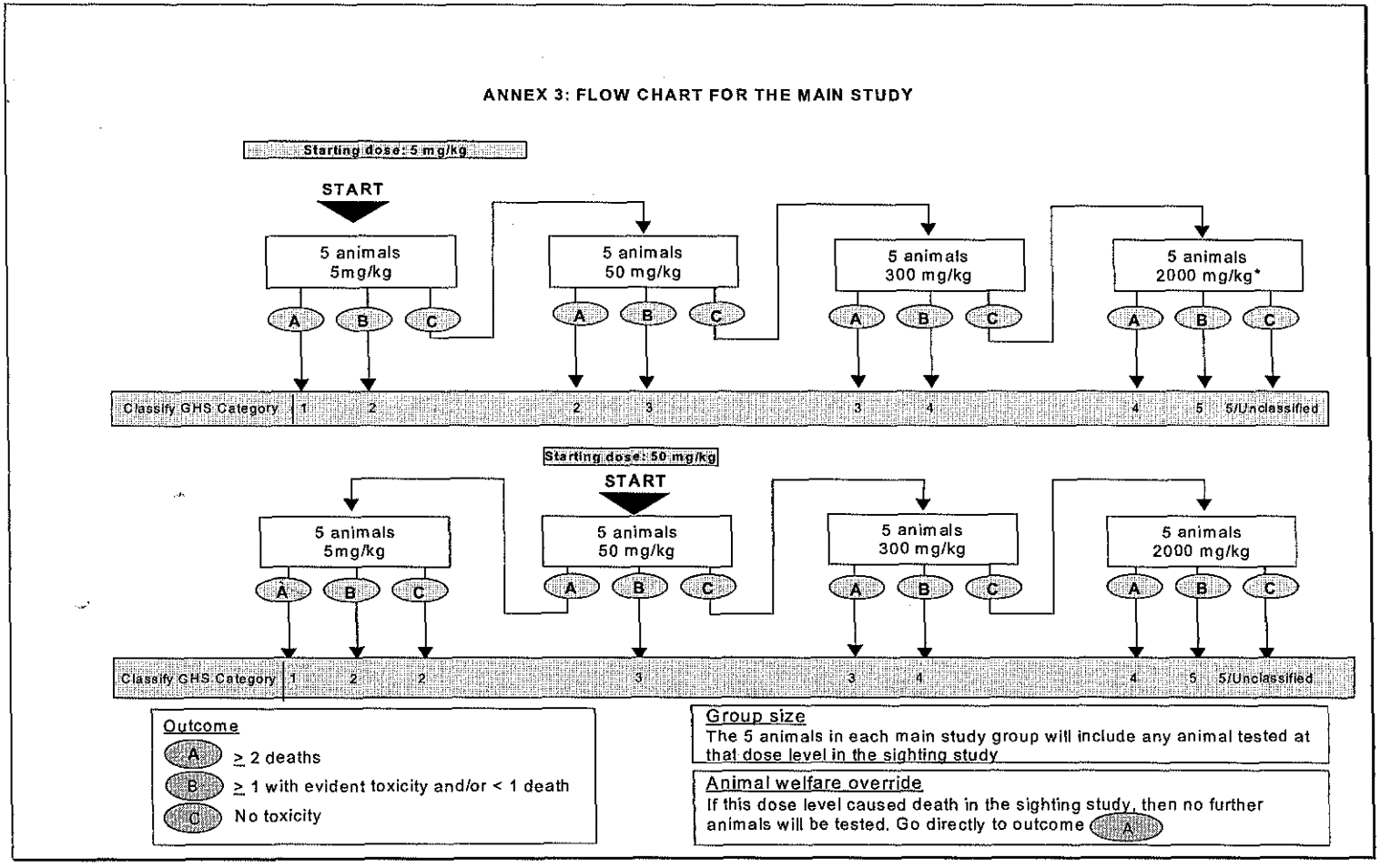
Limit dose refers to a dose at an upper limitation on testing (2000 or 5000 mg/kg).

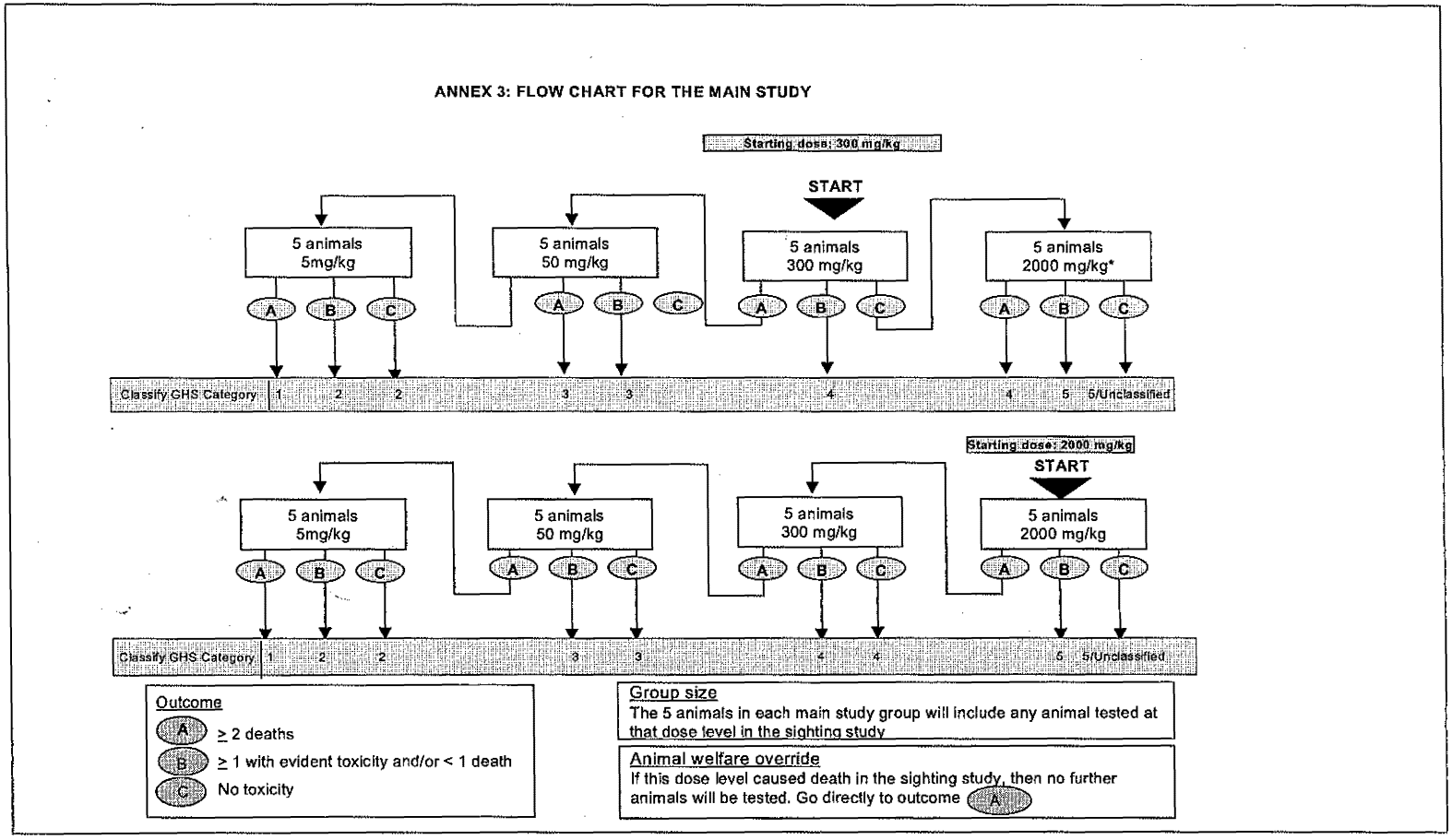
Moribund status: being in a state of dying or inability to survive, even if treated. (See the Humane Endpoint Guidance Document (8) for more details).

Predictable death: presence of clinical signs indicative of death at a known time in the future before the planned end of the experiment, for example: inability to reach water or food. (See the Humane Endpoint Guidance Document (8) for more details).


ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY
Starting dose: 5 mg/kg
START
1 animal 5mg/kg
1 animal 50 mg/kg
1 animal 300 mg/kg
1 animal 2000 mg/kg*
A
B
C
Classify GHS Category 1 *
Main Study starting Dose (mg/kg)
5
5
50
50
300
300
2000
2000
Starting dose: 50 mg/kg
START
1 animal 5mg/kg
1 animal 50 mg/kg
1 animal 300 mg/kg
1 animal 2000 mg/kg
Classify GHS Category 1 *
Main Study starting Dose (mg/kg)
5
5
50
50
300
300
2000
2000
Outcome
A death
B evident toxicity
C No toxicity
* for outcome A at 5 mg/kg there is an optional supplementary procedure to confirm the GHS classification: see paragraph 20.


ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY
Starting dose: 300 mg/kg
START
1 animal 5mg/kg
1 animal 50 mg/kg
1 animal 300 mg/kg
1 animal 2000 mg/kg*
A
B
C
Classify GHS Category 1 *
Main Study starting Dose (mg/kg)
5
5
50
50
300
300
2000
2000
Starting dose: 2000 mg/kg
START
1 animal 5mg/kg
1 animal 50 mg/kg
1 animal 300 mg/kg
1 animal 2000 mg/kg
Classify GHS Category 1 *
Main Study starting Dose (mg/kg)
5
5
50
50
300
300
2000
2000
Outcome
A death
B evident toxicity
C No toxicity
* for outcome A at 5 mg/kg there is an optional supplementary procedure to confirm the GHS classification: see paragraph 20.


ANNEX 3: FLOW CHART FOR THE MAIN STUDY
Starting dose: 5 mg/kg
START
5 animals 5mg/kg
5 animals 50 mg/kg
5 animals 300 mg/kg
5 animals 2000 mg/kg*
A
B
C
Classify GHS Category 1 2 2 3 3 4 4 5 5/Unclassified
Starting dose: 50 mg/kg
START
5 animals 5mg/kg
5 animals 50 mg/kg
5 animals 300 mg/kg
5 animals 2000 mg/kg
Classify GHS Category 1 2 2 3 3 4 4 5 5/Unclassified
Outcome
A ≥ 2 deaths
B ≥ 1 with evident toxicity and/or < 1 death
C No toxicity
Group size
The 5 animals in each main study group will include any animal tested at that dose level in the sighting study
Animal welfare override
If this dose level caused death in the sighting study, then no further animals will be tested. Go directly to outcome A


ANNEX 3: FLOW CHART FOR THE MAIN STUDY
Starting dose: 300 mg/kg
START
5 animals 5mg/kg
5 animals 50 mg/kg
5 animals 300 mg/kg
5 animals 2000 mg/kg*
A B C
Classify GHS Category 1 2 2 3 3 4 4 5 5/Unclassified
Starting dose: 2000 mg/kg
START
5 animals 5mg/kg
5 animals 50 mg/kg
5 animals 300 mg/kg
5 animals 2000 mg/kg
A B C
Classify GHS Category 1 2 2 3 3 4 4 6 5/Unclassified
Outcome
A ≥ 2 deaths
B ≥ 1 with evident toxicity and/or < 1 death
C No toxicity
Group size
The 5 animals in each main study group will include any animal tested at that dose level in the sighting study
Animal welfare override
If this dose level caused death in the sighting study, then no further animals will be tested. Go directly to outcome A

ANNEX 4

CRITERIA FOR CLASSIFICATION OF TEST SUBSTANCES WITH EXPECTED LD50 VALUES EXCEEDING 2000 MG/KG WITHOUT THE NEED FOR TESTING.

1. Criteria for hazard Category 5 are intended to enable the identification of test substances which are of relatively low acute toxicity hazard but which, under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD50 in the range of 2000-5000 mg/kg or equivalent doses for other routes. Test substances could be classified in the hazard category defined by: 2000mg/kg <LD50 < 5000mg/kg (Category 5 in the GHS) in the following cases:

a) if directed to this category by any of the testing schemes of Annex 3, based on mortality incidences;

b) if reliable evidence is already available that indicates the LD50 to be in the range of Category 5 values; or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature;

c) through extrapolation, estimation or measurement of data if assignment to a more hazardous category is not warranted and

- reliable information is available indicating significant toxic effects in humans, or
- any mortality is observed when tested up to category 4 values by the oral route, or
- where expert judgement confirms significant clinical signs of toxicity, when tested up to Category 4 values, except for diarrhoea, piloerection or an ungroomed appearance, or
- where expert judgement confirms reliable information indicating the potential for significant acute effects from the other animal studies.

TESTING AT DOSES ABOVE 2000 MG/KG

2. Exceptionally, and only when justified by specific regulatory needs, the use of an additional upper fixed dose level of 5000 mg/kg may be considered. Recognising the need to protect animal welfare, testing at 5000 mg/kg is discouraged and should only be considered when there is a strong likelihood that the results of such a test would have a direct relevance for protecting animal or human health (9).

Sighting Study

3. The decision rules governing the sequential procedure presented in Annex 2 are extended to include a 5000 mg/kg dose level. Thus, when a sighting study starting dose of 5000 mg/kg is used outcome A (death) will require a second animal to be tested at 2000 mg/kg; outcomes B and C (evident toxicity or no toxicity) will allow the selection of 5000 mg/kg as the main study starting dose. Similarly, if a starting dose other than 5000 mg/kg is used then testing will progress to 5000 mg/kg in the event of outcomes B or C at 2000 mg/kg; a subsequent 5000 mg/kg outcome A will dictate a main study starting dose of 2000 mg/kg and outcomes B and C will dictate a main study starting dose of 5000 mg/kg.

Main Study

4. The decision rules governing the sequential procedure presented in Annex 3 are extended to include a 5000 mg/kg dose level. Thus, when a main study starting dose of 5000 mg/kg is used, outcome A (≥2 deaths) will require the testing of a second group at 2000 mg/kg; outcome B (evident toxicity and/or ≤1 death) or C (no toxicity) will result in the substance being unclassified according to GHS.

Similarly, if a starting dose other than 5000 mg/kg is used then testing will progress to 5000 mg/kg in the event of outcome C at 2000 mg/kg; a subsequent 5000 mg/kg outcome A will result in the substance being assigned to GHS Category 5 and outcomes B or C will lead to the substance being unclassified.

Appendix M5

OECD Guidance on Acute Oral Toxicity Testing

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Unclassified **ENV/JM/MONO(2001)4**

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

23-Jul-2001

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

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GUIDANCE DOCUMENT ON ACUTE ORAL TOXICITY TESTING

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OECD Environment, Health and Safety Publications

Series on Testing and Assessment

N° 24

GUIDANCE DOCUMENT ON ACUTE ORAL TOXICITY TESTING

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris

June 2001

Also published in the Series on Testing and Assessment

No. 1, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals (1993; reformatted 1995)*

No. 2, *Detailed Review Paper on Biodegradability Testing (1995)*

No. 3, *Guidance Document for Aquatic Effects Assessment (1995)*

No. 4, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment (1995)*

No. 5, *Report of the SETAC/OECD Workshop on Avian Toxicity Testing (1996)*

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No. 15, *Detailed Review Document on Classification Systems for Reproductive Toxicity in OECD Member Countries (1998)*

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No. 19, *Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation (2000)*

No. 20, *Revised Draft Guidance Document for Neurotoxcity Testing (2000)*

No. 21, *Detailed Review Paper: Appriasal of Test Methods for Sex Disrupting Chemicals (2000)*

No. .22, *Guidance Document for the Performance of Out-door Monolith Lysimeter Studies (2000)*

No. 23, *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (2000)*

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 29 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised Committees and subsidiary groups composed of Member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's Workshops and other meetings. Committees and subsidiary groups are served by the OECD Secretariat, located in Paris, France, which is organised into Directorates and Divisions.

The work of the OECD related to chemical safety is carried out in the **Environment, Health and Safety Programme**. As part of its work on chemical testing, the OECD has issued several Council Decisions and Recommendations (the former legally binding on Member countries), as well as numerous Guidance Documents and technical reports. The best known of these publications, the **OECD Test Guidelines**, is a collection of methods used to assess the hazards of chemicals and of chemical preparations such as pesticides. These methods cover tests for physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment. The OECD Test Guidelines are recognised world-wide as the standard reference tool for chemical testing.

More information about the Environment, Health and Safety Programme and its publications (including the Test Guidelines) is available on the OECD's World Wide Web site (see next page).

The Environment, Health and Safety Programme co-operates closely with other international organisations. This document was produced within the framework of the Inter-Organisation Programme for the Sound Management of Chemicals (IOMC).

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organisations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. UNITAR joined the IOMC in 1997 to become the seventh Participating Organisation. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The conventional acute oral toxicity test (formerly OECD Test Guideline 401) is the most heavily criticised test in terms of animal welfare and this concern was the driving force behind the development of three alternative tests for acute oral toxicity (Test Guideline 420, 423, 425). Anticipating the presence of validated alternatives, Member countries took the initiative to plan the deletion of Guideline 401.

2. A Nominated Expert Meeting (Rome 1998) and an Expert Consultation Meeting, (Arlington 1999) were convened to determine the acute oral toxicity data requirement needs of Member countries and to assess the capabilities of the alternatives to meet these needs. On the basis of these technical discussions, the 29th Joint Meeting concluded in June 1999 that not all data needs could be met by the alternatives (and not always by Guideline 401). The Joint Meeting decided that Guidelines 420, 423 and 425 should be revised to meet regulatory needs of the Member countries including, where possible, the provision of confidence intervals and the slope of the dose response curve, to support classification and assessment of acute toxicity at 5 and at 5000 mg/kg, and should include the use of a single sex, appropriate statistical methods and, to the extent feasible, a reduction in the number of animals used and the introduction of refinements to reduce the pain and distress of the animals. The guidelines should also be able to allow the classification of substances according to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity (1).

3. The revision of Guidelines 420, 423 and 425 was completed in 2000 following a second Expert Consultation Meeting (Paris, 2000) and the process of deletion of guideline 401 was started.

PURPOSE

4. The purpose of this Guidance Document is to provide information for both the regulated community and regulators to assist with the choice of the most appropriate Guideline to enable particular data requirements to be met while reducing the number of animals used and animal suffering. The Guidance Document also contains additional information on the conduct and interpretation of Guidelines 420, 423 and 425.

DATA NEEDS

5. Acute oral toxicity data are used to satisfy hazard classification and labelling requirements, for risk assessment for human health and the environment, and when estimating the toxicity of mixtures. The provision of either a point estimate of the LD_{50} value or range estimate of the LD_{50} generally meets the acute oral toxicity data requirements for classification for all regulatory authorities in the areas of industrial chemicals, consumer products and for many pesticide applications. OECD document "Revised Analysis of Responses Received from Member Countries to the Questionnaire on Data Requirements for Acute Oral Toxicity" provides an overview of acute toxicity data requirements applicable in 1999 (2). The data needs of the majority of Member countries can also be met with the imposition of a limit dose of 2000 mg/kg. However, several countries have a requirement for information on toxicity at dose levels in the range 2000 to 5000 mg/kg for substances with LD_{50} values in excess of 2000 mg/kg. Although many authorities find it acceptable to use data from observations made at doses of 2000 mg/kg or below, as

described in the GHS classification criteria (which includes a 2000-5000 mg/kg category), testing in this range may be necessary to meet the needs of a few regulatory authorities. For example, some authorities regulating consumer products and pesticides need a point estimate of LD_{50} and confidence intervals, and information on toxicity at levels up to or above 5000 mg/kg. These authorities use LD_{50} data in this way for assessment of risk to humans and also for risk assessments for environmental effects to avoid the need for further animal studies on pesticide products. Furthermore, at least one country has a need for a test at 5000 mg/kg for biological and safer pesticides and products to which the general public are exposed, to provide characterisation of acute toxicity and to support bridging across data sets for structurally related substances, again to eliminate or minimise the requirements for additional animal testing. For reasons of animal welfare concern, testing of animals in GHS category 5 ranges (2000-5000mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.

6. Some national and international regulatory systems estimate the toxicity of mixtures from calculations using weighted averages of the LD_{50} point estimate of the components when actual data on the mixture are not available. The resulting calculated toxicity values are used for hazard classification of mixtures. A dose response curve is also sometimes needed for extrapolation and a reliable identification of hazard and risk posed by mixtures, to avoid testing each mixture and thus to allow a significant saving of animal use. At present, agreed approaches for estimating the toxicity of mixtures using range data are only accepted in the EU and in some other countries. However, the OECD Expert Group on Hazard Classification Criteria for Mixtures has recently agreed that mixtures can be classified using either point or range estimates of the LD50 of each component (3).

7. Acute oral toxicity testing by OECD methods is not required for pharmaceuticals. Pharmaceutical methods are specified by the International Committee on Harmonisation (ICH). In some specific cases such as imaging and antineoplastic agents, estimates of acute toxicity are needed to support single dose studies in man. These studies call for testing to fully characterise the toxicity in the low toxicity region and may involve doses above 2000 mg/kg. However, the study designs for these special purpose studies are different from any of the current OECD acute toxicity guidelines.

COMPARISON OF GUIDELINES 420, 423 AND 425

Outline Of The Methodology

8. All of the guidelines involve the administration of a single bolus dose of test substance to fasted healthy young adult rodents by oral gavage, observation for up to 14 days after dosing, recording of body weight and the necropsy of all animals. Doses may be administered based on a constant volume or a constant concentration depending upon the needs of the toxicologist and the regulatory authorities. Some authorities prefer that substances sold to the public should be tested as constant concentration unless the volumes are too small to administer accurately. Since the effects at the same dose may be different if the materials are diluted, it is important for the toxicologist to consider how the information will be used. If the material will primarily be used diluted in mixtures, then constant volume may be appropriate. On the other hand, if the material is to be used neat, particularly if it may be irritating, the use of constant concentration will be more appropriate (4)(5).

9. Each animal should be selected from the available animals in a random fashion on the day of dosing. In recognition of the fact that most animal suppliers do not indicate littermates, the guidelines do not call for randomizing animals from a single litter across dose groups. Females should be nulliparous

and non-pregnant. At the commencement of its dosing, each animal should be between 8 and 12 weeks old and its weight should fall in an interval within ±20% of the mean weight of all previously dosed animals taken on their day of dosing. As the mean weight will increase as the animals age, this method tends to correct for the change in animals weights with time. In order to conform to these age and weight requirements at the start of dosing of each animal, it may be necessary to order animals sequentially as the tests can sometimes take several weeks to complete. The primary endpoint for Guidelines 423 and 425 is mortality, but for Guideline 420 it is the observation of clear signs of toxicity (termed: evident toxicity).

10. **Guideline 420:** A sighting study is included for Guideline 420 in order to choose an appropriate starting dose and to minimise the number of animals used. Pre-specified fixed doses of 5, 50, 300 or 2000 mg/kg are used both in the sighting study and the main study. There is an option to use an additional dose level of 5000 mg/kg, but only when justified by a specific regulatory need. Groups of animals are dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce some signs of toxicity. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence of signs of toxicity, until the study objective is achieved; that is, the classification of the test substance based on the identification of the dose(s) causing evident toxicity, except when there are no effects at the highest fixed dose.

11. **Guideline 423:** Pre-specified fixed doses of 5, 50, 300 or 2000 mg/kg are used. There is an option to use an additional dose level of 5000 mg/kg, but only when justified by a specific regulatory need. Groups of animals are dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce mortality in some animals. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence of mortality, until the study objective is achieved; that is, the classification of the test substance based on the identification of the dose(s) causing mortality, except when there are no effects at the highest fixed dose.

12. **Guideline 425:** This is also a stepwise procedure, but uses single animals, with the first animal receiving a dose just below the best estimate of the LD_{50}. Depending on the outcome for the previous animal, the dose for the next is increased or decreased, usually by a factor of 3.2. This sequence continues until there is a reversal of the initial outcome (i.e., the point where an increasing dose results in death rather than survival, or decreasing dose results in survival rather than death); then, additional animals are dosed following the up-down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then no more than a specified number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/kg should be taken only when justified by a specific regulatory need.

Animal Welfare Considerations

13. All three Guidelines provide significant improvements in the number of animals used in comparison to Guideline 401, which required 20 animals in a test at least. In addition, they all contain a requirement to follow the OECD Guidance Document on Humane Endpoints (6) which should reduce the overall suffering of animals used in this type of toxicity test. Furthermore, Guideline 420 has as its endpoint evident toxicity rather than mortality and uses a sighting study to minimize the numbers of animals and Guideline 425 has a stopping rule which limits the number of animals in a test.

14. **Guideline 420:** Groups of five young adult animals of one sex are dosed per step in the main study. Single animals are used per step in the sighting study. Regulatory experience and statistical modelling has shown that most tests are likely to be completed with either one or two sighting study steps and one main study step, thus using between 5 and 7 animals. Up to 5 animals are used in a limit test.

15. **Guideline 423:** This test uses groups of 3 animals of one sex per step. Regulatory use of this Guideline demonstrates that the average number of animals used is 7. Up to 6 animals are used in a limit test.

16. **Guideline 425:** This test uses single animals of one sex. Statistical modelling indicates that the average number of animals used in this test is about 6-9. Up to 5 animals are used in a limit test.

17. The following estimates of the number of treatment related deaths for tests conducted on substances with LD_{50} values below 5000 mg/kg are based on practical experience and validation studies using earlier versions of these guidelines and statistical modelling.

- **Guideline 420:** typically 1 animal can be expected to die on test.
- **Guideline 423:** 2-3 animals per test can be expected to die in a full test.
- **Guideline 425:** the expected number of deaths is between 2 and 3.

18. For all three guidelines, careful clinical observations should be made at least twice on the day of dosing or more frequently when indicated by the response of the animals to the treatment, and at least once daily thereafter. Additional observations are made if the animals continue to display signs of toxicity. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Guidance on clinical signs can be found in Chan and Hayes (5). Animals that are moribund or suffering severe pain and distress must be humanely killed. Guidance on clinical signs and objective measurements that are indicative of impending death and/or severe pain and/or distress is available in an OECD Guidance Document (6). Humanely killed animals are considered in the interpretation of the results in the same way as animals that died on test.

Information Provided By Each Method

19. Test Guidelines 420 and 423 provide a range estimate of the LD_{50}; the ranges are defined by cut-off values of the applied classification system and not as a calculated lower and upper level. In the case of Test Guideline 420 this range is inferred from the fixed dose which produces evident toxicity. Guideline 425 provides a point-estimate of the LD_{50} value with confidence intervals.

20. The results of tests conducted according to Guideline 425 will allow a test substance to be classified according to all the systems in current use, including the new GHS. Test Guidelines 420 and 423 have now been revised to allow classification according to the new GHS. However, in order to cover the transition period until the global implementation of the GHS both Guidelines also allow classification according to existing systems as shown in Annex 1 and 2.

Limitations Of The Methods

21. Validations against actual data and statistical simulations identified areas where all three methods may have outcomes which result in a more or less stringent classification than that based on the "true" LD_{50} value (as obtained by the deleted guideline 401). Comparative statistical analysis (see Annex 3) indicated that all are likely to perform poorly for chemicals with shallow dose-response slopes. For all methods, the study outcome is likely to be influenced by the choice of starting dose level(s), relative to the "true" LD_{50} value, especially in the case of shallow slopes. Because Guideline 420 uses evident toxicity as

an endpoint instead of death, information on toxic effects seen only at dose levels close to a lethal dose will not always be obtained (7).

22. Unusually test substances may cause delayed deaths (5 days or more after test substance administration). Substances which cause delayed deaths have an impact on the practicality of conducting a study to Guideline 425 where the duration of testing will be significantly longer compared with other test methods. However, both in Guideline 420 and 423, the finding of a delayed death may require additional lower dose levels to be used or a study to be repeated.

OPTIMISING THE PERFORMANCE OF THE TEST

23. Each guideline provides procedures to assist in selecting the starting dose, particularly in the event that minimal prior information on the substance itself is available. All available information on the test substance must be made available to the testing laboratory and should be considered prior to conducting the study. Such information will include, for example, the identity and chemical structure of the substance; its physico-chemical properties; the result of any other *in vivo* or *in vitro* toxicity tests on the substance; toxicological data on structurally related substances; the anticipated use(s) of the substance; and the likely regulatory data requirements. This information is necessary to satisfy all concerned that the test is relevant for the protection of human and animal health and mammalian wildlife, to select the most appropriate test to satisfy regulatory requirements and will help in the selection of the starting dose.

24. For all three methods the efficiency of the test, in terms of reliability and numbers of animals used, is optimised by the choice of a starting dose close to (423) or just below (425) the actual LD_{50} or the lowest dose producing evident toxicity (420). When this type of information is not available, all three Guidelines include advice on the starting dose level which should be used to minimise the possibility of biased outcome and adverse effects on animal welfare. As a general principle it is suggested that a starting dose is selected that is slightly lower than the best estimate of the LD_{50} based on available evidence.

25. The limit test is an efficient way to characterise substances of low toxicity when there is sufficient information available indicating that the toxic dose is higher that the limit dose. Each method provides a limit test suitable to the design of the main study. A Limit Test should be conducted only when there are strong indications that the test substance is of low or negligible acute toxicity.

USE OF A SINGLE SEX

26. Guidelines 420, 423 and 425 are conducted using a single sex in order to reduce variability and as a means of minimising the number of animals used. Normally females are used. This is because literature surveys of conventional LD_{50} tests show that usually there is little difference in sensitivity between the sexes but, in those cases where differences were observed, females were generally slightly more sensitive (8). Although the use of a single sex (females) also contributes to a further decrease in the use of animals in testing, theoretically this may lead to an oversupply of the other sex (males). However, currently the use of males in experimental animal tests clearly exceeds that of females and, thus, the preference for females in acute toxicity testing may well result in a better overall balance of the use of both genders. For chemicals which are direct acting in their toxic mechanism, this may be because female rats have a lower detoxification capacity than males, as measured by specific activity of phase I and II enzymes. However, all available information should be evaluated, for example on chemical analogues and the results of testing for other toxicological endpoints on the chemical itself, as this may indicate that

males may be more sensitive than females. Knowledge that metabolic activation is required for a chemical's toxicity can also indicate that males may be the more sensitive sex.

27. Occasionally, the results of subsequent testing, for example a sub-chronic test, may raise concerns that the more sensitive sex had not been used. In such cases, and only when considerable differences between the sexes are suspected, it may be necessary to conduct another full acute oral toxicity study in the second sex. This is preferable to conducting confirmatory testing in a small group of animals of the second sex as a late satellite to the original test because there is a strong possibility that this would produce results that are difficult to interpret. The impact of conducting a second full test on the overall number of animals used in acute toxicity testing should be small because re-testing is anticipated to be infrequent and the results of the test in one sex, together with data from any subsequent studies, will greatly assist in the selection of starting doses closer to the LD50 in the second test.

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(7) Van der Heuvel, M.J., Clark, D.G., Fielder, R.J., Koundakjian, P.P., Olivier, G.J.A., Pelling, D., Tomlinson, N.J. and Walker, A.P. (1990). The international validation of a fixed-dose procedure as an alternative to the classical LD_{50} test. Fd. Chem. Toxicol. 28, 469-482.

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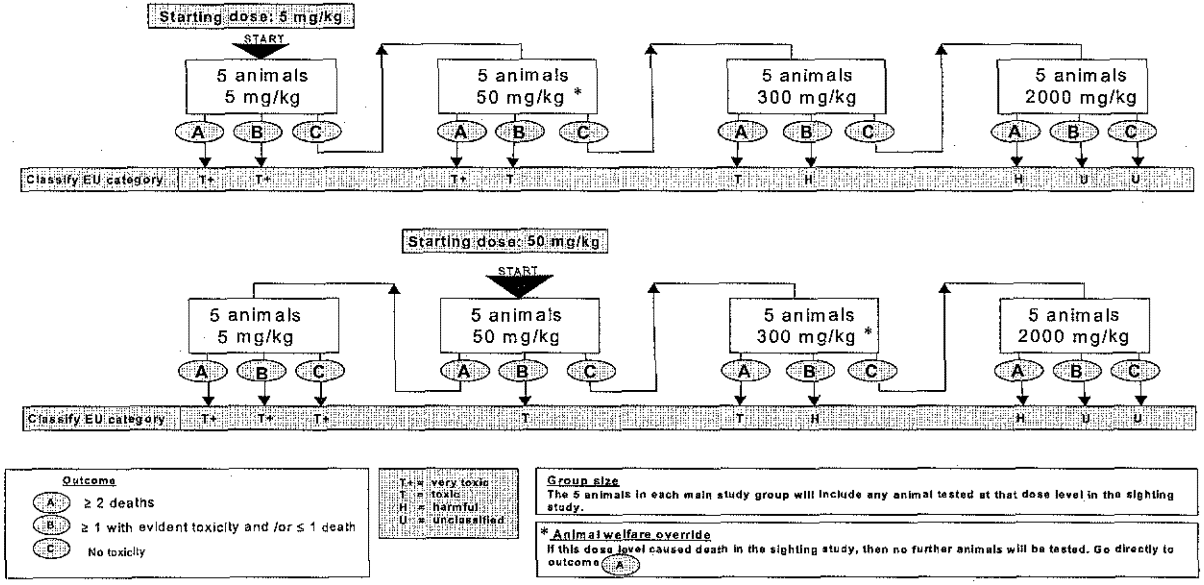
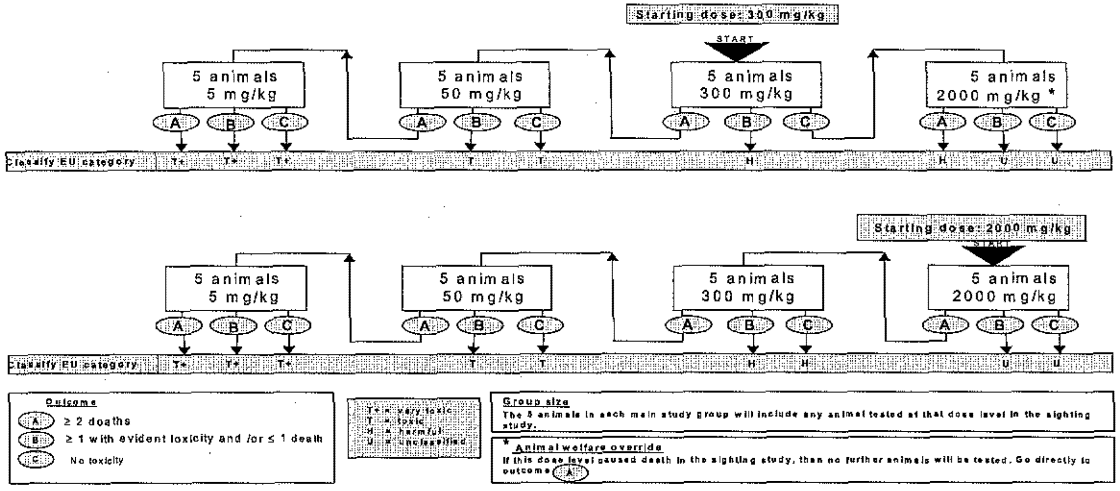
ANNEX 1

TEST GUIDELINE 420 MAIN STUDY: CLASSIFICATION ACCORDING TO THE CURRENTLY STILL APPLICABLE EU SCHEME TO COVER THE TRANSITION PERIOD UNTIL FULL IMPLEMENTATION OF THE GLOBALLY HARMONISED CLASSIFICATION SYSTEM (GHS)



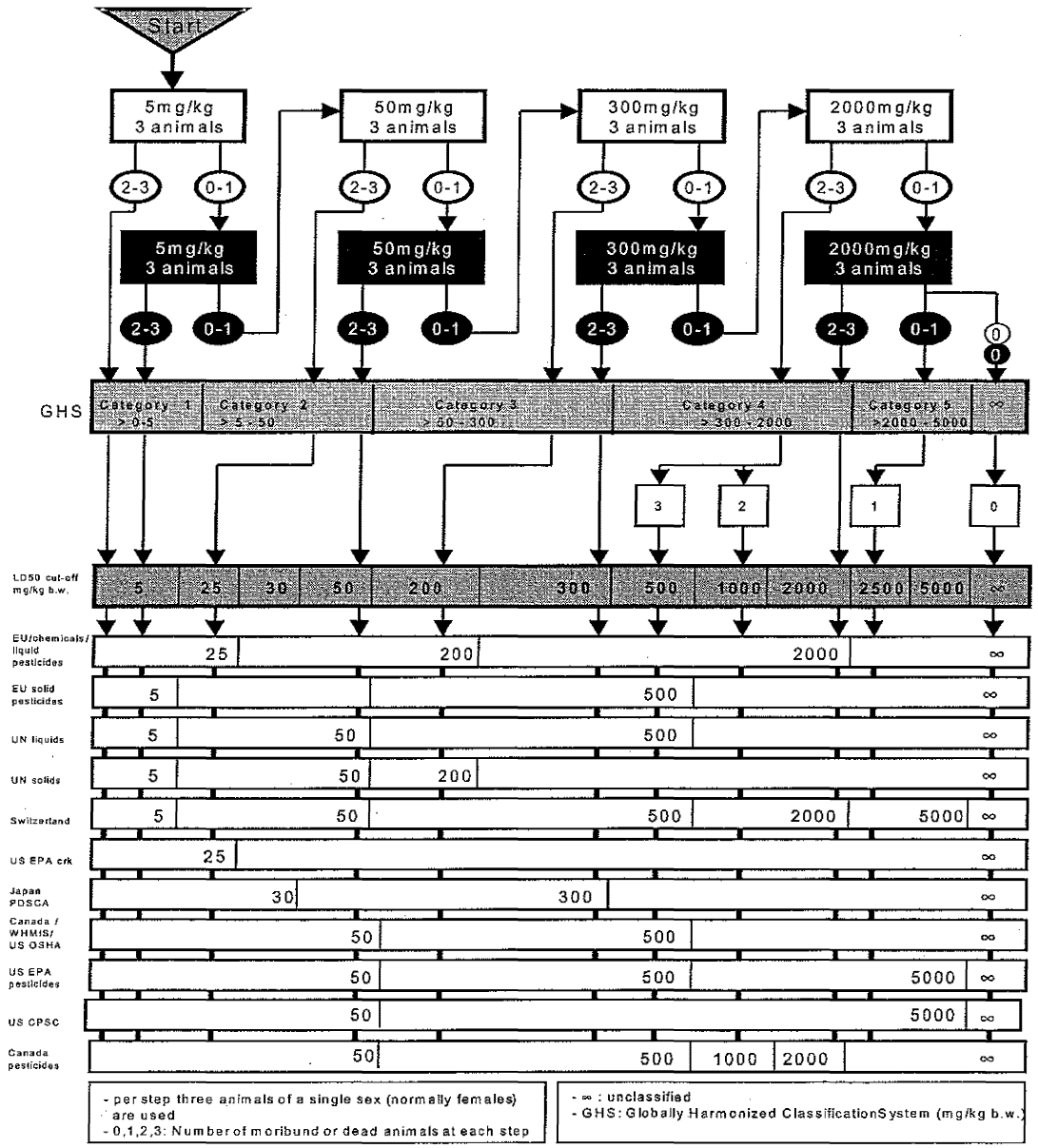
ANNEX 1 (continued)

TEST GUIDELINE 420 MAIN STUDY: CLASSIFICATION ACCORDING TO THE CURRENTLY STILL APPLICABLE EU SCHEME TO COVER THE TRANSITION PERIOD UNTIL FULL IMPLEMENTATION OF THE GLOBALLY HARMONISED CLASSIFICATION SYSTEM (GHS)



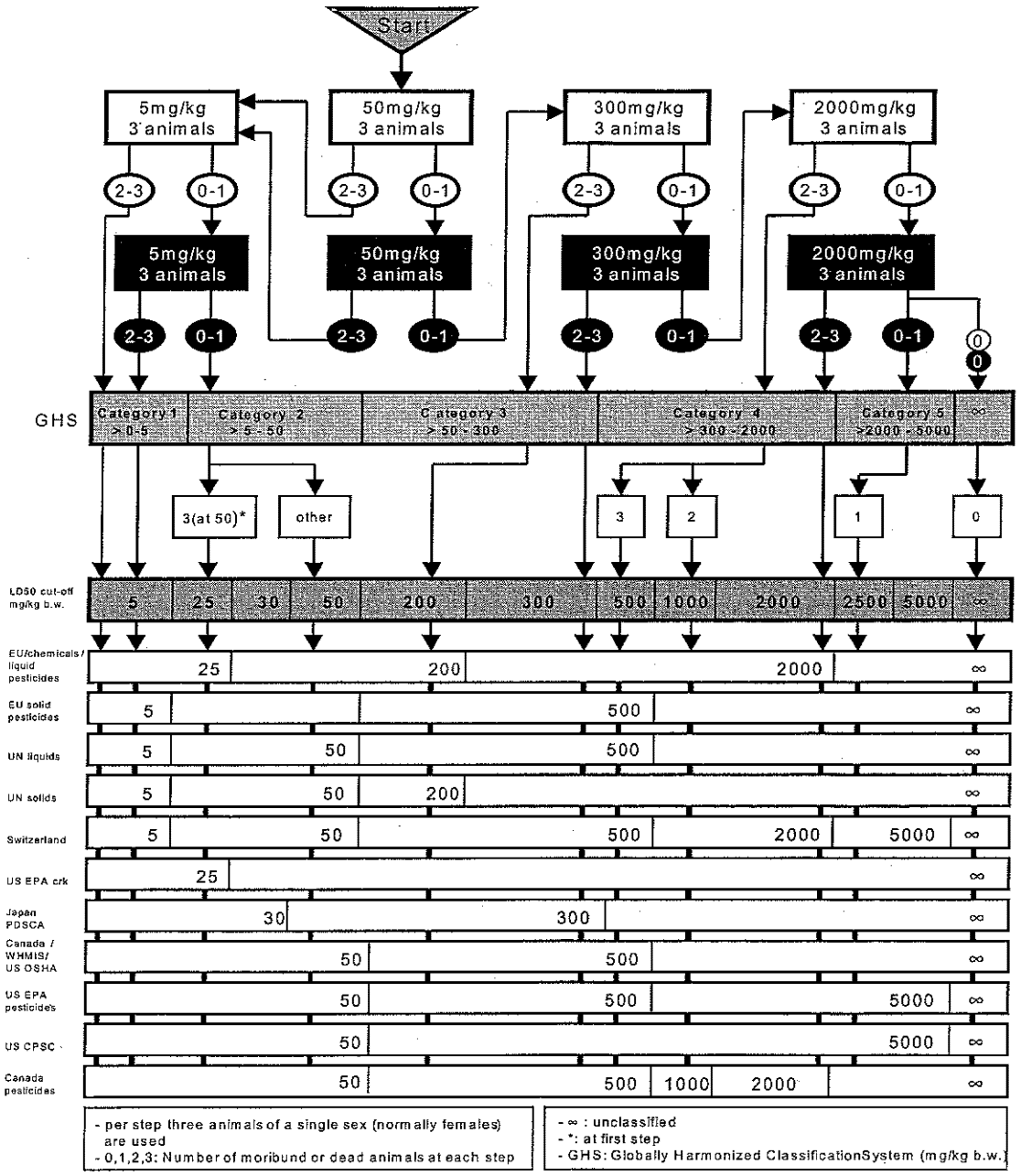
ANNEX 2

TEST GUIDELINE 423: CLASSIFICATION ACCORDING TO CURRENTLY STILL APPLICABLE CLASSIFICATION SCHEMES TO COVER THE TRANSITION PERIOD UNTIL FULL IMPLEMENTATION OF THE GLOBALLY HARMONISED CASSIFICATION SYSTEM (GHS)



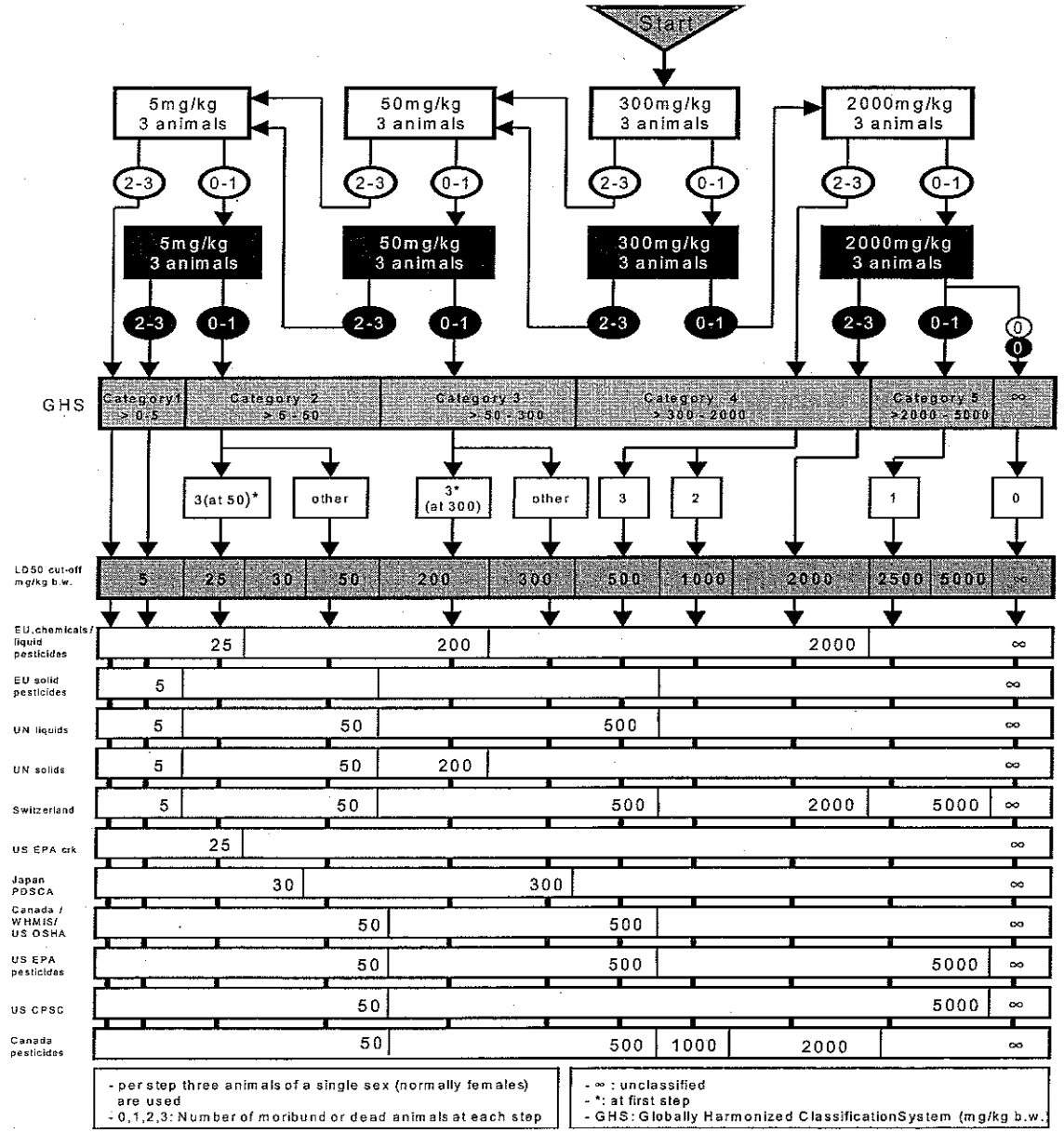
ANNEX 2 (continued 1)

TEST GUIDELINE 423: CLASSIFICATION ACCORDING TO CURRENTLY STILL APPLICABLE CLASSIFICATION SCHEMES TO COVER THE TRANSITION PERIOD UNTIL FULL IMPLEMENTATION OF THE GLOBALLY HARMONISED CASSIFICATION SYSTEM (GHS)



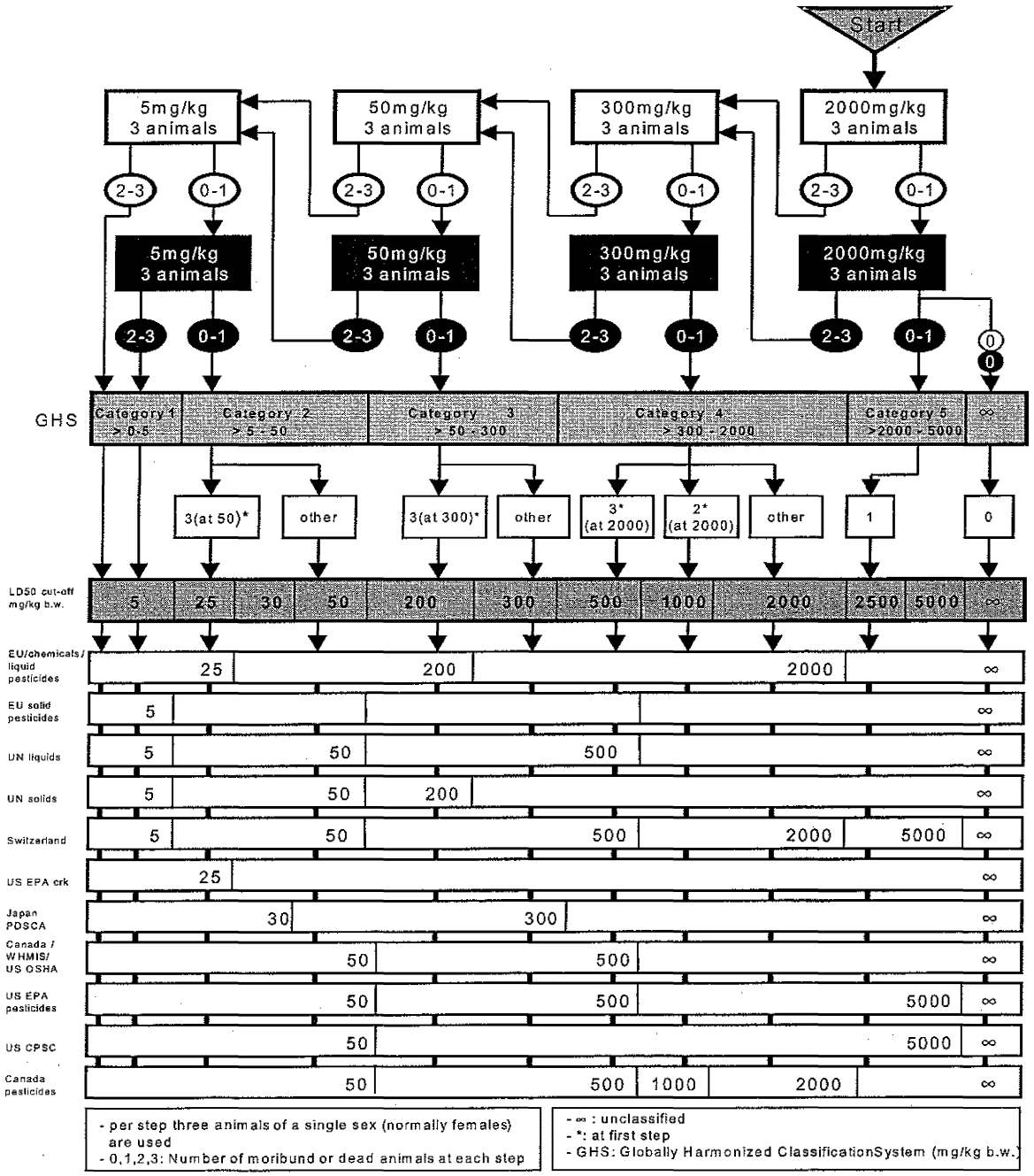
ANNEX 2 (continued 2)

TEST GUIDELINE 423: CLASSIFICATION ACCORDING TO CURRENTLY STILL APPLICABLE CLASSIFICATION SCHEMES TO COVER THE TRANSITION PERIOD UNTIL FULL MPLEMENTATION OF THE GLOBALLY HARMONISED CASSIFICATION SYSTEM (GHS)



ANNEX 2 (continued 3)

TEST GUIDELINE 423: CLASSIFICATION ACCORDING TO CURRENTLY STILL APPLICABLE CLASSIFICATION SCHEMES TO COVER THE TRANSITION PERIOD UNTIL FULL IMPLEMENTATION OF THE GLOBALLY HARMONISED CASSIFICATION SYSTEM (GHS)



ANNEX 3

STATISTICAL BASIS FOR ESTIMATING ACUTE ORAL TOXICITY COMPARISON OF OECD GUIDELINES 420, 423 AND 425

INTRODUCTION

1. This document describes the statistical strengths and limitations of the various methods for accurately determining a point estimate of the LD_{50}, confidence limits around the point estimate of LD_{50}, and information on the dose-effect response. In this context, a dose-response curve applies to the estimation of lethality and a dose-effect response applies to the estimation of the change in the variety and distribution of all other types of toxicological signs with the change in dose. By design not all of the guidelines will provide estimates for all of these endpoints. This document allows the reader to quickly identify the tests that will meet his or her particular needs.

2. The statistical basis for all test methods is that lethality is a quantal response. Its measurement will give rise to a frequency distribution of responses reflecting the composite tolerances of the test population upon exposure to graded doses of the test chemical. In practice, most chemicals give rise to an approximately lognormal distribution of deaths versus dose, skewed toward hypersensitivity. When this frequency population is transformed to a logarithmic abscissa, a (symmetric) normal distribution generally results that can be characterized by two parameters, the median and the standard deviation, SD. The median is the dose at which 50% of the animals are killed by the test chemical and is called the LD_{50}. Not all animals will react in the same way to the chemical and thus SD represents the square root of the variance of the test populations' response to the chemical. The dose-response curve is sigmoidal in nature and represents the cumulative response of the test animals to the chemical. The inflection point of this sigmoidal curve coincides with the LD_{50} for the test population.

3. What follows is a brief description of the mathematical and biological principles underlying each acute oral toxicity method followed by a listing of how each test estimates or does not estimate the specific parameters mentioned above.

GUIDELINE 420 :FIXED DOSE PROCEDURE

Principles Underlying The Test Method

4. The Fixed Dose Procedure (FDP) is a method for assessing acute oral toxicity that involves the identification of a dose level that causes evidence of non-lethal toxicity (termed *evident* toxicity) rather than a dose level that causes lethality. *Evident toxicity* is a general term describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would be expected to result in the development of severe toxic signs and probably mortality.

5. Underpinning the FDP is a belief that the toxic profile of a substance can be characterized with sufficient reliability for most regulatory situations without the need for the identification of a lethal dose. That is, observations made at non-lethal doses will allow substances to be ranked, or classified, according to their acute toxicity, provide information to aid dose level selection for repeat dose studies and provide hazard data for use in a risk assessment. The original FDP was subject to a number of validation and comparison studies, which showed that classification outcome was similar to that based on the outcome of traditional tests for determining an LD_{50} value (1)(2)(3)(4)(5).

6. Fixed dose levels of 5, 50, 300 and 2000 mg/kg and rules for the sequential procedure were adopted following a rigorous analysis using a statistical model (6)(7). The analysis predicted the classification outcome (according to the EU scheme and the lethality-based GHS), numbers of animals used and number of substance-related deaths using a number of FDP design options for substances with a range of LD_{50} values and dose response slopes for lethality. On the basis of this analysis, the design of the FDP was optimised with respect to classification performance and animal welfare.

7. The statistical modelling showed that the FDP produces classification outcomes similar to that based on the LD_{50} value for substances with a steep (greater than 2) dose response curve for mortality. For substances with a relatively shallow (less than 2) dose response curve there is an increasing probability the FDP will produce a more stringent classification than that based on the LD_{50} value; however, the risk of a less stringent classification than that based on the LD_{50} value is negligible. The influence of the choice of starting dose on the classification outcome, which can be a problem with sequential procedures, is negligible.

Point Estimate of LD_{50}

8. The FDP is not designed to determine a point estimate of LD_{50}. However, an approximate LD_{50} range can be inferred from the classification outcome. The ability of the FDP to correctly classify (i.e. assign to an LD_{50} range) is discussed above.

Confidence Limits on the Estimate of LD_{50}

9. The FDP is not designed to determine a point estimate of LD_{50}, or confidence limits on the estimate of the LD_{50}.

Dose-Effect Curve

10. Since lethality is not the preferred endpoint for the FDP, information on toxicological effects seen only at dose levels close to a lethal dose will not always be available. However, it has been shown in a number of validation and comparative studies (1)(2)(3)(4)(5)(6) that while there were instances where clinical signs observed in FDP tests differed from those observed in traditional LD_{50} tests, in only a few cases were these meaningful. In the majority of cases, the clinical signs not observed in the FDP tests were non-specific signs of approaching death.

GUIDELINE 423 : ACUTE TOXIC CATEGORY METHOD

Principles Underlying The Test Method

11. The acute toxic category (ATC) method allows for the allocation of chemical substances to all classification systems currently in use (e.g.. the LD_{50} is between 50 and 500 mg/kg body weight) (8)(9). It is a group sequential procedure using three animals of one sex per step. Four pre-identified starting doses are possible.

12. The ATC Method is based on the probit model; i.e., the dose-response relationship follows the Gaussian distribution for log-dose values with two parameters, the mean (LD_{50}) and the slope in probit units based on the log-scaled dose-axis (logarithm according to base 10). Then, following the test scheme of the method, expected probabilities of a correct, of a lower and of a more stringent classification in dependence on the true oral LD_{50} value of a substance and its slope can be derived.

13. The test doses were selected with respect to the Globally Harmonized Classification system. It

has been shown that the probabilities of correct classification is greatest when test doses and category limits are identical. The minimal distance factor between two neighboring toxic classes has to be 4 for slopes of at least 1 to achieve a probability of correct classification of at least 0.5 for at least one LD_{50} value in each category. For a slope of at least 1 the probability of an allocation to a lower than correct toxic category is limited to 0.256.

14. There is only a low dependence on the starting dose with respect to classification results, especially for slopes of greater than 1. With increasing slopes or increasing LD_{50} values this influence decreases and tends toward zero for an unlimited increase of slope or LD_{50}. Also for infinitely low values of LD_{50} the influence becomes zero.

15. There is a strong dependence on the starting dose with respect to expected numbers of animals used and of moribund/dead animals. Therefore an appropriate starting dose should be near the true LD_{50} of the substance to be tested to minimise the number of animals used.

Point estimate of LD_{50}

16. The ATC was not designed to determine a point estimate of LD_{50}. However, a point estimate of the LD_{50} can be calculated by the maximum likelihood method providing there are at least two doses with mortality rates not equal to 0% or 100%. However, the probability of two such doses is rather low because the distance between two neighboring doses is 6- to 10-fold and up to six animals per dose are used (10).

Confidence Limits On The Estimate Of LD_{50}

17. The ATC was not designed to determine a point estimate of LD_{50}, or confidence limits. Providing there are at least three doses, two of which have mortality rates not equal to 0% or 100%, the maximum likelihood method can be used to calculate and broad confidence limits on the estimated LD_{50}.

Dose-Effect Curve

18. The ATC was not designed to determine a dose-effect curve for the LD_{50}. However, dose-effect curves can be calculated by the maximum likelihood method providing there are at least three doses, two with the specific toxic signs not present in 0% or 100% of the animals.

GUIDELINE 425:UP-AND-DOWN METHOD

Principles Underlying the Test Method

19. The concept of the up-and-down (UDP) testing approach (sometimes called a Staircase Design) was first described by Dixon and Mood (11)(12). There have been papers on such issues as its use with small samples (13) and its use with multiple animals per dose (14). One of the most extensive discussions appears in a draft monograph prepared by W. Dixon and Dixon Statistical Associates for a U.S. National Institutes of Health [NIH] Phase I Final Report, Reduction in Vertebrate Animal Use in Research, produced under SBIR Grant No. 1-R43-RR06151-01(15). This draft monograph is available from its author for a fee or from the National Center for Research Resources of the NIH to individuals under the Freedom of Information Act.

20. In 1985, Bruce proposed the use of the UDP for the determination of acute toxicity of chemicals (16). While there exist several variations of the up-and-down experimental design, Guideline 425 is a modification of the procedure of Bruce as adopted by ASTM in 1987 (17). The guideline provides a main

test, for LD_{50} point estimation and a computational procedure, used together with the main test to calculate confidence intervals. The UDP calls for dosing individual animals of a single sex, usually females, in sequence at 48-hour intervals, with the initial dose set just below "the toxicologist's best estimate of the LD_{50}," or at 175 mg/kg if no such estimate is possible. Following each death (or moribund state) the dose is lowered; following each survival, it is increased, according to a pre-specified dose progression factor. If a death follows an initial direction of increasing doses, or a survival follows an initial direction of decreasing dose, additional animals are tested following the same dose adjustment pattern and testing is ended if certain criteria are met. The OECD 425 protocol calls for a default dose progression factor of 3.2 and default s for maximum likelihood calculations of 0.5 (i.e., log(3.2)). Dosing levels and calculation details are provided in the guideline.

Point Estimate of the LD_{50}

21. From the data a point estimate of the LD_{50} is calculated using the maximum likelihood method (18)(19).

Confidence Limits On The Estimate Of LD_{50}

22. Confidence limits around the LD_{50} value can be calculated using the maximum likelihood method (18)(19), provided a suitable historical or other sound estimate of the standard deviation can be employed. A computational procedure based on profile likelihoods can provide confidence limits for the LD_{50} when no prior estimate of the standard deviation is available. The procedure identifies bounds for LD_{50} from a ratio of likelihood functions optimized over *sigma* (profile likelihoods). Procedures are also included for certain circumstances where no intermediate doses exist (for instance, when testing has proceeded through a wide range of doses with no reversal or where doses are so widely spaced that each animal provides a reversal).

Dose-Effect Curve

23. A dose effect curve can be calculated using a two parameter probit model provided that the response is quantal and there is an overlapping of the range of doses that result in a positive and negative response.

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Appendix N

UDP/ATC Simulation Modeling Results

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Appendix N1

UDP Simulation Results Using Starting Doses One Default Dose Lower than the LD_{50} Predicted by the 3T3 and NHK NRU IC_{50} and the RC Rat-Only Millimole Regression - 5000 mg/kg Upper Limit Dose

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Summary of Animals Used and Animals Dead for UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
			Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3T3	0.12	Cyto	0.196	7.42	0.53	0.0002	0.204	3.43	0.00	0.6675	6.6%	0.1%
		Default	0.176	7.95			0.200	3.44				
	0.25	Cyto	0.189	8.15	0.52	0.0005	0.203	3.76	0.00	0.9311	6.0%	0.1%
		Default	0.178	8.68			0.197	3.76				
	0.50	Cyto	0.169	8.80	0.54	0.0008	0.191	4.09	0.02	0.6341	5.8%	0.5%
		Default	0.163	9.35			0.185	4.11				
	1.25	Cyto	0.135	9.34	0.61	0.0001	0.165	4.48	0.07	0.0238	6.1%	1.5%
		Default	0.131	9.95			0.152	4.55				
	2.00	Cyto	0.112	9.48	0.53	0.0003	0.145	4.60	0.07	0.0506	5.3%	1.5%
		Default	0.096	10.01			0.129	4.67				
			Average Difference		0.55		Average Difference		0.03			
NHK	0.12	Cyto	0.203	7.43	0.49	0.0003	0.215	3.39	-0.01	0.7372	6.2%	-0.2%
		Default	0.176	7.92			0.202	3.39				
	0.25	Cyto	0.197	8.18	0.48	0.0005	0.212	3.72	0.00	0.3125	5.6%	-0.1%
		Default	0.174	8.66			0.198	3.72				
	0.50	Cyto	0.176	8.86	0.50	0.0006	0.199	4.07	0.01	0.2841	5.3%	0.2%
		Default	0.157	9.36			0.183	4.08				
	1.25	Cyto	0.145	9.41	0.55	0.0002	0.173	4.48	0.04	0.0129	5.5%	1.0%
		Default	0.125	9.96			0.150	4.52				
	2.00	Cyto	0.121	9.53	0.49	0.0001	0.151	4.61	0.05	0.0206	4.9%	1.1%
		Default	0.092	10.01			0.127	4.66				
			Average Difference		0.50		Average Difference		0.02			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity..

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean number of animals for the default starting dose and mean number of animals for the NRU-based starting dose.

[4]P-value is from one-sided Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

Summary of Stopping Rules Used for the UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	3 Animals at Limit Dose[2]	5 Reversals[2]	Likelihood Ratio[2]	Maximum Number of Animals Used[2]
3T3	0.12	Cyto	15.6%	56.0%	26.4%	2.0%
		Default	15.4%	56.9%	25.3%	2.4%
	0.25	Cyto	15.0%	33.6%	47.4%	4.0%
		Default	14.7%	34.1%	46.0%	5.3%
	0.5	Cyto	13.4%	19.8%	59.0%	7.8%
		Default	13.0%	20.0%	57.3%	9.7%
	1.25	Cyto	9.8%	13.5%	64.0%	12.7%
		Default	9.1%	13.6%	60.9%	16.4%
	2	Cyto	8.5%	12.3%	65.2%	14.0%
		Default	7.4%	12.5%	62.6%	17.5%
NHK	0.12	Cyto	16.8%	55.3%	26.0%	1.8%
		Default	16.6%	56.0%	25.0%	2.4%
	0.25	Cyto	16.1%	33.3%	46.5%	4.1%
		Default	15.8%	33.5%	45.5%	5.2%
	0.5	Cyto	14.3%	19.7%	58.0%	8.1%
		Default	13.8%	19.9%	56.6%	9.7%
	1.25	Cyto	10.1%	13.5%	63.1%	13.3%
		Default	9.5%	13.5%	60.4%	16.5%
	2	Cyto	8.6%	12.3%	64.6%	14.5%
		Default	7.6%	12.5%	62.3%	17.6%

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 175 mg/kg; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Percentage of the 10,000 test simulations that satisfied the specified condition for completion of testing (see OECD [2001a]; EPA [2002a]).

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
1	3T3	0.12	Cyto	0.431	8.74	0.96	0.6250	0.459	5.58	0.81	0.6250	9.9%	12.7%
			Default	0.277	9.70			0.170	6.39				
		0.25	Cyto	0.660	9.56	1.02	0.6250	0.581	6.06	0.84	0.6250	9.7%	12.2%
			Default	0.179	10.58			0.155	6.90				
		0.50	Cyto	0.697	10.19	1.14	0.6250	0.609	6.46	0.91	0.6250	10.0%	12.3%
			Default	0.201	11.32			0.197	7.37				
		1.25	Cyto	0.664	10.68	1.07	0.6250	0.598	6.70	0.87	0.6250	9.1%	11.5%
			Default	0.156	11.75			0.169	7.57				
		2.00	Cyto	0.548	10.65	0.82	0.6250	0.506	6.54	0.71	0.6250	7.1%	9.8%
			Default	0.146	11.47			0.152	7.24				
				Average Difference		1.00		Average Difference		0.83			
	NHK	0.12	Cyto	0.516	8.95	0.71	0.3750	0.531	5.79	0.58	0.3750	7.3%	9.1%
			Default	0.268	9.66			0.169	6.37				
		0.25	Cyto	0.699	9.77	0.77	0.3750	0.626	6.26	0.61	0.3750	7.3%	8.9%
			Default	0.217	10.53			0.177	6.87				
		0.50	Cyto	0.707	10.47	0.75	0.3750	0.638	6.69	0.63	0.3750	6.7%	8.6%
			Default	0.241	11.21			0.224	7.31				
		1.25	Cyto	0.692	10.92	0.78	0.3750	0.636	6.91	0.65	0.3750	6.7%	8.6%
			Default	0.169	11.70			0.179	7.56				
		2.00	Cyto	0.627	10.81	0.66	0.3750	0.578	6.70	0.53	0.3750	5.7%	7.4%
			Default	0.159	11.47			0.157	7.24				
				Average Difference		0.73		Average Difference		0.60			
2	3T3	0.12	Cyto	0.467	8.54	-0.08	0.8926	0.426	5.16	-0.05	0.9460	-1.0%	-1.0%
			Default	0.278	8.46			0.239	5.11				
		0.25	Cyto	0.426	9.21	-0.13	0.8926	0.404	5.54	-0.07	0.9460	-1.4%	-1.3%
			Default	0.210	9.08			0.202	5.47				
		0.50	Cyto	0.453	9.74	-0.07	1.0000	0.417	5.83	-0.06	1.0000	-0.7%	-1.0%
			Default	0.230	9.68			0.211	5.77				
		1.25	Cyto	0.413	10.25	-0.08	0.9460	0.394	6.06	-0.09	0.8926	-0.8%	-1.5%
			Default	0.236	10.17			0.218	5.97				
		2.00	Cyto	0.328	10.34	-0.14	0.5879	0.335	6.01	-0.10	0.7354	-1.4%	-1.8%
			Default	0.177	10.20			0.178	5.91				
				Average Difference		-0.10		Average Difference		-0.07			
	NHK	0.12	Cyto	0.488	8.77	-0.33	0.3757	0.476	5.26	-0.15	0.5879	-3.9%	-3.0%
			Default	0.260	8.43			0.232	5.11				
		0.25	Cyto	0.428	9.44	-0.36	0.4143	0.444	5.64	-0.17	0.6848	-4.0%	-3.1%
			Default	0.166	9.08			0.187	5.46				
		0.50	Cyto	0.448	9.99	-0.34	0.3757	0.453	5.94	-0.18	0.5417	-3.5%	-3.2%
			Default	0.164	9.65			0.185	5.75				
		1.25	Cyto	0.424	10.46	-0.32	0.3396	0.440	6.16	-0.21	0.4973	-3.2%	-3.5%
			Default	0.183	10.14			0.196	5.95				
		2.00	Cyto	0.348	10.49	-0.32	0.4143	0.381	6.09	-0.20	0.5417	-3.1%	-3.4%
			Default	0.148	10.18			0.166	5.89				
				Average Difference		-0.33		Average Difference		-0.18			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3	3T3	0.12	Cyto	0.189	6.90	-0.29	0.0425	0.149	3.60	-0.23	0.0522	-4.3%	-6.8%
			Default	0.188	6.61			0.125	3.37				
		0.25	Cyto	0.220	7.53	-0.33	0.0522	0.169	3.96	-0.24	0.0640	-4.6%	-6.6%
			Default	0.152	7.20			0.103	3.71				
		0.50	Cyto	0.213	8.18	-0.42	0.0522	0.163	4.31	-0.27	0.0640	-5.5%	-6.7%
			Default	0.101	7.76			0.080	4.04				
		1.25	Cyto	0.141	8.98	-0.35	0.0522	0.123	4.69	-0.22	0.0771	-4.1%	-4.9%
			Default	0.059	8.62			0.057	4.47				
		2.00	Cyto	0.084	9.33	-0.23	0.0522	0.094	4.85	-0.15	0.2036	-2.5%	-3.3%
			Default	0.040	9.10			0.050	4.70				
				Average Difference		-0.33		Average Difference		-0.22			
	NHK	0.12	Cyto	0.190	6.85	-0.28	0.1514	0.133	3.52	-0.16	0.2334	-4.2%	-4.9%
			Default	0.190	6.57			0.127	3.35				
		0.25	Cyto	0.229	7.48	-0.31	0.0425	0.152	3.86	-0.17	0.1099	-4.4%	-4.6%
			Default	0.159	7.17			0.106	3.69				
		0.50	Cyto	0.206	8.12	-0.34	0.0923	0.143	4.20	-0.16	0.2036	-4.4%	-4.1%
			Default	0.109	7.78			0.082	4.04				
		1.25	Cyto	0.120	8.93	-0.28	0.0522	0.108	4.60	-0.12	0.4697	-3.2%	-2.6%
			Default	0.061	8.65			0.060	4.48				
		2.00	Cyto	0.079	9.31	-0.20	0.0923	0.088	4.77	-0.07	0.7334	-2.2%	-1.5%
			Default	0.036	9.11			0.048	4.70				
				Average Difference		-0.28		Average Difference		-0.14			
4	3T3	0.12	Cyto	0.191	7.15	0.31	0.0443	0.063	3.39	0.01	0.9399	4.1%	0.2%
			Default	0.235	7.46			0.066	3.40				
		0.25	Cyto	0.186	7.66	0.28	0.0507	0.032	3.61	-0.003	0.2522	3.5%	-0.1%
			Default	0.201	7.94			0.048	3.60				
		0.50	Cyto	0.210	8.14	0.38	0.1046	0.040	3.80	0.05	0.1591	4.5%	1.4%
			Default	0.212	8.53			0.049	3.86				
		1.25	Cyto	0.180	8.82	0.33	0.0250	0.049	4.10	0.03	0.0934	3.6%	0.8%
			Default	0.145	9.16			0.022	4.13				
		2.00	Cyto	0.133	9.16	0.22	0.0577	0.042	4.26	-0.01	0.8603	2.3%	-0.2%
			Default	0.084	9.38			0.019	4.25				
				Average Difference		0.31		Average Difference		0.02			
	NHK	0.12	Cyto	0.196	7.00	0.49	0.0073	0.064	3.36	0.06	0.1439	6.5%	1.7%
			Default	0.247	7.49			0.071	3.42				
		0.25	Cyto	0.213	7.53	0.45	0.0131	0.036	3.58	0.05	0.0577	5.6%	1.4%
			Default	0.207	7.97			0.048	3.63				
		0.50	Cyto	0.234	8.03	0.52	0.0335	0.041	3.78	0.09	0.0654	6.1%	2.4%
			Default	0.221	8.55			0.052	3.88				
		1.25	Cyto	0.218	8.76	0.41	0.0182	0.051	4.10	0.04	0.1297	4.5%	1.1%
			Default	0.147	9.17			0.023	4.14				
		2.00	Cyto	0.163	9.12	0.27	0.0443	0.042	4.28	-0.02	0.8999	2.9%	-0.4%
			Default	0.086	9.40			0.018	4.26				
				Average Difference		0.43		Average Difference		0.05			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
5	3T3	0.12	Cyto	0.308	7.96	1.21	0.0020	0.042	3.25	0.06	0.0137	13.2%	1.7%
			Default	0.232	9.17			0.034	3.30				
		0.25	Cyto	0.196	9.01	1.33	0.0039	0.049	3.46	0.11	0.0195	12.8%	3.1%
			Default	0.157	10.34			0.062	3.57				
		0.50	Cyto	0.148	9.46	1.28	0.0039	0.051	3.56	0.09	0.0195	11.9%	2.5%
			Default	0.102	10.73			0.059	3.65				
		1.25	Cyto	0.131	9.29	1.38	0.0020	0.038	3.67	0.20	0.0020	12.9%	5.2%
			Default	0.065	10.66			0.030	3.87				
		2.00	Cyto	0.107	9.20	1.16	0.0039	0.032	3.78	0.18	0.0039	11.2%	4.6%
			Default	0.061	10.36			0.013	3.96				
				Average Difference		1.27		Average Difference		0.13			
	NHK	0.12	Cyto	0.285	8.06	1.11	0.0020	0.030	3.25	0.06	0.0273	12.1%	1.7%
			Default	0.233	9.17			0.038	3.31				
		0.25	Cyto	0.241	9.12	1.19	0.0020	0.048	3.47	0.10	0.0273	11.5%	2.8%
			Default	0.152	10.31			0.061	3.56				
		0.50	Cyto	0.200	9.54	1.21	0.0020	0.046	3.55	0.10	0.0098	11.3%	2.7%
			Default	0.082	10.75			0.064	3.65				
		1.25	Cyto	0.167	9.40	1.27	0.0039	0.030	3.68	0.18	0.0039	11.9%	4.7%
			Default	0.052	10.66			0.037	3.86				
		2.00	Cyto	0.131	9.28	1.06	0.0020	0.029	3.79	0.17	0.0020	10.3%	4.2%
			Default	0.037	10.35			0.022	3.96				
				Average Difference		1.17		Average Difference		0.12			
6	3T3	0.12	Cyto	0.685	6.18	1.58	0.0005	0.314	0.88	-0.02	0.0923	20.3%	-2.8%
			Default	0.587	7.76			0.304	0.85				
		0.25	Cyto	0.647	7.10	1.57	0.0005	0.316	1.33	-0.03	0.0342	18.1%	-2.1%
			Default	0.541	8.67			0.309	1.30				
		0.50	Cyto	0.486	8.29	1.58	0.0005	0.255	2.04	-0.01	0.1294	16.0%	-0.4%
			Default	0.342	9.87			0.255	2.03				
		1.25	Cyto	0.301	9.01	1.88	0.0005	0.126	3.00	0.19	0.0005	17.3%	6.0%
			Default	0.058	10.89			0.121	3.19				
		2.00	Cyto	0.246	8.94	1.81	0.0005	0.088	3.33	0.28	0.0005	16.8%	7.7%
			Default	0.030	10.75			0.066	3.60				
				Average Difference		1.68		Average Difference		0.08			
	NHK	0.12	Cyto	0.630	6.19	1.47	0.0002	0.298	0.82	-0.02	0.0281	19.2%	-3.1%
			Default	0.560	7.66			0.289	0.80				
		0.25	Cyto	0.585	7.16	1.47	0.0002	0.295	1.28	-0.02	0.1099	17.0%	-1.7%
			Default	0.499	8.63			0.287	1.26				
		0.50	Cyto	0.440	8.41	1.47	0.0002	0.236	2.03	-0.01	0.0942	14.8%	-0.7%
			Default	0.317	9.87			0.236	2.02				
		1.25	Cyto	0.276	9.14	1.73	0.0002	0.112	3.02	0.16	0.0002	16.0%	5.0%
			Default	0.056	10.87			0.114	3.18				
		2.00	Cyto	0.234	9.06	1.69	0.0002	0.078	3.36	0.25	0.0002	15.7%	7.0%
			Default	0.022	10.74			0.062	3.61				
				Average Difference		1.56		Average Difference		0.07			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); Toxcat=Category from Globally Harmonized System of Classification and Labeling of Chemicals[5] (GHS; UN 2005); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean animals used for the default starting dose and mean animals used for the NRU-based starting dose.

[4]P-value is from one-side Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

[5]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

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Concordance of NRU-Based Starting Dose with Default Starting Dose for GHS Acute Oral Toxicity Category Outcome Based on Simulated UDP LD_{50}[1]

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category[2] Based on LD_{50} Outcome with NHK NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	15	1	0	17	88%	6%	6%
5	0	0	0	0	22	0	22	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	15	23	0	68	96%	1%	3%

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category[2] Based on LD_{50} Outcome with 3T3 NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	0	16	1	0	17	94%	6%	0%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	11	16	22	0	67	97%	1%	1%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; NA=Not applicable; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg. The NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621). The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[2]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

Discordant Substances for GHS Category Outcomes of UDP Simulations[1]

NRU Test Method	Substance	NRU-Based Starting Dose[2]		Default Starting Dose[3]		LD_{50} Difference
		LD_{50}	Toxcat[4]	LD_{50}	Toxcat[4]	
3T3	Acetaminophen	2046.78	5	1765.44	4	-281.34
	Sodium Dichromate Dihydrate	43.70	2	51.87	3	8.17
NHK	Acetaminophen	2173.95	5	1755.26	4	-418.69
	Caffeine	279.63	3	357.17	4	77.55
	Sodium Dichromate Dihydrate	45.09	2	51.77	3	6.69

Abbreviations: Toxcat=Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2005); UDP= Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes.

[1]Substances for which the simulated UDP outcome (in terms of GHS category) at the NRU-based starting dose did not match the simulated UDP outcome at the default starting dose. Simulations were performed with 10,000 runs at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621).

[3]The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[4]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
	$5 < LD_{50} \leq 50$ mg/kg
	$50 < LD_{50} \leq 300$ mg/kg
	$300 < LD_{50} \leq 2000$ mg/kg
2	$2000 < LD_{50} \leq 5000$ mg/kg
3	$LD_{50} > 5000$ mg/kg
4	
5	
6	

Appendix N2

UDP Simulation Results Using Starting Doses One Default Dose Lower than the LD_{50} Predicted by the 3T3 and NHK NRU IC_{50} and the RC Rat-Only Weight Regression - 5000 mg/kg Upper Limit Dose

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Summary of Animals Used and Animals Dead for UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
			Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3T3	0.12	Cyto	0.193	7.32	0.62	0.00003	0.200	3.39	0.04	0.9360	7.8%	1.2%
		Default	0.178	7.94			0.200	3.43				
	0.25	Cyto	0.186	8.04	0.63	0.0001	0.198	3.72	0.04	0.5758	7.2%	1.2%
		Default	0.180	8.67			0.197	3.76				
	0.50	Cyto	0.164	8.70	0.66	0.0001	0.186	4.05	0.06	0.3430	7.0%	1.5%
		Default	0.164	9.36			0.185	4.11				
	1.25	Cyto	0.132	9.26	0.70	0.00003	0.161	4.44	0.11	0.0119	7.0%	2.3%
		Default	0.130	9.96			0.152	4.55				
	2.00	Cyto	0.110	9.41	0.60	0.00005	0.141	4.58	0.10	0.0371	6.0%	2.1%
		Default	0.095	10.01			0.129	4.67				
			Average Difference		0.64		Average Difference		0.07			
NHK	0.12	Cyto	0.195	7.38	0.54	0.0002	0.208	3.35	0.04	0.8066	6.8%	1.1%
		Default	0.176	7.92			0.203	3.39				
	0.25	Cyto	0.189	8.12	0.54	0.0002	0.204	3.67	0.05	0.3274	6.3%	1.2%
		Default	0.175	8.66			0.199	3.72				
	0.50	Cyto	0.169	8.80	0.56	0.0003	0.191	4.02	0.05	0.3154	6.0%	1.3%
		Default	0.159	9.36			0.184	4.08				
	1.25	Cyto	0.136	9.36	0.61	0.0001	0.164	4.43	0.09	0.0044	6.1%	2.0%
		Default	0.125	9.96			0.151	4.52				
	2.00	Cyto	0.114	9.48	0.53	0.0001	0.144	4.56	0.09	0.0089	7.8%	1.9%
		Default	0.092	10.02			0.127	4.66				
			Average Difference		0.56		Average Difference		0.06			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [mg/mL] + 2.024); Default=Default starting dose of 175 mg/kg; Std Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean number of animals for the default starting dose and mean number of animals for the NRU-based starting dose.

[4]P-value is from one-sided Wilcoxon signed rank test for difference in animals between the default starting dose and the NRU-based starting dose. Significant values at $p < 0.05$.

Summary of Stopping Rules Used for the UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	3 Animals at Limit Dose[2]	5 Reversals[2]	Likelihood Ratio[2]	Maximum Number of Animals Used[2]
3T3	0.12	Cyto	15.6%	55.8%	26.8%	1.8%
		Default	15.4%	56.8%	25.3%	2.4%
	0.25	Cyto	15.0%	33.4%	47.9%	3.7%
		Default	14.7%	34.1%	46.0%	5.3%
	0.5	Cyto	13.4%	19.7%	59.6%	7.2%
		Default	13.0%	20.1%	57.3%	9.7%
	1.25	Cyto	9.9%	13.4%	64.6%	12.1%
		Default	9.1%	13.6%	60.8%	16.4%
	2	Cyto	8.6%	12.3%	65.6%	13.5%
		Default	7.4%	12.5%	62.5%	17.6%
NHK	0.12	Cyto	16.8%	55.4%	26.0%	1.8%
		Default	16.6%	55.9%	25.0%	2.4%
	0.25	Cyto	16.2%	33.3%	46.7%	3.8%
		Default	15.8%	33.5%	45.4%	5.2%
	0.5	Cyto	14.3%	19.7%	58.3%	7.7%
		Default	13.8%	19.9%	56.6%	9.7%
	1.25	Cyto	10.2%	13.5%	63.5%	12.8%
		Default	9.5%	13.5%	60.4%	16.5%
	2	Cyto	8.7%	12.3%	64.9%	14.1%
		Default	7.6%	12.5%	62.3%	17.6%

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024); Default=Default starting dose of 175 mg/kg; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Percentage of the 10,000 test simulations that satisfied the specified condition for completion of testing (see OECD [2001a]; EPA [2002a]).

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
1	3T3	0.12	Cyto	0.366	8.92	0.78	0.6250	0.404	5.74	0.65	0.6250	8.0%	10.2%
			Default	0.278	9.70			0.171	6.39				
		0.25	Cyto	0.587	9.75	0.81	0.6250	0.521	6.22	0.66	0.6250	7.7%	9.6%
			Default	0.181	10.55			0.158	6.88				
		0.50	Cyto	0.623	10.38	0.90	0.6250	0.549	6.63	0.72	0.6250	8.0%	9.8%
			Default	0.197	11.29			0.196	7.35				
		1.25	Cyto	0.594	10.86	0.86	0.6250	0.540	6.86	0.70	0.6250	7.3%	9.2%
			Default	0.147	11.72			0.166	7.55				
		2.00	Cyto	0.503	10.80	0.66	0.6250	0.466	6.66	0.57	0.6250	5.7%	7.9%
			Default	0.142	11.45			0.151	7.24				
				Average Difference		0.80		Average Difference		0.66			
	NHK	0.12	Cyto	0.515	8.97	0.69	0.3750	0.531	5.81	0.56	0.3750	7.1%	8.8%
			Default	0.268	9.66			0.169	6.37				
		0.25	Cyto	0.703	9.79	0.74	0.3750	0.629	6.28	0.59	0.3750	7.0%	8.6%
			Default	0.218	10.53			0.178	6.87				
		0.50	Cyto	0.711	10.49	0.72	0.6250	0.641	6.71	0.60	0.6250	6.4%	8.2%
			Default	0.242	11.21			0.224	7.31				
		1.25	Cyto	0.694	10.94	0.76	0.6250	0.638	6.93	0.62	0.6250	6.5%	8.3%
			Default	0.168	11.70			0.179	7.56				
		2.00	Cyto	0.632	10.83	0.63	0.6250	0.581	6.72	0.52	0.6250	5.5%	7.1%
			Default	0.159	11.47			0.157	7.24				
				Average Difference		0.71		Average Difference		0.58			
2	3T3	0.12	Cyto	0.442	8.41	0.06	1.0000	0.398	5.04	0.08	1.0000	0.8%	1.5%
			Default	0.276	8.47			0.240	5.12				
		0.25	Cyto	0.393	9.07	0.05	1.0000	0.370	5.41	0.06	1.0000	0.5%	1.1%
			Default	0.201	9.11			0.202	5.48				
		0.50	Cyto	0.419	9.58	0.13	0.9460	0.381	5.70	0.09	1.0000	1.3%	1.5%
			Default	0.219	9.71			0.210	5.78				
		1.25	Cyto	0.381	10.11	0.08	0.9460	0.359	5.93	0.05	0.9460	0.8%	0.8%
			Default	0.225	10.19			0.214	5.98				
		2.00	Cyto	0.297	10.22	-0.01	0.7354	0.302	5.91	0.00	0.7869	-0.1%	0.0%
			Default	0.170	10.21			0.174	5.91				
				Average Difference		0.06		Average Difference		0.05			
	NHK	0.12	Cyto	0.439	8.59	-0.13	0.3757	0.427	5.10	0.01	0.6848	-1.6%	0.2%
			Default	0.267	8.45			0.235	5.11				
		0.25	Cyto	0.384	9.24	-0.12	0.5879	0.396	5.46	0.01	1.0000	-1.4%	0.2%
			Default	0.178	9.12			0.193	5.47				
		0.50	Cyto	0.413	9.78	-0.07	0.5417	0.409	5.76	0.02	0.8394	-0.8%	0.3%
			Default	0.177	9.70			0.192	5.78				
		1.25	Cyto	0.385	10.28	-0.11	0.4973	0.391	5.99	-0.03	0.8394	-1.1%	-0.4%
			Default	0.187	10.17			0.198	5.96				
		2.00	Cyto	0.306	10.35	-0.16	0.4973	0.334	5.95	-0.05	0.7869	-1.6%	-0.9%
			Default	0.149	10.19			0.166	5.89				
				Average Difference		-0.12		Average Difference		-0.01			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3	3T3	0.12	Cyto	0.181	6.76	-0.18	0.0923	0.136	3.50	-0.14	0.1294	-2.7%	-4.2%
			Default	0.190	6.58			0.127	3.36				
		0.25	Cyto	0.182	7.33	-0.20	0.1514	0.146	3.83	-0.15	0.2061	-2.8%	-4.0%
			Default	0.147	7.13			0.102	3.68				
		0.50	Cyto	0.180	7.99	-0.25	0.1514	0.146	4.18	-0.16	0.1763	-3.3%	-4.0%
			Default	0.100	7.74			0.080	4.02				
		1.25	Cyto	0.119	8.86	-0.22	0.1294	0.112	4.61	-0.13	0.3804	-2.5%	-2.8%
			Default	0.056	8.64			0.057	4.48				
		2.00	Cyto	0.069	9.25	-0.15	0.1294	0.084	4.78	-0.09	0.5186	-1.6%	-1.8%
			Default	0.039	9.11			0.049	4.70				
				Average Difference		-0.20		Average Difference		-0.13			
	NHK	0.12	Cyto	0.205	6.75	-0.18	0.2036	0.137	3.41	-0.06	0.2334	-2.7%	-1.8%
			Default	0.194	6.58			0.129	3.35				
		0.25	Cyto	0.225	7.33	-0.22	0.1099	0.145	3.74	-0.08	0.1763	-3.2%	-2.1%
			Default	0.160	7.11			0.108	3.66				
		0.50	Cyto	0.209	7.99	-0.24	0.1294	0.141	4.09	-0.07	0.1763	-3.1%	-1.9%
			Default	0.110	7.75			0.082	4.01				
		1.25	Cyto	0.123	8.85	-0.18	0.1294	0.106	4.52	-0.03	0.8501	-2.1%	-0.7%
			Default	0.058	8.67			0.060	4.49				
		2.00	Cyto	0.083	9.26	-0.14	0.1294	0.088	4.70	0.00	0.9097	-1.5%	-0.1%
			Default	0.035	9.13			0.048	4.70				
				Average Difference		-0.19		Average Difference		-0.05			
4	3T3	0.12	Cyto	0.176	7.17	0.28	0.0335	0.063	3.39	0.00	0.8999	3.8%	0.0%
			Default	0.236	7.46			0.067	3.39				
		0.25	Cyto	0.173	7.68	0.25	0.0507	0.032	3.61	-0.01	0.1928	3.1%	-0.3%
			Default	0.202	7.93			0.049	3.60				
		0.50	Cyto	0.193	8.16	0.35	0.0577	0.039	3.80	0.05	0.1167	4.1%	1.2%
			Default	0.208	8.52			0.047	3.85				
		1.25	Cyto	0.159	8.83	0.32	0.0250	0.048	4.10	0.03	0.1046	3.5%	0.8%
			Default	0.142	9.15			0.020	4.13				
		2.00	Cyto	0.115	9.17	0.21	0.0335	0.043	4.26	-0.01	0.7820	2.3%	-0.2%
			Default	0.084	9.38			0.020	4.25				
				Average Difference		0.28		Average Difference		0.01			
	NHK	0.12	Cyto	0.160	7.17	0.31	0.0577	0.060	3.38	0.03	0.2744	4.1%	0.8%
			Default	0.234	7.48			0.066	3.41				
		0.25	Cyto	0.194	7.71	0.27	0.0507	0.028	3.62	0.02	0.1591	3.4%	0.4%
			Default	0.189	7.98			0.042	3.63				
		0.50	Cyto	0.223	8.20	0.34	0.0833	0.040	3.82	0.05	0.1167	3.9%	1.3%
			Default	0.206	8.54			0.046	3.87				
		1.25	Cyto	0.196	8.86	0.28	0.0577	0.050	4.11	0.02	0.2744	3.1%	0.4%
			Default	0.141	9.14			0.022	4.13				
		2.00	Cyto	0.143	9.18	0.19	0.0654	0.043	4.28	-0.02	0.7820	2.1%	-0.6%
			Default	0.083	9.38			0.019	4.25				
				Average Difference		0.28		Average Difference		0.02			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	Cell Type	Sigma	Method	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
5	3T3	0.12	Cyto	0.365	7.61	1.59	0.0020	0.046	3.20	0.12	0.0059	17.3%	3.6%
			Default	0.235	9.20			0.037	3.31				
		0.25	Cyto	0.285	8.67	1.72	0.0020	0.056	3.41	0.18	0.0098	16.6%	5.1%
			Default	0.159	10.39			0.065	3.59				
		0.50	Cyto	0.242	9.14	1.64	0.0039	0.055	3.52	0.16	0.0137	15.2%	4.2%
			Default	0.106	10.78			0.063	3.67				
		1.25	Cyto	0.204	9.08	1.61	0.0020	0.044	3.65	0.23	0.0020	15.0%	6.0%
			Default	0.071	10.69			0.031	3.88				
		2.00	Cyto	0.161	9.05	1.33	0.0039	0.037	3.77	0.21	0.0039	12.8%	5.2%
			Default	0.064	10.38			0.015	3.97				
				Average Difference		1.58		Average Difference		0.18			
	NHK	0.12	Cyto	0.326	7.90	1.28	0.0020	0.035	3.23	0.08	0.0273	14.0%	2.5%
			Default	0.234	9.18			0.038	3.31				
		0.25	Cyto	0.307	8.93	1.41	0.0020	0.052	3.43	0.15	0.0098	13.6%	4.2%
			Default	0.146	10.34			0.066	3.58				
		0.50	Cyto	0.251	9.40	1.38	0.0020	0.047	3.54	0.13	0.0098	12.8%	3.5%
			Default	0.084	10.77			0.067	3.66				
		1.25	Cyto	0.194	9.30	1.37	0.0020	0.033	3.67	0.19	0.0020	12.8%	5.0%
			Default	0.055	10.67			0.038	3.86				
		2.00	Cyto	0.155	9.20	1.15	0.0020	0.031	3.79	0.18	0.0020	11.1%	4.4%
			Default	0.038	10.36			0.023	3.96				
				Average Difference		1.32		Average Difference		0.15			
6	3T3	0.12	Cyto	0.686	6.14	1.63	0.0005	0.316	0.88	-0.03	0.1294	21.0%	-3.1%
			Default	0.587	7.76			0.304	0.85				
		0.25	Cyto	0.653	7.05	1.62	0.0005	0.317	1.33	-0.03	0.0210	18.7%	-2.2%
			Default	0.542	8.67			0.309	1.30				
		0.50	Cyto	0.484	8.23	1.65	0.0005	0.254	2.04	-0.01	0.3394	16.7%	-0.4%
			Default	0.343	9.87			0.256	2.03				
		1.25	Cyto	0.305	8.93	1.96	0.0005	0.126	2.99	0.20	0.0005	18.0%	6.3%
			Default	0.058	10.89			0.122	3.20				
		2.00	Cyto	0.251	8.87	1.88	0.0005	0.089	3.32	0.29	0.0005	17.5%	8.0%
			Default	0.028	10.75			0.067	3.61				
				Average Difference		1.75		Average Difference		0.09			
	NHK	0.12	Cyto	0.625	6.12	1.53	0.0005	0.298	0.82	-0.02	0.0398	20.0%	-3.1%
			Default	0.560	7.66			0.289	0.80				
		0.25	Cyto	0.581	7.10	1.53	0.0002	0.296	1.28	-0.02	0.1099	17.7%	-1.8%
			Default	0.500	8.63			0.287	1.26				
		0.50	Cyto	0.435	8.34	1.54	0.0002	0.236	2.03	-0.01	0.1677	15.6%	-0.6%
			Default	0.318	9.88			0.236	2.02				
		1.25	Cyto	0.277	9.07	1.81	0.0002	0.112	3.01	0.17	0.0002	16.7%	5.4%
			Default	0.057	10.88			0.114	3.18				
		2.00	Cyto	0.235	8.99	1.75	0.0002	0.078	3.34	0.27	0.0005	16.3%	7.4%
			Default	0.022	10.74			0.062	3.61				
				Average Difference		1.63		Average Difference		0.08			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); Toxcat=Category from Globally Harmonized System of Classification and Labeling of Chemicals[5] (GHS; UN 2005); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mmol/kg] = 0.372 log IC_{50} [mM] + 2.024); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean animals used for the default starting dose and mean animals used for the NRU-based starting dose.

[4]P-value is from one-side Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

[5]GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

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Concordance of NRU-Based Starting Dose with Default Starting Dose for GHS Acute Oral Toxicity Category Outcome Based on Simulated UDP LD_{50}[1]

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with NHK NRU-Based Starting Dose									
	d	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	16	0	0	17	94%	0%	6%
5	0	0	0	0	22	0	22	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	16	22	0	68	97%	0%	3%

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with 3T3 NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	16	0	0	17	94%	0%	6%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	16	21	0	67	97%	0%	3%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; NA=Not applicable; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg. The NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024). The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[2]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

Discordant Substances for GHS Category Outcomes of UDP Simulations[1]

NRU Test Method	Substance	NRU-Based Starting Dose[2]		Default Starting Dose[3]		LD_{50} Difference
		LD_{50}	Toxcat[4]	LD_{50}	Toxcat[4]	
3T3	Caffeine	271.54	3	338.16	4	66.62
	Sodium dichromate dihydrate	43.70	47.97	2	50.66	3
NHK	Caffeine	269.85	3	339.43	4	69.59
	Sodium dichromate dihydrate	48.52	2	50.64	3	2.12

Abbreviations: Toxcat=Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2005); UDP= Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes.

[1]Substances for which the simulated UDP outcome (in terms of GHS category) at the NRU-based starting dose did not match the simulated UDP outcome at the default starting dose. Simulations were performed with 10,000 runs at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [μg/mL] + 2.024).

[3]The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[4]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
	$5 < LD_{50} \leq 50$ mg/kg
	$50 < LD_{50} \leq 300$ mg/kg
	$300 < LD_{50} \leq 2000$ mg/kg
2	$2000 < LD_{50} \leq 5000$ mg/kg
3	$LD_{50} > 5000$ mg/kg
4	
5	
6	

Appendix N3

ATC Simulation Results Starting at the Next Fixed Dose Below the LD_{50} Predicted by the 3T3 and NHK NRU IC_{50} and the RC Rat-Only Millimole Regression - 2000 mg/kg Upper Limit Dose

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Summary of Animals Used and Animals Dead for ATC Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
			Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
NHK	0.12	Cyto	0.290	9.96	0.70	0.0113	0.286	2.67	0.48	0.1061	6.6%	15.3%
		Default	0.169	10.67			0.334	3.15				
	0.25	Cyto	0.269	9.98	0.77	0.0127	0.283	2.88	0.50	0.5613	7.1%	14.7%
		Default	0.149	10.75			0.324	3.38				
	0.50	Cyto	0.239	10.11	0.80	0.0005	0.261	3.19	0.53	0.0002	7.3%	14.2%
		Default	0.114	10.91			0.297	3.72				
	1.25	Cyto	0.183	10.31	0.79	0.0035	0.201	3.86	0.55	0.0002	7.1%	12.4%
		Default	0.068	11.10			0.228	4.40				
	2.00	Cyto	0.163	10.43	0.82	0.0003	0.168	4.20	0.53	0.0012	7.3%	11.2%
		Default	0.050	11.25			0.189	4.73				
			Average Difference		0.78		Average Difference		0.52			
3T3	0.12	Cyto	0.273	10.13	0.51	0.0226	0.291	2.77	0.43	0.0283	4.8%	13.4%
		Default	0.170	10.64			0.335	3.20				
	0.25	Cyto	0.257	10.15	0.58	0.0075	0.281	2.99	0.45	0.0139	5.4%	13.0%
		Default	0.151	10.73			0.325	3.43				
	0.50	Cyto	0.238	10.27	0.62	0.0038	0.257	3.31	0.46	0.0237	5.7%	12.2%
		Default	0.115	10.89			0.299	3.77				
	1.25	Cyto	0.193	10.46	0.64	0.0154	0.201	3.96	0.48	0.00003	5.8%	10.8%
		Default	0.067	11.10			0.228	4.43				
	2.00	Cyto	0.166	10.56	0.69	0.0002	0.168	4.28	0.47	0.00002	6.1%	9.9%
		Default	0.049	11.25			0.190	4.76				
			Average Difference		0.61		Average Difference		0.46			

Abbreviations: ATC=Acute Toxic Class method (OECD 2001d); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 300 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 2000 ATC simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]Mean number of animals for 2000 simulations.

[3]Difference between mean number of animals for the default starting dose and mean number of animals for the NRU-based starting dose.

[4]P-value is from one-sided Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
1	NHK	0.12	Cyto	1.228	6.09	2.99	0.2500	1.209	5.87	2.99	0.1250	33.0%	33.8%
			Default	0.083	9.09			0.080	8.86				
		0.25	Cyto	1.284	6.37	2.99	0.2500	1.183	5.68	2.99	0.1250	31.9%	34.5%
			Default	0.178	9.35			0.070	8.67				
		0.50	Cyto	1.311	6.78	2.96	0.2500	1.192	5.52	2.98	0.2500	30.4%	35.0%
			Default	0.158	9.74			0.060	8.50				
		1.25	Cyto	1.247	7.48	2.91	0.2500	1.052	5.20	2.72	0.1250	28.0%	34.4%
			Default	0.111	10.39			0.066	7.92				
		2.00	Cyto	1.285	7.86	2.99	0.2500	0.973	5.05	2.46	0.2500	27.6%	32.7%
			Default	0.066	10.85			0.052	7.51				
				Average Difference		2.97		Average Difference		2.83			
	3T3	0.12	Cyto	1.088	6.38	2.70	0.1250	1.163	6.15	2.71	0.1250	29.7%	30.5%
			Default	0.081	9.08			0.081	8.86				
		0.25	Cyto	1.068	6.68	2.68	0.1250	1.089	6.01	2.66	0.1250	28.7%	30.7%
			Default	0.174	9.36			0.073	8.67				
		0.50	Cyto	1.087	7.09	2.68	0.1250	1.073	5.85	2.65	0.1250	27.4%	31.2%
			Default	0.170	9.77			0.049	8.50				
		1.25	Cyto	1.106	7.75	2.67	0.1250	0.975	5.49	2.43	0.1250	25.6%	30.7%
			Default	0.093	10.42			0.081	7.93				
		2.00	Cyto	1.113	8.16	2.68	0.1250	0.887	5.32	2.22	0.1250	24.7%	29.4%
			Default	0.060	10.84			0.058	7.54				
				Average Difference		2.68		Average Difference		2.54			
2	NHK	0.12	Cyto	0.448	10.42	1.33	0.0322	0.702	5.21	1.34	0.0266	11.4%	20.5%
			Default	0.165	11.76			0.256	6.55				
		0.25	Cyto	0.395	10.35	1.29	0.0171	0.764	5.40	1.31	0.0327	11.1%	19.5%
			Default	0.180	11.64			0.313	6.71				
		0.50	Cyto	0.352	10.38	1.18	0.0398	0.739	5.66	1.22	0.0479	10.2%	17.7%
			Default	0.212	11.56			0.312	6.88				
		1.25	Cyto	0.400	10.26	1.28	0.0479	0.590	5.85	1.06	0.0681	11.1%	15.3%
			Default	0.156	11.54			0.191	6.91				
		2.00	Cyto	0.478	10.21	1.41	0.0398	0.526	5.77	1.01	0.0479	12.1%	14.9%
			Default	0.089	11.62			0.142	6.77				
				Average Difference		1.30		Average Difference		1.19			
	3T3	0.12	Cyto	0.433	10.60	1.15	0.0479	0.645	5.39	1.16	0.0479	9.8%	17.7%
			Default	0.163	11.75			0.255	6.56				
		0.25	Cyto	0.471	10.46	1.19	0.0398	0.662	5.52	1.21	0.0398	10.2%	17.9%
			Default	0.189	11.64			0.314	6.72				
		0.50	Cyto	0.522	10.39	1.17	0.0479	0.647	5.71	1.18	0.0398	10.2%	17.1%
			Default	0.214	11.56			0.313	6.89				
		1.25	Cyto	0.550	10.30	1.21	0.0681	0.538	5.90	0.99	0.0681	10.5%	14.3%
			Default	0.148	11.51			0.194	6.89				
		2.00	Cyto	0.555	10.31	1.27	0.0574	0.474	5.82	0.95	0.0398	10.9%	14.0%
			Default	0.083	11.58			0.146	6.77				
				Average Difference		1.20		Average Difference		1.10			

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3	NHK	0.12	Cyto	0.489	9.63	-0.20	0.3750	0.073	3.12	0.29	0.2749	-2.1%	8.4%
			Default	0.264	9.44			0.217	3.41				
		0.25	Cyto	0.407	9.86	0.03	0.3013	0.138	3.37	0.44	0.0098	0.3%	11.5%
			Default	0.275	9.89			0.237	3.80				
		0.50	Cyto	0.288	10.39	0.44	0.1514	0.160	3.59	0.67	0.0015	4.0%	15.8%
			Default	0.207	10.83			0.171	4.26				
		1.25	Cyto	0.254	10.80	0.93	0.0122	0.201	4.19	0.85	0.0049	7.9%	16.8%
			Default	0.083	11.73			0.119	5.03				
		2.00	Cyto	0.290	10.63	1.19	0.0015	0.217	4.51	0.90	0.0015	10.0%	16.6%
			Default	0.038	11.82			0.091	5.41				
				Average Difference		0.48		Average Difference		0.63			
	3T3	0.12	Cyto	0.110	9.27	0.15	0.7520	0.102	3.18	0.23	0.9097	1.6%	6.6%
			Default	0.258	9.42			0.213	3.40				
		0.25	Cyto	0.153	9.65	0.25	0.1475	0.171	3.49	0.31	0.0830	2.5%	8.1%
			Default	0.271	9.90			0.237	3.80				
		0.50	Cyto	0.172	10.39	0.42	0.0522	0.170	3.81	0.45	0.0425	3.9%	10.5%
			Default	0.202	10.81			0.169	4.26				
		1.25	Cyto	0.237	11.05	0.69	0.0425	0.202	4.45	0.59	0.0361	5.8%	11.8%
			Default	0.084	11.73			0.119	5.04				
		2.00	Cyto	0.261	11.03	0.77	0.0640	0.198	4.82	0.59	0.0522	6.5%	10.9%
			Default	0.037	11.80			0.095	5.41				
				Average Difference		0.45		Average Difference		0.43			
4	NHK	0.12	Cyto	0.625	10.11	-0.85	0.1133	0.069	3.05	-0.01	0.1627	-9.2%	-0.2%
			Default	0.098	9.26			0.067	3.04				
		0.25	Cyto	0.560	10.14	-0.71	0.1089	0.093	3.14	-0.02	0.0013	-7.5%	-0.7%
			Default	0.095	9.43			0.092	3.12				
		0.50	Cyto	0.494	10.37	-0.60	0.7960	0.062	3.18	-0.001	0.9229	-6.1%	0.1%
			Default	0.062	9.77			0.057	3.18				
		1.25	Cyto	0.290	10.88	-0.31	0.0730	0.051	3.66	0.04	0.5520	-2.9%	1.2%
			Default	0.043	10.57			0.067	3.70				
		2.00	Cyto	0.095	11.13	-0.03	0.6051	0.061	4.08	0.08	0.5871	-0.3%	1.9%
			Default	0.048	11.10			0.080	4.16				
				Average Difference		-0.50		Average Difference		0.02			
	3T3	0.12	Cyto	0.619	10.56	-1.30	0.0210	0.070	3.05	-0.01	0.5520	-14.0%	-0.2%
			Default	0.102	9.26			0.068	3.04				
		0.25	Cyto	0.543	10.52	-1.09	0.0806	0.093	3.13	0.00	0.4690	-11.6%	0.0%
			Default	0.098	9.42			0.095	3.13				
		0.50	Cyto	0.483	10.67	-0.92	0.0262	0.060	3.20	-0.02	0.0787	-9.5%	-0.7%
			Default	0.065	9.75			0.056	3.18				
		1.25	Cyto	0.283	10.99	-0.42	0.0038	0.057	3.64	0.07	0.0787	-4.0%	1.9%
			Default	0.040	10.57			0.069	3.71				
		2.00	Cyto	0.099	11.11	0.00	0.8040	0.062	4.02	0.15	0.0832	0.0%	3.6%
			Default	0.047	11.11			0.077	4.17				
				Average Difference		-0.75		Average Difference		0.04			

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
5	NHK	0.12	Cyto	0.148	11.89	-0.02	0.6328	0.185	0.39	0.001	0.6953	-0.2%	0.2%
			Default	0.101	11.87			0.189	0.39				
		0.25	Cyto	0.138	11.65	-0.02	0.6250	0.157	1.10	-0.02	0.0625	-0.2%	-1.5%
			Default	0.119	11.63			0.157	1.08				
		0.50	Cyto	0.119	11.25	-0.03	0.3750	0.096	1.82	0.00	1.0000	-0.3%	0.0%
			Default	0.083	11.22			0.097	1.82				
		1.25	Cyto	0.062	10.81	-0.04	0.7695	0.040	2.89	-0.01	0.6426	-0.4%	-0.2%
			Default	0.038	10.77			0.041	2.89				
		2.00	Cyto	0.041	10.87	-0.04	0.2422	0.033	3.36	-0.01	0.6250	-0.3%	-0.2%
			Default	0.011	10.83			0.026	3.35				
				Average Difference		-0.03		Average Difference		-0.01			
	3T3	0.12	Cyto	0.096	11.77	0.11	0.0781	0.188	0.39	0.005	0.2324	0.9%	1.2%
			Default	0.103	11.88			0.188	0.39				
		0.25	Cyto	0.113	11.53	0.09	0.4316	0.155	1.10	-0.01	0.3848	0.8%	-0.8%
			Default	0.117	11.62			0.158	1.09				
		0.50	Cyto	0.080	11.14	0.08	0.0645	0.098	1.82	-0.002	0.8457	0.7%	-0.1%
			Default	0.083	11.22			0.093	1.82				
		1.25	Cyto	0.039	10.75	0.02	0.6953	0.043	2.87	0.01	1.0000	0.2%	0.2%
			Default	0.037	10.77			0.041	2.88				
		2.00	Cyto	0.018	10.84	0.01	0.6953	0.032	3.36	-0.005	0.6250	0.1%	-0.1%
			Default	0.010	10.85			0.027	3.35				
				Average Difference		0.06		Average Difference		-0.001			
6	NHK	0.12	Cyto	0.804	9.34	2.66	0.0195	0.0002	0.0004	0.00004	1.0000	22.2%	9.1%
			Default	0.000	12.00			0.0002	0.0004				
		0.25	Cyto	0.801	9.35	2.65	0.0322	0.033	0.11	-0.002	0.4824	22.1%	-1.6%
			Default	0.002	11.99			0.033	0.10				
		0.50	Cyto	0.732	9.43	2.43	0.0024	0.099	0.73	0.01	0.0398	20.5%	1.2%
			Default	0.034	11.86			0.099	0.73				
		1.25	Cyto	0.462	9.70	1.53	0.0024	0.106	2.14	0.12	0.0479	13.6%	5.5%
			Default	0.058	11.23			0.086	2.27				
		2.00	Cyto	0.288	10.06	0.92	0.0105	0.080	2.85	0.08	0.1465	8.4%	2.7%
			Default	0.031	10.98			0.053	2.93				
				Average Difference		2.04		Average Difference		0.04			
	3T3	0.12	Cyto	0.842	9.81	2.19	0.0195	0.0002	0.004	-0.001	0.7500	18.3%	-25.0%
			Default	0.000	12.00			0.0002	0.003				
		0.25	Cyto	0.839	9.80	2.19	0.0137	0.034	0.11	0.003	0.2334	18.3%	2.3%
			Default	0.002	11.99			0.035	0.12				
		0.50	Cyto	0.779	9.82	2.03	0.0210	0.107	0.74	-0.002	0.6773	17.1%	-0.3%
			Default	0.035	11.85			0.106	0.74				
		1.25	Cyto	0.509	9.98	1.24	0.0522	0.110	2.17	0.11	0.0923	11.0%	4.6%
			Default	0.060	11.22			0.096	2.27				
		2.00	Cyto	0.332	10.19	0.79	0.0425	0.091	2.86	0.09	0.0425	7.2%	3.0%
			Default	0.029	10.99			0.057	2.95				
				Average Difference		1.69		Average Difference		0.04			

Abbreviations: ATC=Acute Toxic Class method (OECD 2001d); Toxcat=Category from Globally Harmonized System of Classification and Labeling of Chemicals[5] (GHS; UN 2005); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 300 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity..

[1]For 2000 ATC simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]Mean number of animals for 2000 simulations.

[3]Difference between mean animals used for the default starting dose and mean animals used for the NRU-based starting dose.

[4]P-value is from one-side Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

[5]GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

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Concordance of NRU-Based Starting Dose with Default Starting Dose for GHS Acute Oral Toxicity Category Outcome for ATC Simulations[1]

GHS Category Outcome with Default Starting Dose	GHS Category Outcome with NHK NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	8	0	0	0	0	0	8	100%	0%	0%
2	0	11	0	0	0	0	11	100%	0%	0%
3	0	1	13	0	0	0	14	93%	0%	7%
4	0	0	0	13	0	0	13	100%	0%	0%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	1	1	100%	0%	0%
Total	8	12	13	13	21	1	68	99%	0%	1%

GHS Category Outcome with Default Starting Dose	GHS Category Outcome with 3T3 NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	8	0	0	0	0	0	8	100%	0%	0%
2	0	11	0	0	0	0	11	100%	0%	0%
3	0	0	15	0	0	0	15	100%	0%	0%
4	0	0	1	11	0	0	12	92%	0%	8%
5	0	0	0	0	20	0	20	100%	0%	0%
6	0	0	0	0	0	1	1	100%	0%	0%
Total	8	11	16	11	20	1	67	99%	0%	1%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); ATC=Acute Toxic Class method (OECD 2001d); NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; RC=Registry of Cytotoxicity.

[1]For 2000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg. The NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621). The default starting dose = 300 mg/kg. Shaded cells are those containing the correct predictions.

[2]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

Discordant Substances[1] for GHS Category[2] Outcomes of ATC Simulations

NRU Test Method	Substance	NRU-Based Starting Dose[3] Toxicity Category	Default Starting Dose[4] Toxicity Category
3T3	Cupric sulfate pentahydrate	3	4
NHK	Hexachlorophene	2	3

Abbreviations: GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); ATC= Acute Toxic Class method (OECD 2001d): NRU=Neutral red uptake; 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes.

[1]Substances for which the simulated ATC outcome for the NRU-based starting dose did not match the simulated ATC outcome for the default starting dose. Simulations were performed with 2000 runs at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
6	$LD_{50} > 5000$ mg/kg

[3]NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621).

[4]The default starting dose = 300 mg/kg.

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Appendix N4

ATC Simulation Results Starting at the Next Fixed Dose Below the LD_{50} Predicted by the 3T3 and NHK NRU IC_{50} and the RC Rat-Only Weight Regression - 2000 mg/kg Upper Limit Dose

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Summary of Animals Used and Animals Dead for ATC Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
			Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
NHK	0.12	Cyto	0.297	9.75	0.91	0.0025	0.280	2.67	0.48	0.0999	8.6%	15.2%
		Default	0.169	10.67			0.334	3.15				
	0.25	Cyto	0.274	9.77	0.98	0.0015	0.276	2.88	0.50	0.3451	9.1%	14.7%
		Default	0.149	10.75			0.324	3.38				
	0.50	Cyto	0.242	9.95	0.96	0.0000	0.254	3.21	0.52	0.0030	8.8%	13.8%
		Default	0.114	10.91			0.297	3.72				
	1.25	Cyto	0.180	10.24	0.86	0.0005	0.193	3.86	0.54	0.0000	7.8%	12.3%
		Default	0.068	11.10			0.228	4.40				
	2.00	Cyto	0.152	10.39	0.86	0.0000	0.160	4.19	0.53	0.0001	7.6%	11.3%
		Default	0.050	11.25			0.189	4.73				
			Average Difference		0.91		Average Difference		0.51			
3T3	0.12	Cyto	0.293	9.55	1.09	0.0006	0.283	2.73	0.47	0.0001	10.2%	14.6%
		Default	0.170	10.64			0.335	3.20				
	0.25	Cyto	0.273	9.61	1.11	0.0002	0.275	2.95	0.48	0.0024	10.4%	14.1%
		Default	0.151	10.73			0.325	3.43				
	0.50	Cyto	0.244	9.85	1.04	0.0001	0.251	3.27	0.50	0.0007	9.6%	13.2%
		Default	0.115	10.89			0.299	3.77				
	1.25	Cyto	0.187	10.22	0.88	0.0000	0.192	3.93	0.51	0.0000	7.9%	11.4%
		Default	0.067	11.10			0.228	4.43				
	2.00	Cyto	0.153	10.43	0.81	0.0000	0.160	4.27	0.49	0.0000	7.2%	10.3%
		Default	0.049	11.25			0.190	4.76				
			Average Difference		0.99		Average Difference		0.49			

Abbreviations: ATC=Acute Toxic Class method (OECD 2001d); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [□g/mL] + 2.024); Default=Default starting dose of 300 mg/kg; Std. Error=Standard error for number of animals; NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; RC=Registry of Cytotoxicity.

[1]For 2000 ATC simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]Mean number of animals for 2000 simulations.

[3]Difference between mean number of animals for the default starting dose and mean number of animals for the NRU-based starting dose.

[4]P-value is from one-sided Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
1	NHK	0.12	Cyto	1.195	6.18	2.91	0.1250	1.180	5.96	2.91	0.1250	32.0%	32.8%
			Default	0.083	9.09			0.080	8.86				
		0.25	Cyto	1.250	6.45	2.91	0.2500	1.156	5.77	2.90	0.2500	31.1%	33.5%
			Default	0.178	9.35			0.070	8.67				
		0.50	Cyto	1.277	6.87	2.87	0.2500	1.157	5.61	2.89	0.1250	29.4%	34.0%
			Default	0.158	9.74			0.060	8.50				
		1.25	Cyto	1.215	7.52	2.87	0.2500	1.033	5.26	2.66	0.1250	27.6%	33.6%
			Default	0.111	10.39			0.066	7.92				
		2.00	Cyto	1.225	7.94	2.90	0.2500	0.940	5.10	2.41	0.1250	26.8%	32.1%
			Default	0.066	10.85			0.052	7.51				
				Average Difference		2.89		Average Difference		2.75			
	3T3	0.12	Cyto	0.987	6.85	2.24	0.1250	1.057	6.62	2.24	0.1250	24.6%	25.3%
			Default	0.081	9.08			0.081	8.86				
		0.25	Cyto	0.980	7.12	2.24	0.1250	1.008	6.44	2.23	0.1250	23.9%	25.7%
			Default	0.174	9.36			0.073	8.67				
		0.50	Cyto	1.029	7.56	2.21	0.1250	0.998	6.28	2.22	0.1250	22.6%	26.1%
			Default	0.170	9.77			0.049	8.50				
		1.25	Cyto	1.011	8.16	2.26	0.1250	0.911	5.89	2.04	0.1250	21.7%	25.7%
			Default	0.093	10.42			0.081	7.93				
		2.00	Cyto	1.017	8.61	2.22	0.1250	0.841	5.68	1.86	0.1250	20.5%	24.7%
			Default	0.060	10.84			0.058	7.54				
				Average Difference		2.23		Average Difference		2.12			
2	NHK	0.12	Cyto	0.333	10.40	1.36	0.0049	0.618	5.20	1.36	0.0144	11.5%	20.7%
			Default	0.165	11.76			0.256	6.55				
		0.25	Cyto	0.266	10.31	1.33	0.0034	0.690	5.38	1.33	0.0046	11.5%	19.8%
			Default	0.180	11.64			0.313	6.71				
		0.50	Cyto	0.192	10.31	1.25	0.0061	0.668	5.66	1.22	0.0134	10.8%	17.8%
			Default	0.212	11.56			0.312	6.88				
		1.25	Cyto	0.261	10.21	1.33	0.0105	0.504	5.82	1.09	0.0046	11.5%	15.8%
			Default	0.156	11.54			0.191	6.91				
		2.00	Cyto	0.344	10.21	1.41	0.0034	0.438	5.74	1.03	0.0012	12.1%	15.3%
			Default	0.089	11.62			0.142	6.77				
				Average Difference		1.33		Average Difference		1.21			
	3T3	0.12	Cyto	0.329	10.27	1.48	0.0061	0.597	5.06	1.49	0.0024	12.6%	22.8%
			Default	0.163	11.75			0.255	6.56				
		0.25	Cyto	0.350	10.13	1.51	0.0024	0.645	5.19	1.53	0.0024	13.0%	22.8%
			Default	0.189	11.64			0.314	6.72				
		0.50	Cyto	0.384	10.06	1.51	0.0061	0.630	5.41	1.48	0.0022	13.0%	21.5%
			Default	0.214	11.56			0.313	6.89				
		1.25	Cyto	0.425	9.98	1.52	0.0061	0.486	5.64	1.25	0.0061	13.2%	18.2%
			Default	0.148	11.51			0.194	6.89				
		2.00	Cyto	0.445	10.03	1.55	0.0046	0.426	5.60	1.17	0.0024	13.4%	17.3%
			Default	0.083	11.58			0.146	6.77				
				Average Difference		1.51		Average Difference		1.39			

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3	NHK	0.12	Cyto	0.489	9.63	-0.20	0.4688	0.073	3.12	0.28	0.0713	-2.1%	8.3%
			Default	0.264	9.44			0.217	3.41				
		0.25	Cyto	0.407	9.86	0.04	0.3013	0.139	3.35	0.45	0.0210	0.4%	11.8%
			Default	0.275	9.89			0.237	3.80				
		0.50	Cyto	0.279	10.41	0.42	0.1099	0.155	3.62	0.64	0.0093	3.8%	15.1%
			Default	0.207	10.83			0.171	4.26				
		1.25	Cyto	0.248	10.91	0.82	0.0342	0.193	4.27	0.76	0.0210	7.0%	15.1%
			Default	0.083	11.73			0.119	5.03				
		2.00	Cyto	0.302	10.74	1.09	0.0034	0.218	4.60	0.81	0.0342	9.2%	15.1%
			Default	0.038	11.82			0.091	5.41				
				Average Difference		0.43		Average Difference		0.59			
	3T3	0.12	Cyto	0.099	9.20	0.22	0.2647	0.104	3.17	0.23	0.1294	2.4%	6.7%
			Default	0.258	9.42			0.213	3.40				
		0.25	Cyto	0.155	9.60	0.31	0.0449	0.165	3.50	0.30	0.1060	3.1%	7.9%
			Default	0.271	9.90			0.237	3.80				
		0.50	Cyto	0.176	10.35	0.47	0.0225	0.160	3.83	0.43	0.0522	4.3%	10.1%
			Default	0.202	10.81			0.169	4.26				
		1.25	Cyto	0.228	11.11	0.62	0.0210	0.180	4.51	0.52	0.0210	5.3%	10.4%
			Default	0.084	11.73			0.119	5.04				
		2.00	Cyto	0.253	11.09	0.71	0.0449	0.186	4.88	0.53	0.0640	6.0%	9.8%
			Default	0.037	11.80			0.095	5.41				
				Average Difference		0.47		Average Difference		0.40			
4	NHK	0.12	Cyto	0.645	10.23	-0.97	0.1445	0.068	3.045	-0.005	0.2444	-10.4%	-0.1%
			Default	0.098	9.26			0.067	3.040				
		0.25	Cyto	0.565	10.23	-0.80	0.1089	0.093	3.13	-0.01	0.0229	-8.5%	-0.4%
			Default	0.095	9.43			0.092	3.12				
		0.50	Cyto	0.500	10.46	-0.69	0.6416	0.058	3.20	-0.02	0.0744	-7.1%	-0.6%
			Default	0.062	9.77			0.057	3.18				
		1.25	Cyto	0.296	10.91	-0.34	0.0256	0.057	3.64	0.06	0.3259	-3.3%	1.6%
			Default	0.043	10.57			0.067	3.70				
		2.00	Cyto	0.093	11.12	-0.02	0.4851	0.070	4.04	0.13	0.6791	-0.2%	3.0%
			Default	0.048	11.10			0.080	4.16				
				Average Difference		-0.57		Average Difference		0.03			
	3T3	0.12	Cyto	0.664	10.65	-1.39	0.0762	0.068	3.04	0.00	0.8160	-15.0%	0.0%
			Default	0.102	9.26			0.068	3.04				
		0.25	Cyto	0.586	10.56	-1.14	0.0437	0.094	3.13	0.00	0.5871	-12.1%	0.0%
			Default	0.098	9.42			0.095	3.13				
		0.50	Cyto	0.496	10.67	-0.93	0.0229	0.057	3.18	-0.01	0.4691	-9.5%	-0.2%
			Default	0.065	9.75			0.056	3.18				
		1.25	Cyto	0.279	10.95	-0.38	0.0928	0.053	3.62	0.08	0.1208	-3.6%	2.2%
			Default	0.040	10.57			0.069	3.71				
		2.00	Cyto	0.105	11.12	-0.01	0.4212	0.061	4.00	0.17	0.1089	-0.1%	4.2%
			Default	0.047	11.11			0.077	4.17				
				Average Difference		-0.77		Average Difference		0.05			

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
5	NHK	0.12	Cyto	0.604	11.03	0.84	0.8867	0.188	0.393	-0.003	0.1055	7.1%	-0.9%
			Default	0.101	11.87			0.189	0.390				
		0.25	Cyto	0.528	10.88	0.75	0.3223	0.156	1.10	-0.02	0.0098	6.4%	-1.8%
			Default	0.119	11.63			0.157	1.08				
		0.50	Cyto	0.365	10.69	0.53	0.1523	0.098	1.819	-0.002	0.7891	4.7%	-0.1%
			Default	0.083	11.22			0.097	1.817				
		1.25	Cyto	0.175	10.52	0.24	0.1934	0.041	2.87	0.01	0.3223	2.2%	0.5%
			Default	0.038	10.77			0.041	2.89				
		2.00	Cyto	0.094	10.69	0.14	0.1934	0.034	3.37	-0.01	0.7695	1.3%	-0.3%
			Default	0.011	10.83			0.026	3.35				
				Average Difference		0.50		Average Difference		0.00			
	3T3	0.12	Cyto	0.876	9.44	2.43	0.0742	0.184	0.38	0.01	0.1856	20.5%	2.1%
			Default	0.103	11.88			0.188	0.39				
		0.25	Cyto	0.751	9.54	2.08	0.1934	0.150	1.09	-0.01	0.5566	17.9%	-0.5%
			Default	0.117	11.62			0.158	1.09				
		0.50	Cyto	0.514	9.80	1.43	0.0273	0.095	1.80	0.02	0.0488	12.7%	1.1%
			Default	0.083	11.22			0.093	1.82				
		1.25	Cyto	0.260	10.08	0.69	0.0273	0.052	2.87	0.01	0.6953	6.4%	0.4%
			Default	0.037	10.77			0.041	2.88				
		2.00	Cyto	0.127	10.49	0.36	0.0273	0.046	3.41	-0.06	0.1055	3.3%	-1.6%
			Default	0.010	10.85			0.027	3.35				
				Average Difference		1.40		Average Difference		0.00			
6	NHK	0.12	Cyto	0.853	8.75	3.25	0.0068	0.00022	0.0005	-0.0001	0.5313	27.1%	-27.3%
			Default	0.000	12.00			0.00021	0.0004				
		0.25	Cyto	0.847	8.75	3.25	0.0105	0.033	0.11	-0.0004	0.1099	27.1%	-3.8%
			Default	0.002	11.99			0.033	0.10				
		0.50	Cyto	0.776	8.91	2.94	0.0081	0.099	0.72	0.02	0.0327	24.8%	2.3%
			Default	0.034	11.86			0.099	0.73				
		1.25	Cyto	0.481	9.44	1.78	0.0085	0.106	2.12	0.15	0.0085	15.9%	6.4%
			Default	0.058	11.23			0.086	2.27				
		2.00	Cyto	0.318	9.89	1.09	0.0266	0.090	2.82	0.11	0.0288	9.9%	3.8%
			Default	0.031	10.98			0.053	2.93				
				Average Difference		2.46		Average Difference		0.05			
	3T3	0.12	Cyto	0.912	8.67	3.33	0.0098	0.00020	0.0005	-0.00017	0.2500	27.7%	-50.0%
			Default	0.000	12.00			0.00018	0.003				
		0.25	Cyto	0.912	8.68	3.31	0.0068	0.034	0.11	0.01	0.0210	27.6%	4.4%
			Default	0.002	11.99			0.035	0.12				
		0.50	Cyto	0.833	8.83	3.02	0.0068	0.106	0.74	0.00	0.8057	25.5%	-0.3%
			Default	0.035	11.85			0.106	0.74				
		1.25	Cyto	0.542	9.41	1.81	0.0122	0.117	2.12	0.15	0.0269	16.1%	6.6%
			Default	0.060	11.22			0.096	2.27				
		2.00	Cyto	0.346	9.86	1.12	0.0161	0.095	2.83	0.12	0.0269	10.2%	4.0%
			Default	0.029	10.99			0.057	2.95				
				Average Difference		2.52		Average Difference		0.05			

Abbreviations: ATC=Acute Toxic Class method (OECD 2001d); Toxcat=Category from Globally Harmonized System of Classification and Labeling of Chemicals[5] (GHS; UN 2005); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024); Default=Default starting dose of 300 mg/kg; Std. Error=Standard error for number of animals; NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; RC=Registry of Cytotoxicity.

[1]For 2000 ATC simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]Mean number of animals for 2000 simulations.

[3]Difference between mean animals used for the default starting dose and mean animals used for the NRU-based starting dose.

[4]P-value is from one-side Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

[5]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

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Concordance of NRU-Based Starting Dose with Default Starting Dose for GHS Acute Oral Toxicity Category Outcome for ATC Simulations[1]

GHS Category Outcome with Default Starting Dose	GHS Category Outcome with NHK NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	8	0	0	0	0	0	8	100%	0%	0%
2	0	11	0	0	0	0	11	100%	0%	0%
3	0	1	13	0	0	0	14	93%	0%	7%
4	0	0	1	12	0	0	13	92%	0%	8%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	1	1	100%	0%	0%
Total	8	12	14	12	21	1	68	97%	0%	3%

GHS Category Outcome with Default Starting Dose	GHS Category Outcome with 3T3 NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	8	0	0	0	0	0	8	100%	0%	0%
2	0	11	0	0	0	0	11	100%	0%	0%
3	0	0	15	0	0	0	15	100%	0%	0%
4	0	0	1	11	0	0	12	92%	0%	8%
5	0	0	0	0	20	0	20	100%	0%	0%
6	0	0	0	0	0	1	1	100%	0%	0%
Total	8	11	16	11	20	1	67	99%	0%	1%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); ATC=Acute Toxic Class method (OECD 2001d); NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; RC=Registry of Cytotoxicity.

[1]For 2000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg. The NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024).. The default starting dose = 300 mg/kg. Shaded cells are those containing the correct predictions.

[2]

GHS Toxicity Category	Oral LD_{50} Limit
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

Discordant Substances[1] for GHS Category[2] Outcomes of ATC Simulations

NRU Test Method	Substance	NRU-Based Starting Dose[3] Toxicity Category	Default Starting Dose[4] Toxicity Category
3T3	Cupric sulfate pentahydrate	3	4
NHK	Hexachlorophene	2	3
NHK	Propranolol	3	4

Abbreviations: GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); ATC= Acute Toxic Class method (OECD 2001d); 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; NRU=Neutral red uptake.

[1]Substances for which the simulated ATC outcome for the NRU-based starting dose did not match the simulated ATC outcome for the default starting dose. Simulations were performed with 2000 runs at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
6	$LD_{50} > 5000$ mg/kg

[3]NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [μg/mL] + 2.024).

[4]The default starting dose = 300 mg/kg.

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Appendix O

Federal Register Notices

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committee (NMQAAC). Concurrently, nomination materials for prospective candidates should be sent to FDA by April 21, 2005. A nominee may either be self-nominated or nominated by an organization to serve as a nonvoting industry representative.

ADDRESSES: All letters of interest and nominations should be sent to the contact person listed in the **FOR FURTHER INFORMATION** section of this notice.

FOR FURTHER INFORMATION CONTACT: Kathleen L. Walker, Center for Devices and Radiological Health (HFZ–17), Food and Drug Administration, 2098 Gaither Rd., Rockville, MD 20850, 240–276–0450, ext. 114.

SUPPLEMENTARY INFORMATION: The Mammography Quality Standards Reauthorization Act of 2004 (Public Law 108–365) requires the addition of at least two industry representatives with expertise in mammography equipment to the National Mammography Quality Assurance Advisory Committee.

I. Functions of NMQAAC

The functions of the NMQAAC are to advise FDA on: (1) Developing appropriate quality standards and regulations for mammography facilities, (2) developing appropriate standards and regulations for bodies accrediting mammography facilities under this program, (3) developing regulations with respect to sanctions, (4) developing procedures for monitoring compliance with standards, (5) establishing a mechanism to investigate consumer complaints, (6) reporting new developments concerning breast imaging which should be considered in the oversight of mammography facilities, (7) determining whether there exists a shortage of mammography facilities in rural and health professional shortage areas and determining the effects of personnel on access to the services of such facilities in such areas, (8) determining whether there will exist a sufficient number of medical physicists after October 1, 1999, and (9) determining the costs and benefits of compliance with these requirements.

II. Selection Procedure

Any organization representing the mammography device industry wishing to participate in the selection of a nonvoting member to represent industry should send a letter stating that interest to the FDA contact (see **FOR FURTHER INFORMATION CONTACT**) within 30 days of publication of this notice. Persons who nominate themselves as industry representatives will not participate in the selection process. It is, therefore, recommended that nominations be made by someone within an organization, trade association or firm who is willing to participate in the selection process. Within the subsequent 30 days, FDA will send a letter to each organization and a list of all nominees along with their resumes. The letter will state that the interested organizations are responsible for conferring with one another to select a candidate, within 60 days after receiving the letter, to serve as the nonvoting member representing the a particular committee. If no individual is selected within the 60 days, the Commissioner of Food and Drugs (the Commissioner) may select the nonvoting member to represent industry interests.

III. Qualifications

Persons nominated for membership on the committee as an industry representative must meet the following criteria:(1) Demonstrate expertise in mammography equipment and (2) be able to discuss equipment specifications and quality control procedures affecting mammography equipment. The industry representative must be able to represent the industry perspective on issues and actions before the advisory committee; serve as liaison between the committee and interested industry parties; and facilitate dialogue with the advisory committee on mammography equipment issues.

IV. Application Procedure

Individuals may nominate themselves, or an organization representing the mammography device industry may nominate one or more individuals to serve as nonvoting industry representatives. A current curriculum vitae (which includes the nominee's business address, telephone number, and e-mail address) and the name of the committee of interest should be sent to the FDA contact person. FDA will forward all nominations to the organizations that have expressed interest in participating in the selection process for the committee.

FDA has a special interest in ensuring that women, minority groups, individuals with disabilities, and small businesses are adequately represented on its advisory committees. Therefore, the agency encourages nominations for appropriately qualified candidates from these groups.

This notice is issued under the Federal Advisory Committee Act (5 U.S.C. app. 2) and 21 CFR part 14 relating to advisory committees.

Dated: March 14, 2005.

Sheila Dearybury Walcoff,

Associate Commissioner for External Relations.

[FR Doc. 05–5551 Filed 3–21–05; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Toxicology Program; National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Request for Nominations for an Independent Peer Review Panel To Evaluate In Vitro Testing Methods for Estimating Acute Oral Systemic Toxicity and Request for In Vivo and In Vitro Data

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), HHS.

ACTION: Request for nominations for an independent peer review panel and request for *in vivo* and *in vitro* data.

SUMMARY: The NTP Interagency Center for Evaluation of Alternative Toxicological Methods (NICEATM) in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is planning to convene an independent peer review panel (hereafter, Panel) to evaluate the validation status of two *in vitro* cytotoxicity assays for estimating *in vivo* acute oral toxicity. The Panel will evaluate the usefulness, limitations, accuracy, and reliability of these test methods for their intended purpose. NICEATM requests nominations of expert scientists for consideration as potential Panel members. ICCVAM will consider the conclusions and recommendations from the Panel in developing test method recommendations and performance standards for these test methods. Data from standard *in vivo* acute oral toxicity testing and *in vitro* cytotoxicity testing also is requested.

DATES: Nominations and data should be received by noon on May 6, 2005.

ADDRESSES: Nominations and data should be sent by mail, fax, or e-mail to Dr. William S. Stokes, Director of NICEATM, at NICEATM, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov*. Courier address: NICEATM, 79 T.W. Alexander Drive,

Building 4401, Room 3128, Research Triangle Park, NC 27709.

FOR FURTHER INFORMATION CONTACT: NICEATM, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov.*

SUPPLEMENTARY INFORMATION:

Background

NICEATM and the European Committee on the Validation of Alternative Methods (ECVAM) conducted a collaborative validation study to independently evaluate the usefulness of two *in vitro* basal cytotoxicity assays proposed for estimating *in vivo* rat acute oral toxicity. Neutral red uptake assays using both a mouse cell line (*i.e.*, BALB/c 3T3 fibroblasts) and a primary human cell type (*i.e.*, normal human epithelial keratinocytes) were evaluated in a multi-laboratory validation study. Cytotoxicity results are proposed for use in predicting starting doses for *in vivo* acute oral lethality assays, which may reduce the number of animals required for such determinations.

NICEATM is preparing Background Review Documents on the two *in vitro* test methods that will contain comprehensive summaries of available data, an analysis of the accuracy and reliability of standardized test method protocols, and related information characterizing the current validation status of these assays. Once completed, the Background Review Documents will be provided to the Panel and made available to the public. Meeting information, including date and location, and public availability of the Background Review Documents will be announced in a future **Federal Register** notice and posted on the ICCVAM/NICEATM Web site (*http://iccvam.niehs.nih.gov*).

Request for the Nomination of Scientists for the Peer Review Panel

NICEATM invites nominations of scientists with relevant knowledge and experience to serve on the Panel. Areas of relevant expertise include, but are not limited to: physiology and pharmacology, acute systemic toxicity testing in animals, evaluation and treatment of acute toxicity in humans, development and use of *in vitro* methodologies, biostatistical data analysis, knowledge of chemical data sets useful for validation of acute toxicity studies, and hazard classification of chemicals and products. Each nomination should include the person's name, affiliation, contact information (*i.e.* mailing address, e-mail address, telephone and fax numbers), and a brief summary of relevant experience and qualifications. Nominations should be sent to NICEATM by mail, fax, or e-mail within 45 days of the publication of this notice. Correspondence should be directed to Dr. William Stokes, Director, NICEATM, at the address given above.

Request for Data

NICEATM invites the submission of data from standard *in vivo* acute oral toxicity testing and *in vitro* cytotoxicity testing. Two previous requests for existing *in vivo* and *in vitro* acute toxicity data have been made (**Federal Register**, Vol. 69, No. 201, pp. 61504–5, October 19, 2004 and Vol. 65, No. 115, pp. 37400–3, June 14, 2000). *In vivo* and *in vitro* acute toxicity testing data for chemicals or products should be sent to NICEATM by mail, fax, or e-mail to the address given above. Data submitted by the deadline listed in this notice will be considered during an evaluation of the validation status of the two cytotoxicity methods, anticipated in late 2005; however, data will be accepted at any time. Chemical and protocol information/test data submitted in response to this notice may be incorporated in future NICEATM and ICCVAM reports and publications as appropriate.

When submitting chemical and protocol information/test data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

NICEATM prefers data to be submitted as copies of pages from study notebooks and/or study reports, if available. Raw data and analyses available in electronic format may also be submitted. Each submission for a chemical should preferably include the following information, as appropriate:

- Common and trade name.
- Chemical Abstracts Service Registry Number (CASRN).
- Chemical class.
- Product class.
- Commercial source.
- *In vitro* basal cytotoxicity test protocol used.
- *In vitro* cytotoxicity test results.
- *In vivo* acute oral toxicity test protocol used.
- Individual animal responses at each observation time (if available).
- The extent to which the study complied with national or international Good Laboratory Practice (GLP) guidelines.
- Date and testing organization.

Those persons submitting data on chemicals tested for *in vitro* basal cytotoxicity are referred to the standard test-reporting template recommended for the High Production Volume (HPV) program at *http://www.epa.gov/chemrtk/toxprtow.htm* or at *http://iccvam.niehs.nih.gov/methods/invitro.htm. In vivo* data for the same chemicals should be reported as recommended in the test reporting section of the current Environmental Protection Agency (EPA) guideline for acute oral toxicity (EPA, 2002).

Submitted data will be used to further evaluate the usefulness and limitations of *in vitro* cytotoxicity data for estimating acute oral toxicity and will be included in a database to support the investigation of other test methods necessary to improve the accuracy of *in vitro* assessments of acute systemic toxicity.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 (Pub. L. 106–545, available at *http://iccvam.niehs.nih.gov/about/PL106545.htm*) establishes ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM can be found at the following Web site: *http://iccvam.niehs.nih.gov.*

Dated: March 11, 2005.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 05–5564 Filed 3–21–05; 8:45 am]

BILLING CODE 4140–01–P

and Eukaryotic Genetics and Molecular Biology.

Date: November 3–5, 2004.

Time: 7 a.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: Hyatt Regency Bethesda, One Bethesda Metro Center, 7400 Wisconsin Avenue, Bethesda, MD 20814.

Contact Person: Mary P. McCormick, PhD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 2208, MSC 7890, Bethesda, MD 20892, (301) 435–1047, *mccormim@csr.nih.gov.*

Name of Committee: Center for Scientific Review Special Emphasis Panel, Fetal Basis for Adult Disease.

Date: November 3–4, 2004.

Time: 7 a.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: Bethesda Marriott Suites, 6711 Democracy Boulevard, Bethesda, MD 20817.

Contact Person: Ray Bramhall, PhD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 6046 F, MSC 7892, Bethesda, MD 20892, (910) 458–1871, *bramhalr@csr.nih.gov.*

(Catalogue of Federal Domestic Assistance Program Nos. 93.306, Comparative Medicine; 93.333, Clinical Research, 93.306, 93.333, 93.337, 93.393–93.396, 93.837–93.844, 93.846–93.878, 93.892, 93.893, National Institutes of Health, HHS.)

Dated: October 7, 2004.

LaVerne Y. Stringfield,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. 04–23350 Filed 10–18–04; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS); National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM): Availability of Updated Standardized *In Vitro* Cytotoxicity Test Method Protocols for Estimating Acute Oral Systemic Toxicity; Request for Existing *In Vivo* and *In Vitro* Acute Toxicity Data

Summary: NICEATM announces the availability of two updated standardized in vitro cytotoxicity test method protocols to estimate acute oral systemic toxicity in rodents. These two test methods were previously recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for selecting starting doses for in vivo acute oral systemic toxicity tests (**Federal Register** Vol. 66, No. 189, pages 49686–49687, September 28, 2001). This approach can reduce the number of animals required for acute oral toxicity testing. NICEATM also requests the submission of existing and future data on chemicals and products tested for both acute oral systemic toxicity and in vitro cytotoxicity using the standardized test method protocols mentioned in this notice. These data will be used to further evaluate the usefulness and limitations of cytotoxicity methods for estimating in vivo acute oral toxicity. The data will also be used to establish a database to support the investigation of other test methods necessary to improve the accuracy of in vitro assessments of acute systemic toxicity.

Availability of Standardized Test Method Protocols for Estimating Starting Doses for *In Vivo* Acute Oral Toxicity Tests

Updated standardized protocols for two neutral red uptake assays using either BALB/c 3T3 cells or normal human keratinocytes are now available at: *http://iccvam.niehs.nih.gov/methods/invitro.htm.* These test method protocols have been improved to maximize intra- and inter-laboratory reproducibility and are currently being used for the final phase of a joint NICEATM-European Center for the Validation of Alternative Methods (ECVAM) validation study. NICEATM recommends that these updated test method protocols be used in place of standard operating procedures previously recommended by ICCVAM for two cytotoxicity test methods to estimate starting doses for in vivo acute oral toxicity tests (ICCVAM, 2001b).

Submission of Chemical and Protocol Information/Test Data

In vivo and in vitro acute toxicity testing data for chemicals or products should be sent by mail, fax or e-mail to NICEATM [Dr. William S. Stokes, Director, NICEATM, NIEHS, PO Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *iccvam@niehs.nih.gov*]. Data will be accepted at any time. Data submitted within the next 9 months will be considered during an evaluation of the validation status of the two cytotoxicity methods anticipated in late 2005. Chemical and protocol information/test data submitted in response to this notice may be incorporated in future NICEATM and ICCVAM reports and publications as appropriate.

When submitting chemical and protocol information/test data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

NICEATM prefers data to be submitted as copies of pages from study notebooks and/or study reports, if available. Raw data and analyses available in electronic format may also be submitted. Each submission for a chemical should preferably include the following information, as appropriate:

- Common and trade name
- Chemical Abstracts Service Registry Number (CASRN)
- Chemical and/or product class
- Commercial source
- In vitro basal cytotoxicity test protocol used
- In vitro cytotoxicity test results
- In vivo acute oral toxicity test protocol used
- Individual animal responses at each observation time (if available)
- The extent to which the study complied with national or international Good Laboratory Practice (GLP) guidelines
- Date and testing organization

Those persons submitting data on chemicals tested for in vitro basal cytotoxicity are referred to the standard test-reporting template recommended for the High Production Volume (HPV) program at *http://www.epa.gov/chemrtk/toxprtow.htm* or at *http://iccvam.niehs.nih.gov/methods/invitro.htm.* In vivo data for the same chemicals should be reported as recommended in the test reporting section of the current Environmental Protection Agency (EPA) guideline for acute oral toxicity (EPA, 2002).

Submitted data will be used to further evaluate the usefulness and limitations of in vitro cytotoxicity data for estimating acute oral toxicity, and will be included in a database to support the investigation of other test methods necessary to improve the accuracy of in vitro assessments of acute systemic toxicity.

History

In September 2001, the ICCVAM recommended that in vitro cytotoxicity test methods be considered as a tool for estimating starting doses for in vivo acute systemic toxicity testing studies (**Federal Register** Vol. 66, No. 189, pages 49686–49687, September 28, 2001.) The recommendations were based on the Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity (ICCVAM, 2001a). The Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity (ICCVAM, 2001b) was

also made available at that time. The guidance document provided standard operating procedures for two cytotoxicity test methods and instructions for using these assays to estimate starting doses for in vivo testing.

Federal agency responses to the ICCVAM test method recommendations were announced on March 10, 2004 (**Federal Register** Vol. 69, No. 47, pages 11448–11449). Federal agencies agreed to encourage, to the extent applicable, the use of in vitro tests for determining starting doses for acute systemic toxicity testing. Furthermore, EPA specifically encouraged those participating in the HPV Challenge Program to consider using the recommended in vitro tests as a supplemental component in conducting any new in vivo acute oral toxicity studies for the program (*http://www.epa.gov/chemrtk/toxprtow.htm*).

A NICEATM–ECVAM validation study was initiated in 2002 to evaluate the usefulness of the two neutral red uptake cytotoxicity assays currently available for predicting starting doses for in vivo acute oral toxicity tests. During the pre-validation phases of the study, the test method protocols were further standardized and revised to improve their intra- and inter-laboratory reproducibility. NICEATM recommends using the revised test method protocols rather than the standard operating procedures outlined in the guidance document (ICCVAM, 2001b.) The guidance document should be consulted for the procedure for calculating starting doses using in vitro cytotoxicity data.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from fifteen Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM promotes the development, validation, regulatory acceptance, and national and international harmonization of toxicological test methods that more accurately assess the safety or hazards of chemicals and products, and test methods that refine, reduce and replace animal use. The ICCVAM Authorization Act of 2000 (available at *http://iccvam.niehs.nih.gov/about/PL106545.htm*) established ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific support for ICCVAM and ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM can be found at the following Web site: *http://iccvam.niehs.nih.gov/*.

References

EPA. 2002. Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, EPA 712–C–02–190. Available at: *http://www.epa.gov/opptsfrs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870–1100.pdf*.

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). 2001a. Report of the international workshop on in vitro methods for assessing acute systemic toxicity. NIH Publication 01–4499. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available at: *http://iccvam.niehs.nih.gov/*.

ICCVAM. 2001b. Guidance document on using in vitro data to estimate in vivo starting doses for acute toxicity. NIH Publication 01–4500. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available at: *http://iccvam.niehs.nih.gov/*.

Dated: October 6, 2004.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 04–23335 Filed 10–18–04; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HOMELAND SECURITY

Coast Guard

[CGD17–04–002]

Cook Inlet Regional Citizen's Advisory Committee; Charter Renewal

AGENCY: Coast Guard, DHS.

ACTION: Notice of recertification.

SUMMARY: The Coast Guard has recertified the Cook Inlet Regional Citizen's Advisory Council for the period covering September 1, 2004 through August 31, 2005. Under the Oil Terminal and Oil Tanker Environmental Oversight Act of 1990, the Coast Guard may certify on an annual basis an alternative voluntary advisory group in lieu of a regional citizens' advisory council for Cook Inlet, Alaska. This advisory group monitors the activities of terminal facilities and crude oil tankers under the Cook Inlet Program established by the statute.

DATES: The Cook Inlet Regional Citizen's Advisory Council is certified through August 31, 2005.

ADDRESSES: You may request a copy of the recertification letter by writing to Commander, Seventeenth Coast Guard District (mor), P.O. Box 25517, Juneau, AK 99802–5517.

FOR FURTHER INFORMATION CONTACT: Lieutenant Andrew Vanskike, Seventeenth Coast Guard District (mor), 907–463–2818.

SUPPLEMENTARY INFORMATION:

Background And Purpose

On September 1, 2004, the Coast Guard recertified the Cook Inlet Regional Citizen's Advisory Council (CIRCAC) through August 31, 2005. Under the Oil Terminal and Oil Tanker Environmental Oversight Act of 1990 (33 U.S.C. 2732), the Coast Guard may certify, on an annual basis, an alternative voluntary advisory group in lieu of a regional citizens' advisory council for Cook Inlet, Alaska. This advisory group monitors the activities of terminal facilities and crude oil tankers under the Cook Inlet Program established by Congress, 33 U.S.C. 2732 (b).

On September 16, 2002, the Coast Guard published a notice of policy on revised recertification procedures for alternative voluntary advisory groups in lieu of councils at Prince William Sound and Cook Inlet, AK (67 FR 58440, 58441). This revised policy indicated that applicants seeking recertification in 2003 and 2004 need only submit a streamlined application and public comments would not be solicited prior to recertification.

Dated: September 24, 2004.

James C. Olson,

Rear Admiral, U.S. Coast Guard, Commander, Seventeenth Coast Guard District.

[FR Doc. 04–23370 Filed 10–18–04; 8:45 am]

BILLING CODE 4910–15–M

DEPARTMENT OF HOMELAND SECURITY

Federal Emergency Management Agency

Notice of Adjustment of Countywide Per Capita Impact Indicator

AGENCY: Federal Emergency Management Agency, Emergency Preparedness and Response Directorate, Department of Homeland Security.

ACTION: Notice.

SUMMARY: FEMA gives notice that the countywide per capita impact indicator under the Public Assistance program for disasters declared on or after October 1, 2004 will be increased.

DATES: Effective October 1, 2004 and applies to major disasters declared on or after October 1, 2004.

FOR FURTHER INFORMATION CONTACT: James A. Walke, Recovery Division, Federal Emergency Management

Natives (AI/AN) tribal governments to all available programs in the Department of Health and Human Services (HHS), and coordinate the tribal consultation activities associated with formulation of the IHS annual budget request. The application is for a five year project which will commence with an initial award on March 15, 2004. The initial budget period will be awarded at $227,00.00 and the entire project is expected to be awarded at $1,135,000.00.

The award is issued under the authority of the Public Health Service Act, section 301(a) and is included under the Catalog of Federal Domestic Assistance number 93.933. The specific objectives of the project are to:

1. Provide ongoing technical advice and consultation as the national Indian organization that is representative of all tribal governments in the area of health care policy analysis and program development.

2. Assure that health care advocacy is based on tribal input through a broad-based consumer network involving the Area Indian Health Boards or Health Board Representatives from each of the 12 IHS Areas.

3. Establish relationships with other national Indian organizations, with professional groups and with Federal, State and local entities to serve as advocates for AI/AN health programs. As a recipient of a grant/cooperative agreement, the NIHB is prohibited from conducting lobbying activities using Federal funding.

4. Improve and expand access for AI/AN tribal governments to all available programs in the HHS.

5. Publish, at least three times a year, a newsletter featuring articles on health promotion/disease prevention activities and models of best or improving practices, health policy and funding information relevant to AI/AN, *etc.*

6. Disseminate timely health care information to tribal governments, AI/AN Health Boards, other national Indian organizations, professional groups, Federal, State, and local entities.

7. Coordinate the tribal consultation activities associated with formulation of the IHS annual budget request.

Justification for Single Source: This project has been awarded on a non-competitive single source basis. NIHB is the only national AI/AN organization with health expertise that represents the interest of all federally recognized tribes.

Use of Cooperative Agreement: A non-competitive single source Cooperative Agreement Award will involve:

1. IHS staff will review articles concerning the Agency for accuracy and may, as requested by the NIHB, provide articles.

2. IHS staff will have aproval over the hiring of key personnel as defined by regulation or provision in the cooperative agreement.

3. IHS will provide technical assistance to the NIHB as requested and attend and participate in all NIHB Board meetings.

FOR FURTHER INFORAMTION CONTACT: Douglas Black, Director, Office of Tribal Programs, Office of the Director, Indian Health Service, 801 Thompson Avenue, Reyes Building, Suite 220, Rockville, Maryland 20852, telephone (301) 443–1104. For grants information, contact Sylvia Tyan, Grants Management Specialist, Division of Acquisition and Grants Management Branch, 1200 Twinbrook Parkway, Room 450A, Rockville, Maryland 20852, telephone (301) 443–5204.

Dated: March 1, 2004.

Charles W. Grim,

Assistant Surgeon General, Director, Indian Health Service.

[FR Doc. 04–5305 Filed 3–9–04; 8:45 am]

BILLING CODE 4160–16–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Office of the Director; Notice of Meeting

The Office of the Director, National Institutes of Health (NIH), announces a meeting of the NIH Blue Ribbon Panel on Conflict of Interest Policies, a working group of the Advisory Committee to the director, NIH. The meeting is scheduled for March 12–13, 2004. The meeting will be held at the NIH, 9000 Rockville Pike, Bethesda, Maryland, Building 31C, Conference Room 6. Attendance will be limited to space available. In the interest of security, NIH has instituted stringent procedures for entrance into the building by non-government employees. Persons without a government I.D. will need to shop a photo I.D. and sign in at the security desk upon entering the building.

On March 12, the Panel will meet in closed, Executive Session, from 8:30–10 a.m., and in public session, from 10 a.m.–6:15 p.m. On March 13, the Panel will meet in closed, Executive Session, from 8:30 a.m.–2 p.m. The agenda will be posted on the NIH Web site *(http://www.nih.gov)* prior to the meeting.

During the public session, time will be set aside for oral presentations by the public. Any person wishing to take a presentation should notify Charlene French, Office of Science Policy, National Institutes of Health, Building 1, Room 103, Bethesda, Maryland 20892, telephone (301) 496–2122 by March 11, 2004 or by e-mail: *blueribbonpanel@mail.nih.gov.*

Oral comments will be limited to 5 minutes. Due to time constraints, only one representative from each organization will be allotted time for oral testimony. The number of speakers and the time allotment may also be limited by the number of presentations. The opportunity to speak will be based on a first come first served basis. All requests to present oral comments should include the name, addresses, telephone number, and business or professional affiliation of the interested party, and should indicate the areas of interest or issue to be addressed. Please provide, if possible, an electronic copy of your comments.

Any person attending the meeting who has not registered to speak in advance of the meeting will be allowed to make a brief oral statement during the time set aside for public comment, if time permits and at the discretion of the co-chairs.

Individuals who plan to attend the meeting and need special assistance, such as sign language interpretation or other reasonable accommodations, should notify Charlene French at the address listed earlier in this notice in advance of the meeting.

Dated: March 5, 2004.

LaVerne Stringfield,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. 04–5504 Filed 3–8–04; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS); National Toxicology Program (NTP); Notice of the Availability of Agency Responses to ICCVAM Test Recommendations for the Revised Up-and-Down Procedure for Determining Acute Oral Toxicity and In Vitro Methods for Assessing Acute Systemic Toxicity

Summary

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) announces the availability of Federal agency responses to Interagency Coordinating Committee on the Validation of Alternative Methods

(ICCVAM) test recommendations for: (1) The revised Up-and-Down Procedure (UDP) for determining acute oral toxicity and (2) *in vitro* methods for assessing acute systemic toxicity. Pursuant to sections 3 of the ICCVAM Authorization Act of 2000 [Pub. L. 106–545 (42 U.S.C. 285l–4)], ICCVAM is required to make final ICCVAM test recommendations and the responses from agencies regarding such recommendations available to the public.

Availability of Agency Responses

The agency responses to the ICCVAM test recommendations and other current information relevant to these test recommendations are available electronically (PDF and HTML formats) on the NICEATM/ICCVAM Web site at *http://iccvam.niehs.nih.gov*. Hard copy versions of these responses can be requested by contacting NICEATM at P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709 (mail), 919–541–2384 (telephone), 919–541–0947 (fax), or *niceatm@niehs.nih.gov*.

In summary, the Federal agencies agreed that the UDP had been adequately validated as a replacement for the conventional LD50 test and indicated to the extent applicable, that they will encourage the use of *in vitro* tests for determining starting doses for acute systemic toxicity testing.

ICCVAM Recommmendations

NICEATM announced availability of the ICCVAM recommendations for the UDP on February 7, 2002 (**Federal Register** Vol. 67, No. 26, pages 5842–5844). ICCVAM recommends based upon the report, *The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals; Results of an Independent Peer Review Evaluation Organized by the ICCVAM and NICEATM*, NIH Publication No. 02–4501, that the UDP be used instead of the conventional LD50 test to determine the acute oral toxicity hazard of chemicals for hazard classification and labeling purposes.

NICEATM announced availability of the ICCVAM recommendations for the *in vitro* methods for assessing acute systemic toxicity on September 28, 2001 (**Federal Register** Vol. 66, No. 189, pages 49686–49687). ICCVAM recommends based upon the reports, *Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity*, NIH Publication No. 01–4499, and the *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity*, NIH Publication No. 01–4500, that the *in vitro* methods be considered as a tool for estimating starting doses for animal tests of acute systemic toxicity.

Background Information on ICCVAM and NICEATM

The NIEHS established the ICCVAM in 1997 to coordinate the interagency technical review of new, revised, and alternative test methods of interagency interest, and to coordinate cross-agency issues relating to the validation, acceptance, and national/international harmonization of toxicological testing methods. ICCVAM was established as a permanent interagency committee of the NIEHS under the NICEATM on December 19, 2000, by the ICCVAM Authorization Act of 2000 (Pub. L. 106–545, available at *http://iccvam.niehs.nih.gov/about/PL106545.pdf*). The Committee is composed of representatives from fifteen Federal regulatory and research agencies that use or generate toxicological information. ICCVAM promotes the scientific validation and regulatory acceptance of toxicological test methods that will improve agencies' ability to accurately assess the safety or hazards of chemicals and various types of products, while refining (less pain and distress), reducing, and replacing animal use wherever possible. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM and ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM can be found at the following Web site: *http://iccvam.niehs.nih.gov*.

Dated: March 2, 2004.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 04–5321 Filed 3–9–04; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HOMELAND SECURITY

Coast Guard

[USCG–2000–7848]

Inland Tank Barge Certificates of Inspection; Administrative Changes

AGENCY: Coast Guard, DHS.

ACTION: Notice of results.

SUMMARY: The Coast Guard commissioned a one-year tank barge Certificate of Inspection (COI) pilot program to test administrative changes to inland tank barge COIs. Under the old Marine Safety Information System, a regulatory change would have been required had any changes been made to the COIs. Use of the new Marine Information for Safety and Law Enforcement information system allows easy access to the COIs; therefore no change in the regulations is needed.

DATES: No further actions are planned.

FOR FURTHER INFORMATION CONTACT: For questions on this Notice, contact Commander Robert Hennessy, U.S. Coast Guard Headquarters, 2100 Second Street, SW., Washington, DC 20593–0001, telephone: 202–267–0103, facsimile: 202–267–4570, e-mail: *RHennessy@comdt.uscg.mil* or Lieutenant Raymond Lechner, U.S. Coast Guard Marine Safety Center, 400 7th Street, SW., Washington, DC 20590, telephone: 202–366–6462, e-mail: *RLechner@msc.uscg.mil*.

SUPPLEMENTARY INFORMATION: A pilot program was initiated to evaluate a Chemical Transportation Advisory Committee (CTAC) recommendation. The pilot program assessed the benefits of shifting the vessel cargo authority and conditions of carriage information from one required document (the vessel's Certificate of Inspection (COI)) to another required document (the vessel's cargo transfer procedures). Background information about the pilot program conducted by the Marine Safety Office, New Orleans, LA, in cooperation with the Marine Safety Center, American Commercial Barge Lines, and the Petroleum Services Corporation, can be found in the August 31, 2000, **Federal Register** Notice (65 FR 53071).

Since the pilot program was initiated, the Coast Guard now has the Marine Information for Safety and Law Enforcement (MISLE) information system in use. MISLE allows for a different presentation of cargo information than the old Marine Safety Information System. A Certificate of Inspection for inland tank barges and a newly developed Cargo Authority Attachment are now easily accessible from the MISLE; therefore, no changes in the regulations are required. Additional information can be found on the Marine Safety Center's Web site: *http://www.uscg.mil/hq/msc/T2.misle.htm* under "T2: Tank Vessel Cargo and Vapor Control Authority Under MISLE."

Dated: February 27, 2004.

Joseph J. Angelo,

Director of Standards, Marine Safety, Security and Environmental Protection.

[FR Doc. 04–5300 Filed 3–9–04; 8:45 am]

BILLING CODE 4910–15–P

valid for use as replacements for the animal test and were ready to be considered for regulatory acceptance (Balls and Corcelle, 1998; Balls and Hellsten, 2000). The European Scientific Committee for Cosmetic Products and Non-food Products (SCCNFP) evaluated the EPISKIN™ and Rat Skin TER and concluded that they were applicable for the safety evaluation of cosmetic ingredients or mixtures of ingredients (Anon., 1999). The European Commission subsequently adopted EpiDerm™, EPISKIN™, and Rat Skin TER (Anon., 2000).

Proposed ICCVAM Recommendations

ICCVAM proposes that these assays can be used to assess the dermal corrosion potential of chemicals in a weight-of-evidence approach in an integrated testing scheme [e.g., OECD Globally Harmonised Classification System (OECD, 1998); OECD Revised Proposals for Updated Test Guidelines 404 and 405: Dermal and Eye Corrosion/Irritation Studies (OECD, 2001a)]. These integrated testing schemes for dermal irritation/corrosion allow for the use of validated and accepted in vitro methods. In this approach, positive in vitro corrosivity responses do not generally require further testing and can be used for classification and labeling. Negative in vitro corrosivity responses shall be followed by in vivo dermal corrosion/irritation testing. (Note: The first animal used in the irritation/corrosivity assessment would be expected to identify any chemical corrosives that were false negatives in the in vitro test). Furthermore, as is appropriate for any in vitro assay, there is the opportunity for confirmatory testing if false positive results are indicated on a weight of evidence evaluation of supplemental information, such as pH, structure activity relationships (SAR), and other chemical and testing information.

Additional Information About ICCVAM and NICEATM

ICCVAM, with 15 participating Federal agencies, was established in 1997 to coordinate interagency issues on toxicological test method development, validation, regulatory acceptance, and national and international harmonization. The ICCVAM Authorization Act of 2000 (Public Law 106–545) formally authorized and designated ICCVAM as a permanent committee administered by the NIEHS with specific duties that include the technical evaluation of new and alternative testing methods. ICCVAM is charged with developing test recommendations based on those technical evaluations, and forwarding these to Federal agencies for their consideration. The NICEATM was established in 1998 to coordinate and facilitate ICCVAM activities, to provide peer review for validation activities and to promote communication with stakeholders. The NICEATM is located at the NIEHS, Research Triangle Park, NC. Additional information concerning ICCVAM and NICEATM can be found on the ICCVAM/NICEATM web site at *http://iccvam.niehs.nih.gov.*

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Anon. EU Commission Directive 2000/33/EC of 25 April 2000 (Official Journal of the European Communities), Skin Corrosion, Rat Skin TER and Human Skin Model Assay. OJ L 136, June 8, 2000. Available: *http://embryo.ib.amwaw.edu.pl/invittox/prot/1_13620000608en00010089.pdf* [cited July 19, 2001].

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Organization for Economic Co-operation and Development (OECD). Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, OECD, Paris, France. (November 1998) *http://www.oecd.org/ehs/Class/HCL6.htm*

OECD. OECD Revised Proposals for Updated Test Guidelines 404 and 405: Dermal and Eye Corrosion/Irritation Studies. [OECD ENV/JM/TG (2001)2]. OECD Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. Test Guidelines Programme. Circulated in preparation for the 13th Meeting of the Working Group of the National Coordinators of the Test Guidelines Programme, OECD, Paris, France. (2001a)

Dated: September 21, 2001.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 01–24371 Filed 9–27–01; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS); National Toxicology Program (NTP)

Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity; Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity: Notice of Availability and Request for Public Comment.

Summary

Notice is hereby given of the availability of the reports entitled, "Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity" NIH Publication 01–4499 and "Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity" NIH Publication 01–4500. The Report provides conclusions and recommendations from expert scientists based on their review of current in vitro methods for assessing acute toxicity at an October 17–20, 2000 workshop. The workshop was organized by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The Guidance Document

provides Standard Operating Procedures (SOPs) for performing two in vitro basal cytotoxicity assays and describes how to use this in vitro data to predict starting doses for in vivo acute oral toxicity studies.

Availability of the Documents

To receive a copy of either report, please contact NICEATM at P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709 (mail), 919–541–3398 (phone), 919–541–0947 (fax), or *niceatm@niehs.nih.gov* (email). The reports are also available on the ICCVAM/NICEATM website at *http://iccvam.niehs.nih.gov.*

Request for Public Comments

NICEATM invites written public comments on the Workshop Report and the Guidance Document. Comments should be sent to NICEATM by November 13, 2001. Comments submitted via e-mail are preferred; the acceptable file formats are MS Word (Office 98 or older), plain text, or PDF. Comments should be sent to Dr. William S. Stokes, Director, NICEATM, NIEHS, MD EC–17, PO Box 12233, Research Triangle Park, NC, 27709; telephone 919–541–2384; fax 919–541–0947; e-mail *niceatm@niehs.nih.gov.* Persons submitting written comments should include their contact information (name, affiliation, address, telephone and fax numbers, and e-mail) and sponsoring organization, if any. Public comments received in response to this **Federal Register** notice will be posted on the NICEATM/ICCVAM web site (*http://iccvam.niehs.nih.gov*).

Background

The International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity was held October 17–20, 2000, at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA 22202. The workshop was organized by the NICEATM and ICCVAM, and sponsored by the NIEHS, the NTP, and U.S. EPA. The objectives of the workshop were (1) to assess the current validation status of in vitro test methods that might be useful for assessing the acute systemic toxicity potential of chemicals and (2) to develop recommendations for future research, development, and validation studies that might further enhance the use of in vitro methods for this purpose.

A **Federal Register** notice (Vol. 65, No. 115, pp. 37400–37403, June 14, 2000) requested information and data that should be considered at the workshop, and nominations of expert scientists to participate in the workshop. A second **Federal Register** notice (Vol. 65, No. 184, pp. 57203–57205, September 21, 2000) announced availability of the workshop agenda, registration information, and a background summary of available in vitro methods.

At the workshop, the invited expert scientists were divided into four breakout groups as follows:

Breakout Group 1: In Vitro Screening Methods for Assessing Acute Toxicity
Breakout Group 2: In Vitro Methods for Toxicokinetic Determinations
Breakout Group 3: In Vitro Methods for Predicting Organ-Specific Toxicity
Breakout Group 4: Chemical Data Sets for Validation of In Vitro Acute Toxicity Test Methods

Each breakout group subsequently prepared a written report that represented the consensus of the invited scientists assigned to that group and these reports are included in the Workshop Report. It also includes as appendices: A detailed workshop agenda; summary minutes of plenary sessions and public comments; the background document for workshop participants; a NICEATM summary of the Multicenter Evaluation of In Vitro Cytotoxicity (MEIC); a summary of Federal regulations on acute toxicity; related **Federal Register** notices; and ICCVAM test method recommendations. The ICCVAM test recommendations were developed following the workshop to forward to Federal agencies in accordance with Pub. L. 106–545.

The Breakout Group on In Vitro Screening Methods recommended preparation of a document that would provide guidance on how to use in vitro data to estimate starting doses for in vivo acute toxicity studies. Three scientists subsequently collaborated with the NICEATM to develop a "Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity". The Guidance Document provides SOPs for conducting two in vitro cytotoxicity tests (the BALB/c 3T3 Neutral Red Uptake (NRU) and the Normal Human Keratinocyte (NHK) NRU assays) and instruction for using these assays to estimate starting doses for in vivo testing. The Guidance Document also includes the ZEBET (German National Centre for the Documentation and Evaluation of Alternatives to Animal Experimentation) Registry of Cytotoxicity (RC) Regression Analysis that provides a mathematical relationship between acute oral systemic rodent toxicity and in vitro basal cytotoxicity using data for 347 chemicals (Halle, 1998; Spielmann et al., 1999). The Guidance Document expands on an approach suggested by Spielmann and colleagues that—as an initial step—the relationship found with the RC data be used to predict starting doses for subsequent in vivo acute lethality assays.

Additional Information About ICCVAM and NICEATM

ICCVAM, with 15 participating Federal agencies, was established in 1997 to coordinate interagency issues on toxicological test method development, validation, regulatory acceptance, and national and international harmonization. The ICCVAM Authorization Act of 2000 (Pub. L. 106–545) formally authorized and designated ICCVAM as a permanent committee administered by the NIEHS with specific duties that include the technical evaluation of new and alternative testing methods. ICCVAM is charged with developing test recommendations based on those technical evaluations, and forwarding these to Federal agencies for their consideration. The NICEATM was established in 1998 to coordinate and facilitate ICCVAM activities, to provide peer review for validation activities and to promote communication with stakeholders. The NICEATM is located at the NIEHS, Research Triangle Park, NC. Additional information concerning ICCVAM and NICEATM can be found on the ICCVAM/NICEATM web site at *http://iccvam.niehs.nih.gov.* In accordance with Public Law 106–545, the Workshop Report and the Guidance Document will be forwarded with ICCVAM test recommendations to Federal agencies for their consideration.

References

Halle, W. 1998. Toxizitätsprüfungen in Zellkulturen für eine Vorhersage der akuten Toxizität (LD_{50}) zur Einsparung von Tierversuchen. Life Sciences/Lebenswissenschaften, Volume 1, 94 pp., Jülich: Forschungszentrum Jülich.

Spielmann, H., E. Genschow, M. Liebsch, and W. Halle. 1999. Determination of the starting dose for acute oral toxicity (LD_{50}) testing in the up and down procedure (UDP) from cytotoxicity data. ATLA 27: 957–966.

Dated: September 18, 2001.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 01–24370 Filed 9–27–01; 8:45 am]

BILLING CODE 4140–01–P

signed Confidential Disclosure Agreement will be required to receive a copy of any pending patent applications.

SUPPLEMENTARY INFORMATION: Gaucher Disease is a rare inborn error of metabolism which affects between 10,000 and 20,000 people worldwide, 40% in the United States. Gaucher Disease is the most common lipid storage disease. The symptoms associated with Gaucher Disease result from the accumulation of a lipid called glucocerebroside. This lipid is a byproduct of the normal recycling of red blood cells. When the gene with the instructions for producing an enzyme to break down this byproduct is defective, the lipid accumulates. The lipid is found in many places in the body, but most commonly in the macrophages in the bone marrow. There it interferes with normal bone marrow functions, such as production of platelets (leading to bleeding and bruising) and red blood cells (leading to anemia) and potentially death. The presence of glucocerebroside seems to also trigger the loss of minerals in the bones, causing the bones to weaken, and can interfere with the bone's blood supply.

The field of use is directed to the development of therapies for remedying enzyme deficiencies in the treatment of Gaucher Disease.

The prospective exclusive license will be royalty-bearing and will comply with the terms and conditions of 35 U.S.C. 209 and 37 CFR 404.7. The prospective exclusive license may be granted unless, within ninety (90) days from the date of this published notice, NIH receives written evidence and argument that establishes that the grant of the license would not be consistent with the requirements of 35 U.S.C. 209 and 37 CFR 404.7.

Applications for a license filed in response to this notice will be treated as objections to the grant of the contemplated license. Comments and objections submitted in response to this notice will not be made available for public inspection, and, to the extent permitted by law, will not be released under the Freedom of Information Act, 5 U.S.C. 552.

Dated: September 11, 2000.

Jack Spiegel,

Director, Division of Technology Development and Transfer, Office of Technology Transfer.

[FR Doc. 00–24241 Filed 9–20–00; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), National Toxicology Program (NTP); Notice of an International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity, co-sponsored by NIEHS, NTP and the U.S. Environmental Protection Agency (EPA): Workshop Agenda and Registration Information

SUMMARY: Pursuant to Public Law 103–43, notice is hereby given of a public meeting sponsored by NIEHS, the NTP, and the EPA, and coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The agenda topic is a scientific workshop to assess the current status of *in vitro* test methods for evaluating the acute systemic toxicity potential of chemicals and to develop recommendations for future research, development, and validation studies. The workshop will take place on October 17–20, 2000, at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA, 22202. The meeting will be open to the public.

In a previous **Federal Register** notice (Vol. 65, No. 115, pp. 37400–37403), ICCVAM requested information and data that should be considered at the Workshop and nominations of expert scientists to participate in the Workshop. A preliminary list of relevant studies to be considered for the Workshop was also provided. As a result of this request, an ICCVAM interagency Workshop Organizing Committee has selected an international group of scientific experts to participate in this Workshop. NICEATM, in collaboration with ICCVAM, has developed a background summary of data and performance characteristics for available *in vitro* methods. This summary will be made available to invited expert scientists and the public before the Workshop. Requests for the summary can be made to the address given below. This notice provides an agenda, registration information, and updated details about the Workshop.

Workshop Background and Scope

A. Background

Acute toxicity testing is conducted to determine the hazards of various chemicals and products. This information is used to properly classify and label materials as to their lethality in accordance with an internationally harmonized system (OECD, 1998). Non-lethal endpoints may also be evaluated to identify potential target organ toxicity, toxicokinetic parameters, and dose-response relationships. While animals are currently used to evaluate acute toxicity, recent studies suggest that *in vitro* methods may also be helpful in predicting acute toxicity.

Studies by Spielmann *et al.* (1999) suggest that *in vitro* cytotoxicity methods may be useful in predicting a starting dose for *in vivo* studies, and thus may potentially reduce the number of animals necessary for such determinations. Other studies (*e.g.*, Ekwall *et al.*, 2000) have indicated an association between chemical concentrations leading to *in vitro* cytotoxicity and human lethal blood concentrations. A program to assess toxicokinetics and target organ toxicity utilizing *in vitro* methods has been proposed that may provide enhanced predictions of toxicity and potentially reduce or replace animal use for some tests (Ekwall *et al.*, 1999). However, many of the necessary *in vitro* methods for this program have not yet been developed. Other methods have not been evaluated in validation studies to determine their usefulness and limitations for generating information to meet regulatory requirements for acute toxicity testing. Development and validation of *in vitro* methods which can establish accurate dose-response relationships will be necessary before such methods can be considered for the reduction or replacement of animal use for acute toxicity determinations.

This workshop will examine the status of available *in vitro* methods for assessing acute toxicity. This includes screening methods for acute toxicity, such as methods that may be used to predict the starting dose for *in vivo* animal studies, and methods for generating information on toxicokinetics, target organ toxicity, and mechanisms of toxicity. The workshop will develop recommendations for validation efforts necessary to characterize the usefulness and limitations of these methods. Recommendations will also be developed for future mechanism-based research and development efforts that might further improve *in vitro* assessments of acute systemic lethal and non-lethal toxicity.

B. Objectives of the Workshop

Four major topics will be addressed:

- *In Vitro* Screening Methods for Assessing Acute Toxicity;

- *In Vitro* Methods for Toxicokinetic Determinations;
- *In Vitro* Methods for Predicting Organ Specific Toxicity; and
- Chemical Data Sets for Validation of *In Vitro* Acute Toxicity Test Methods.

The objectives of the meeting are to:

1. Review the status of *in vitro* methods for assessing acute systemic toxicity:

a. Review the validation status of available *in vitro* screening methods for their usefulness in estimating *in vivo* acute toxicity,

b. Review *in vitro* methods for predicting toxicokinetic parameters important to acute toxicity (*i.e.*, absorption, distribution, metabolism, elimination), and

c. Review *in vitro* methods for predicting specific target organ toxicity;

2. Recommend candidate methods for further evaluation in prevalidation and validation studies;

3. Recommend validation study designs that can be used to characterize adequately the usefulness and limitations of proposed *in vitro* methods;

4. Identify reference chemicals that can be used for development and validation of *in vitro* methods for assessing *in vivo* acute toxicity; and

5. Identify priority research efforts necessary to support the development of mechanism-based *in vitro* methods to assess acute systemic toxicity. Such efforts might include incorporation and evaluation of new technologies, such as gene microarrays, and development of methods necessary to generate dose response information.

Workshop Information

A. Workshop Agenda

Tuesday, October 17, 2000

8:30 a.m.—Opening Plenary Session
- Workshop Introduction
- Welcome from the National Toxicology Program (NTP)
- Overview of ICCVAM and NICEATM
- Acute Toxicity: Historical and Current Regulatory Perspectives
- Acute Toxicity Data: A Clinical Perspective

10:30 a.m.—*In Vitro* Approaches to Estimate the Acute Toxicity Potential of Chemicals
- Estimating Starting Doses for *In Vivo* Studies using *In Vitro* Data
- An Integrated Approach for Predicting Systemic Toxicity
- Opportunities for Future Progress

Public Comment
Breakout Groups' Charges
12:30 p.m.—Lunch Break

1:45 p.m.—Breakout Groups: Identifying What Is Needed from *In Vitro* Methods
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

5:30 p.m.—Adjourn for the Day

Wednesday, October 18, 2000

8:00 a.m.—Plenary Session—Status Reports by Breakout Group Co-Chairs
9:00 a.m.—Breakout Groups: Current Status of *In Vitro* Methods for Acute Toxicity
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

12:00 p.m.—Lunch Break
1:30 p.m.—Breakout Groups: Current Status of *In Vitro* Methods for Acute Toxicity (Cont'd)
5:30 p.m.—Adjourn for the Day

Thursday, October 19, 2000

8:00 a.m.—Plenary Session—Status Reports by Breakout Group Co-Chairs
9:00 a.m.—Breakout Groups: Future Directions for *In Vitro* Methods for Acute Toxicity
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

12:00 p.m.—Lunch Break
1:30 p.m.—Breakout Groups: Future Directions for *In Vitro* Methods for Acute Toxicity (Cont'd)
5:30 p.m.—Adjourn for the Day

Friday, October 20, 2000

8:00 a.m.—Closing Plenary Session—Reports by Breakout Group Co-Chairs
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

Public Comment
Closing Comments
12:15 p.m.—Adjourn

B. Workshop Registration

The Workshop meeting will be open to the public, limited only by the space available. Due to space limitations, advance registration is requested by October 13, 2000. Registration forms can be obtained by contacting NICEATM at the address given below or by accessing the on-line registration form at: *http://iccvam.niehs.nih.gov/invi_reg.htm*. Other relevant Workshop information (*i.e.*, accommodations, transportation, etc.) is also provided at this website.

C. Public Comment

The Public is invited to attend the Workshop and the number of observers will be limited only by the space available. Two formal public comment sessions on Tuesday, October 17th and Friday, October 20th will provide an opportunity for interested persons or groups to present their views and comments to the Workshop participants (please limit to one speaker per group). Additionally, time will be allotted during each of the Breakout Group sessions for general discussion and comments from observers and other participants. The Public is invited to present oral comments or to submit comments in writing for distribution to the Breakout Groups to NICEATM at the address given below by October 13, 2000. Oral presentations will be limited to seven minutes per speaker to allow for a maximum number of presentations. Individuals presenting oral comments are asked to provide a hard copy of their statement at registration. For planning purposes, persons wishing to give oral comments are asked to check the box provided on the Registration Form, although requests for oral presentations will also be accepted on-site (subject to availability of time). Persons registering for oral comments or submitting written remarks are asked to include their contact information (name, address, affiliation, telephone, fax, and e-mail).

Guidelines for Requesting Registration Form and Submission of Public Comment

Requests for registration information and submission of public comments should be directed to the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, Environmental Toxicology Program, NIEHS/NTP, MD EC–17, PO Box 12233, Research Triangle Park, NC 27709; 919–541–3398 (phone); 919–541–0947 (fax); *iccvam@niehs.nih.gov* (e-mail). Public comments should be accompanied by complete contact information including name, (affiliation, if applicable), address, telephone number, and e-mail address.

References

- OECD (Organisation for Economic Cooperation and Development). (1998). Harmonized integrated hazard classification system for human health and environmental effects of chemical substances. OECD, Paris. (website: *http://www.oecd.org//ehs/Class/HCL6.HTM*)
- Spielmann, H., Genschow, E., Leibsch, M., and Halle, W. (1999) Determination of the starting dose for

acute oral toxicity (LD50) testing in the up and down procedure (UDP) from cytotoxicity data. ATLA, 27(6), 957–966.

- Ekwall, B., Ekwall, B., and Sjorstrom, M. (2000) MEIC evaluation of acute systemic toxicity: Part VIII. Multivariate partial least squares evaluation, including the selection of a battery of cell line tests with a good prediction of human acute lethal peak blood concentrations for 50 chemicals. ATLA, 28, Suppl. 1, 201–234.
- Ekwall, B., Clemedson, C., Ekwall, B., Ring, P., and Romert, L. (1999) EDIT: A new international multicentre programme to develop and evaluate batteries of *in vitro* tests for acute and chronic systemic toxicity. ATLA 27, 339–349.

Dated: September 12, 2000.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 00–24244 Filed 9–20–00; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

[Docket No. FR–4463–N–04]

Notice of FHA Debenture Call

AGENCY: Office of the Assistant Secretary for Housing-Federal Housing Commissioner, HUD.

ACTION: Notice.

SUMMARY: This Notice announces a debenture recall of certain Federal Housing Administration debentures, in accordance with authority provided in the National Housing Act.

FOR FURTHER INFORMATION CONTACT: Richard Keyser, Room 3119P, L'Enfant Plaza, Department of Housing and Urban Development, 451 Seventh Street, SW., Washington, DC 20410, telephone (202) 755–7510 x137. This is not a toll-free number.

SUPPLEMENTARY INFORMATION: Pursuant to Sections 204(c) and 207(j) of the National Housing Act, 12 U.S.C. 1710(c), 1713(j), and in accordance with HUD's regulation at 24 CFR 203.409 and § 207.259(e)(3), the Federal Housing Commissioner, with approval of the Secretary of the Treasury, announces the call of all Federal Housing Administration debentures, with a coupon rate of 6.625 percent or above, except for those debentures subject to "debenture lock agreements", that have been registered on the books of the Federal Reserve Bank of Philadelphia, and are, therefore, "outstanding" as of September 30, 2000. The date of the call is January 1, 2001.

The debentures will be redeemed at par plus accrued interest. Interest will cease to accrue on the debentures as of the call date. Final interest on any called debentures will be paid with the principal at redemption.

During the period from the date of this notice to the call date, debentures that are subject to the call may not be used by the mortgagee for a special redemption purchase in payment of a mortgage insurance premium.

No transfer of debentures covered by the foregoing call will be made on the books maintained by the Treasury Department on or after October 1, 2000. This does not affect the right of the holder of a debenture to sell or assign the debenture on or after this date. Payment of final principal and interest due on January 1, 2001, will be made automatically to the registered holder.

Dated: September 15, 2000.

William C. Apgar,

Assistant Secretary for Housing-Federal Housing Commissioner.

[FR Doc. 00–24288 Filed 9–20–00; 8:45 am]

BILLING CODE 4210–27–M

DEPARTMENT OF THE INTERIOR

Fish and Wildlife Service

Notice of Receipt of Applications for Permit

Endangered Species

The following applicants have applied for a permit to conduct certain activities with endangered species. This notice is provided pursuant to Section 10(c) of the Endangered Species Act of 1973, *as amended* (16 U.S.C. 1531, *et seq.*):

PRT–841026

Applicant: Thane Wibbels, University of Alabama at Birmingham, Birmingham, AL

The applicant requests a permit to import up to 1000 blood samples and up to 500 tissue samples taken from Kemp's Ridley sea turtles (*Lepidochelys kempii*) in Mexico for enhancement of the species through scientific research. This notification covers activities conducted by the applicant over a five year period.

PRT–032758

Applicant: Exotic Feline Breeding Compound, Inc., Rosamond, CA

The applicant requests a permit to import 1 captive-born male Amur leopard (*Panthera pardus orientalis*) from the Novosibirsk Zoo, Russia for the purpose of propagation for the enhancement of the survival of the species.

PRT–032757

Applicant: Omaha's Henry Doorly Zoo, Omaha, NE

The applicant requests a permit to import 1 captive-born female Sumatran tiger (*Panthera tigris sumatrae*) from the Surabaya Zoo, Indonesia for the purpose of propagation for the enhancement of the survival of the species.

PRT–031061

Applicant: Susan E. Aronoff, Tampa, FL, 33624

The applicant requests a permit to import 1 captive-born male cheetah (*Acinonyx jubatus*) from the Endangered Animal Foundation, Driftweg, the Netherlands to enhance the survival of the species through conservation education.

PRT–830414

Applicant: Duke University Primate Center, Durham, NC

The applicant requests re-issuance of a permit to import two male and three female wild-caught golden-crowned sifakas (*Propithecus tattersalli*) from Dariana, Madagascar for the purpose of propagation for the enhancement of the survival of the species. This notification covers requests for re-issuances of the permit by the applicant over a five year period.

PRT–808256

Applicant: Duke University Primate Center, Durham, NC

The applicant requests re-issuance of a permit to import one male and two female wild-caught diademed sifakas (*Propithecus diadema*) from the Department of Water and Forest, Maramize, Madagascar for the purpose of propagation for the enhancement of the survival of the species. This notification covers requests for re-issuances of the permit by the applicant over a five year period.

PRT–031796

Applicant: Larry Edward Johnson, Boerne, TX

The applicant requests a permit to export two male and two female captive-born ring-tailed lemurs (*Catta lemur*) to Munchi's Zoo, Buenos Aires, Argentina to enhance the survival of the species through conservation education and captive propagation.

PRT–026102

Applicant: Elizabeth G. Stone/University of Georgia, Athens, GA

The applicant requests a permit to import salvaged specimens, non-viable eggs, and biological samples from Thick-billed parrots (*Rhynchopsitta pachyrhyncha*) collected in the wild in Mexico, for scientific research. This

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is hereby given of the following meeting.

The meeting will be closed to the public in accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6), Title 5 U.S.C., as amended. The grant applications and the discussions could disclose confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: National Institute of Diabetes and Digestive and Kidney Diseases Special Emphasis Panel, ZDK1 GRB 4 (01).

Date: June 16, 2000.

Time: 8:00 am to 2:00 pm.

Agenda: To review and evaluate grant applications.

Place: Embassy Suites Hotel, 1300 Concourse Drive, Linthicum, Maryland 21090.

Contact Person: William E. Elzinga, Scientific Review Administrator, Review Branch, DEA, NIDDK, Room 647, 6707 Democracy Boulevard, National Institutes of Health, Bethesda, MD 20892–6600, (301) 594–8895.

This notice is being published less than 15 days prior to the meeting due to the timing limitations imposed by the review and funding cycle.

(Catalogue of Federal Domestic Assistance Program Nos. 93.847, Diabetes, Endocrinology and Metabolic Research; 93.848, Digestive Diseases and Nutrition Research; 93.849, Kidney Diseases, Urology and Hematology Research, National Institutes of Health, HHS)

Dated: June 8, 2000.

LaVerne Y. Stringfield,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. 00–14960 Filed 6–13–00; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institute of Health

National Institute of Nursing Research; Notice of Closed Meeting

Pursuant to section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of the following meeting.

The meeting will be closed to the public in accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6). Title 5 U.S.C., as amended. The grant applications and the discussions could disclose confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: National Institute of Nursing Research Special Emphasis Panel, NINR Career Transitional Award Applications (K22s).

Date: June 21, 2000.

Time: 3:00 PM to 5:00 PM.

Agenda: To review and evaluate grant applications.

Place: Bethesda Holiday Inn, 8120 Wisconsin Avenue, Bethesda, MD 20852.

Contact Person: Mary J. Stephens-Frazier, Scientific Review Administrator, National Institute of Nursing Research, National Institutes of Health, Natcher Building, Room 3AN32, (301) 594–5971.

This notice is being published less than 15 days prior to the meeting due to the timing limitations imposed by the review and funding cycle.

(Catalogue of Federal Domestic Assistance Program Nos. 93.361, Nursing Research, National Institute of Health, HHS)

Dated: June 8, 2000.

LaVerne Y. Stringfield,

Director, Office of Federal Advisory Committee Policy

[FR Doc. 00–14963 Filed 6–13–00; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institute of Nursing Research; Notice of Closed Meeting

Pursuant to section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of the following meeting.

The meeting will be closed to the public in accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6), Title 5 U.S.C., as amended. The grant applications and the discussions could disclose confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: National Institute of Nursing Research Special Emphasis Panel, NINR/ORMH Mentored Research Scientist Development Award for Minority Investigators (KO1s).

Date: June 21, 2000.

Time: 8:30 a.m. to 2 p.m.

Agenda: To review and evaluate grant applications.

Place: Bethesda Holiday Inn, 8120 Wisconsin Avenue, Bethesda, MD 20814.

Contact Person: Mary J. Stephens-Frazier, Scientific Review Administrator, National Institute of Nursing Research, National Institutes of Health, Natcher Building, Room 3AN32, Bethesda, MD 20892, (301) 594–5971.

This notice is being published less than 15 days prior to the meeting due to the timing limitations imposed by the review and funding cycle.

(Catalogue of Federal Domestic Assistance Program Nos. 93.361, Nursing Research, National Institutes of Health, HHS)

Dated: June 8, 2000.

LaVerne Y. Stringfield,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. 00–14964 Filed 6–13–00; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), National Toxicology Program (NTP); Notice of an International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity, co-sponsored by NIEHS, NTP and the U.S. Environmental Protection Agency (EPA): Request for Data and Suggested Expert Scientists

SUMMARY: Pursuant to Public Law 103–43, notice is hereby given of a public meeting sponsored by NIEHS, the NTP, and the EPA, and coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The agenda topic is a scientific workshop to assess the current status of in vitro test methods for evaluating the acute systemic toxicity potential of chemicals, and to develop recommendations for future development and validation studies. The workshop will take place on October 17–20, 2000 at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA, 22202. The meeting will be open to the public.

In preparing for this Workshop, ICCVAM is requesting: (1) Information and data that should be considered at the Workshop, including relevant data on currently available in vitro methods for assessing acute systemic toxicity; and (2) nominations of expert scientists to participate in the Workshop. An agenda, registration information, and other details will be provided in a subsequent **Federal Register** notice.

Background

ICCVAM, with participation by 14 Federal regulatory and research agencies and programs, was established in 1997 to coordinate issues relating to the development, validation, acceptance, and national/international harmonization of toxicological test methods. ICCVAM seeks to promote the scientific validation and regulatory acceptance of new and improved test methods applicable to Federal agencies, including methods that may reduce or replace animal use, or that refine protocols to lessen animal pain and distress. The Committee's functions include the coordination of interagency reviews of toxicological test methods and communication with stakeholders throughout the process of test method development and validation. The following Federal regulatory and research agencies participate:

Consumer Product Safety Commission
Department of Defense
Department of Energy
Department of Health and Human Services
  Agency for Toxic Substances and Disease Registry
  Food and Drug Administration
  National Institute for Occupational Safety and Health/CDC
  National Institutes of Health
  National Cancer Institute
  National Institute of Environmental Health Sciences
  National Library of Medicine
Department of the Interior
Department of Labor
  Occupational Safety and Health Administration
Department of Transportation
  Research and Special Programs Administration
Environmental Protection Agency

NICEATM was established in 1998 and provides operational support for the ICCVAM. NICEATM and ICCVAM collaborate to carry out activities associated with the development, validation, and regulatory acceptance of proposed new and improved test methods. These activities may include:

• Test Method Workshops, which are convened as needed to evaluate the adequacy of current methods for assessing specific toxicities, to identify areas in need of improved or new testing methods, to identify research efforts that may be needed to develop new test methods, and to identify appropriate development and validation activities for proposed new methods.

• Expert Panel Meetings, which are typically convened to evaluate the validation status of a method following the completion of initial development and pre-validation studies. Expert Panels are asked to recommend additional validation studies that might be helpful in further characterizing the usefulness of a method, and to identify any additional research and development efforts that might enhance the effectiveness of a method.

• Independent Peer Review Panel Meetings, which are typically convened following the completion of comprehensive validations studies on a test method. Peer Review Panels are asked to develop scientific consensus on the usefulness and limitations of test methods to generate information for specific human health and/or ecological risk assessment purposes. Following the independent peer review of a test method, ICCVAM forwards recommendations on its usefulness to agencies for their consideration. Federal agencies then determine the regulatory acceptability of a method according to their mandates.

Additional information about ICCVAM and NICEATM can be found at the website: *http://iccvam.niehs.nih.gov.*

Workshop Background and Scope

A. Background

Federal regulatory agencies require toxicity testing to determine the safety or hazard of various chemicals and products prior to human exposure. Agencies use this information to properly classify and label products as to their hazard potential. Acute oral toxicity determinations are currently made using animals. However, recent studies (e.g., Spielmann et al., 1999) suggest that in vitro cytotoxicity methods may be useful in predicting a starting dose for in vivo studies, and thus may potentially reduce the number of animals necessary for such determinations.

Other studies (e.g., Ekwall et al., 2000) have indicated an association between in vitro cytotoxicity and human lethal blood concentrations. However, these in vitro methods have not yet been evaluated in validation studies to determine their usefulness and limitations for generating acute toxicity testing information necessary to meet regulatory testing requirements. Additionally, other in vitro methods would likely be necessary to establish accurate dose-response relationships before such methods could substantially reduce or replace animal use for acute toxicity determinations.

This workshop will examine the status of available in vitro methods and develop recommendations for validation efforts necessary to characterize the usefulness and limitations of existing methods. Recommendations for future research and development efforts that might further enhance the usefulness of in vitro assessments of acute systemic lethal toxicity will also be developed.

B. Objectives of the Workshop

Four major topics will be addressed:

1. General cytotoxicity methods predictive of acute lethal toxicity;

2. Toxicokinetic and organ specific toxicity methods;

3. Reference chemicals for validation of the above methods; and

4. The use of quantitative structure activity relationships (QSAR) and chemical/physical properties for predicting acute lethal toxicity.

The objectives of the meeting are to:

1 a. Identify and review the status of in vitro general cytotoxicity screening methods that may reduce animal use for assessing acute systemic toxicity;

b. Identify information from in vitro methods necessary to predict acute systemic toxicity and review the status of relevant methods (*e.g.*, in vitro methods to assess gut absorption, metabolism, blood-brain barrier penetration, volume distribution to critical target organs, and specific target organ toxicity);

2. Identify candidate methods for further evaluation in prevalidation and validation studies;

3. Identify reference chemicals useful for development and validation of in vitro methods for assessing acute systemic toxicity;

4. Identify validation study designs needed to adequately characterize the proposed methods in 2.; and

5. Identify priority research efforts necessary to support the development of in vitro methods to adequately assess acute systemic toxicity. Such efforts might include incorporation and evaluation of new technologies such as gene microarrays, and development of methods necessary to generate dose response information.

C. Methods for Consideration

Given the breadth of the workshop topics, many methods are likely to be considered relevant to the discussion. Methods will include but are not limited to those proposed in the Multicentre Evaluation of In Vitro Cytotoxicity (MEIC) battery (*http://www.ctlu.se*). A background document summarizing the data and performance characteristics for available methods is being prepared by NICEATM in collaboration with the ICCVAM interagency organizing committee. Information received as a result of this **Federal Register** notice will be

considered for inclusion in the background document. In formulating its recommendations, the Workshop participants will evaluate information in the background document and relevant information from other sources.

D. Test Method Data and Information Sought

Data are sought from completed, ongoing, or planned studies that provide comparative performance data for in vitro methods compared to currently accepted in vivo methods for determining acute lethal toxicity and hazard classification. Data from test methods that provide toxicokinetic and specific target organ toxicity information are also sought. Submissions should describe the extent to which established criteria for validation and regulatory acceptance have been addressed. These criteria are provided in "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods," NIH publication 97–3981 (*http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/iccvam.html*). Where possible, submitted data and information should adhere to the guidance provided in the document, "Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to ICCVAM," NIH Publication 99–4496, (*http://iccvam.niehs.nih.gov/doc1.htm*). Both publications are also available on request from NICEATM at the address provided below. Relevant information submitted in response to this request will be incorporated into the background material provided to Workshop participants. A preliminary list of relevant studies is provided at the end of this announcement, and public comment and suggestions for additions are invited.

NICEATM and the ICCVAM interagency workshop organizing committee will compile information on the studies to be considered at the Workshop. All data should be submitted by July 15, 2000 in order to ensure full consideration.

E. Request for Nomination of Expert Scientists for the Test Method Workshop

NICEATM is soliciting nominations for expert scientists to participate in the Workshop. (See Guidelines for Submission of Comments below). Types of expertise likely to be relevant include acute toxicity testing in animals, evaluation and treatment of acute toxicity in humans, development and use of in vitro methodologies, statistical data analysis, knowledge of chemical data sets useful for validation of acute toxicity studies, and hazard classification of chemicals and products. Expertise need not be limited to these areas, nor will these areas necessarily be included on the Panel. An appropriate breadth of expertise will be sought. If other areas of scientific expertise are recommended, the rationale should be provided.

Nominations should be accompanied by complete contact information including name, address, institutional affiliation, telephone number, and e-mail address. The rationale for nomination should be provided. If possible, a biosketch or a curriculum vitae should be included. To avoid the potential for candidates being contacted by a large number of nominators, candidates need not be contacted prior to nomination.

Workshop experts will be selected by an ICCVAM interagency workshop organizing committee after considering all nominations received from the public as well as nominations developed internally. All nominees will be contacted for interest and availability, and curricula vitae will be solicited from the nominees. Candidates will be required to disclose potential conflicts of interest.

Schedule for the Workshop

The Workshop will take place on October 17–20, 2000 at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA 22202. The Workshop meeting will be open to the public, limited only by space available.

Submitted methods and supporting data will be reviewed during the July to August 2000 timeframe and a background review document will be prepared by NICEATM in collaboration with the ICCVAM interagency organizing committee. The background information will be made available to Workshop experts for discussion at the meeting and will be available to the Public in advance of the Workshop.

Public Input Invited

As described above, ICCVAM invites comments on the scope and process for the review; comments on the ICCVAM preliminary list of studies for consideration; the submission of other test methods for consideration; and the nomination of experts to participate in the Workshop. Nominations must be submitted within 30 days of the publication date of this notice, and other information should be submitted by July 15, 2000.

Guidelines for Submission of Public Comment

Correspondence should be directed to Dr. William S. Stokes, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, Environmental Toxicology Program, NIEHS/NTP, MD EC–17, PO Box 12233, Research Triangle Park, NC 27709; 919–541–3398 (phone); 919–541–0947 (fax); *iccvam@niehs.nih.gov* (e-mail). Public comments should be accompanied by complete contact information including name, (affiliation, if applicable), address, telephone number, and e-mail address.

Preliminary List of Studies to be Considered for the Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity

ICCVAM has compiled a preliminary list of relevant studies. The public is invited to comment on this list, and suggestions for additions may be submitted. (See Section of this **Federal Register** announcement on Guidelines for Submission of Public Comments).

Studies that may be completed but not published are not included here. This list provides examples of studies and information that may be appropriate for consideration by the Workshop experts.

Balls, M., Blaauboer, B.J., Fentem, J.H., Bruner, L., Combes, R.D., Ekwall, B., Fielder, R.J., Guillouzo, A., Lewis, R.W., Lovell, D.P., Reinhardt, C.A., Repetto, G., Sladowski, D., Spielmann, H., and Zucco, F. (1995) Practical aspects of the validation of toxicity test procedures—The report and recommendations of ECVAM Workshop 5. ATLA 23, 129–147.

Bernson, V., Bondesson, I., Ekwall, B., Stenberg, K., and Walum, E. (1987) A multicenter evaluation study of *in vitro* cytotoxicity. ATLA, 14, 144–145.

Bondesson, I., Ekwall, B., Stenberg, K., Romert, L., and Walum, E. (1988) Instruction for participants in the multicenter evaluation study of *in vitro* cytotoxicity (MEIC). ATLA, 15, 191–193.

Bondesson, I., Ekwall, B., Hellberg, S., Romert, L., Stenberg, K., and Walum, E. (1989) MEIC—A new international multicenter project to evaluate the relevance to human toxicity of *in vitro* cytotoxicity tests. Cell Biol. Toxicol., 5, 331–347.

Clemedson, C., and Ekwall, B. (1999) Overview of the final MEIC results: I. The *in vitro-in vivo* evaluation. Toxicology In vitro, 13, 657–663.

Clemedson, C., McFarlane-Abdulla, E., Andersson, M., Barile, F.A., Calleja, M.C., Chesnea, C., Clothier, R., Cottin, M., Curren, R., Daniel-Szolgay, E., Dierickx, P., Ferro, M., Fiskesj", G., Garza-Ocanas, L., Goamez-Lechoan, M.J., Gualden, M., Isomaa, B., Janus, J., Judge, P., Kahru, A., Kemp, R.B., Kerszman, G., Kristen, U., Kunimoto, M., Karenlampi, S., Lavrijsen, K., Lewan L., Lilius, H., Ohno, T., Persoone, G., Roguet, R.,

Romert, L., Sawyer, T., Seibert, H., Shrivastava, R., Stammati, A., Tanaka, N., Torres Alanis, O., Voss, J–U., Wakuri, S., Walum, E., Wang, X., Zucco, F., and Ekwall, B. (1996) MEIC evaluation of acute systemic toxicity. Part I. Methodology of 68 *in vitro* toxicity assays used to test the first 30 reference chemicals. ATLA, 24, Suppl. 1, 249–272.

Clemedson, C., McFarlane-Abdulla, E., Andersson, M., Barile, F.A., Calleja, M.C., Chesne, C., Clothier, R., Cottin, M., Curren, R., Dierickx, P., Ferro, M., Fiskesja,.G., Garza-Ocanas, L., Gomez-Lechon, M.J., Gulden, M., Isomaa, B., Janus, J., Judge, P., Kahru, A., Kemp, R.B., Kerszman, G., Kristen, U., Kunimoto, M., Karenlampi, S., Lavrijsen, K., Lewan L., Lilius, H., Malmsten, A., Ohno, T., Persoone, G., Pettersson, R., Roguet, R., Romert, L., Sandberg,.M.,.Sawyer, T., Seibert, H., Shrivastava, R., Sjostrom, M., Stammati, A., Tanaka, N., Torres Alanis, O., Voss, J–U., Wakuri, S., Walum, E., Wang, X., Zucco, F. and, Ekwall, B. (1996) MEIC evaluation of acute systemic toxicity. Part II. *In vitro* results from 68 toxicity assays used to test the first 30 reference chemicals and a comparative cytotoxicity analysis. ATLA, 24, Suppl. 1, 273–311.

.Clemedson, C., Barile, F.A., Ekwall, B., Gomez-Lechon, M.J., Hall, T., Imai, K., Kahru, A., Logemann, P., Monaco, F., Ohno, T., Segner, H., Sjostrom, M., Valentino, M., Walum, E., Wang, X., and Ekwall, B. (1998). MEIC evaluation of acute systemic toxicity: Part III. *In vitro* results from 16 additional methods used to test the first 30 reference chemicals and a comparative cytotoxicity analysis. ATLA 26, Suppl. 1, 91–129.

Clemedson, C., Aoki, Y., Andersson, M., Barile, F.A., Bassi, A.M., Calleja, M.C., Castano, A., Clothier, R.H., Dierickx, P., Ekwall, B., Ferro, M., Fiskeso, G., Garza-Ocanas, L. Gomez-Lechoan, M.J., Gulden, M., Hall, T., Imai, K., Isomaa, B., Kahru, A., Kerszman, G., Kjellstrand, P., Kristen, U., Kunimoto, M., Karenlampi, S., Lewan, L., Lilius, H., Loukianov, A., Monaco, F., Ohno, T., Persoone, G., Romert, L., Sawyer, T.W., Shrivastava, R., Segner, H., Seibert, H., Sjostrom, M., Stammati, A., Tanaka, N., Thuvander, A., Torres-Alanis, O., Valentino, M., Wakuri, S., Walum, E., Wieslander, A., Wang, X., Zucco, F., and Ekwall, B. (1998). MEIC evaluation of acute systemic toxicity. Part IV. *In vitro* results from 67 toxicity assays used to test reference chemicals 31–50 and a comparative cytotoxicity analysis. ATLA 26, Suppl. 1, 131–183.

Clemedson, C., Barile, F.A., Chesne, C., Cottin, M., Curren, R., Ekwall, B., Ferro, M., Gomez-Lechon, M.J., Imai, K., Janus, J., Kemp, R.B., Kerszman, G., Kjellstrand, P., Lavrijsen, K., Logemann, P., McFarlane-Abdulla, E., Roguet, R., Segner, H., Seibert, H., Thuvander, A., Walum, E., and Ekwall, Bj. (2000) MEIC evaluation of acute systemic toxicity: Part VII. Prediction of human toxicity by results from testing of the first 30 reference chemicals with 27 further *in vitro* assays. ATLA 28, Suppl. 1, 161–200.

Ekwall, B. (1995) The basal cytotoxicity concept, pp 721–725. In Proceedings of the World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing. Alternative Methods in Toxicology and the Life Sciences, Vol. 11. Mary Ann Liebert, New York, 1995.

Ekwall, B. (1999) Overview of the Final MEIC Results: II. The *In vitro/in vivo* evaluation, including the selection of a practical battery of cell tests for prediction of acute lethal blood concentrations in humans. Toxicol. *In vitro*, 13, 665–673.

Ekwall, B., Gomez-Lechon, M.J., Hellberg, S., Bondsson, I., Castell, J.V., Jover, R., Hogberg, J., Ponsoda, X., Stenberg, K., and Walum, E. (1990) Preliminary results from the Scandinavian multicentre evaluation of *in vitro* cytotoxicity (MEIC). Toxicol. *In vitro*, 4, 688–691.

Ekwall, B., Clemedson, C., Crafoord, B., Ekwall, Ba., Hallander, S., Walum E., and Bondesson, I. (1998) MEIC evaluation of acute systemic toxicity. Part V. Rodent and human toxicity data for the 50 reference chemicals. ATLA 26, Suppl. 2, 569–615.

Ekwall, B., Barile., F.A., Castano, A., Clemedson, C., Clothier, R.H., Dierickx, P., Ekwall, B., Ferro, M., Fiskesjo;, G., Garza-Ocanas, L., Gomez-Lechon, M-J., Gulden, M., Hall, T., Isomaa, B., Kahru, A, Kerszman, G., Kristen, U., Kunimoto, M., Karenlampi, S., Lewan, L, Loukianov, A., Ohno, T., Persoone, G., Romert, L., Sawyer, T.W., Segner, H., Shrivastava, R., Stammati, A., Tanaka, N., Valentino, M., Walum, E., and Zucco, F. (1998) MEIC evaluation of acute systemic toxicity. Part VI. Prediction of human toxicity by rodent LD50 values and results from 61 *in vitro* tests. ATLA 26, Suppl. 2, 617–658.

Ekwall, B., Clemedson, C., Ekwall, B., Ring, P., and Romert, L. (1999) EDIT: A new international multicentre programme to develop and evaluate batteries of *in vitro* tests for acute and chronic systemic toxicity. ATLA 27, 339–349.

Ekwall, B., Ekwall, B., and Sjostrom, M. (2000) MEIC evaluation of acute systemic toxicity: Part VIII. Multivariate partial least squares evaluation, including the selection of a battery cell line tests with a good prediction of human acute lethal peak blood concentrations for 50 chemicals. ATLA 28, Suppl. 1, 201–234.

Hellberg, S., Bondesson, I., Ekwall, B., Gomez-Lechon, M.J., Jover, R., Hogberg, J., Ponsoda, X., Romert, L., Stenberg, K., and Walum, E. (1990) Multivariate validation of cell toxicity data: The first ten MEIC chemicals. ATLA, 17, 237–238.

Hellberg, S., Eriksson, L., Jonsson, J., Lindgren, F., Sjostrom, M., Wold, S., Ekwall, B., Gomez-Lechon, J.M., Clothier, R., Accomando, N.J., Gimes, G., Barile, F.A., Nordin, M., Tyson, C.A., Dierickx, P., Shrivastava, R.S., Tingsleff-Skaanild, M., Garza-Ocanas, L., and Fiskesjo;, G. (1990) Analogy models for prediction of human toxicity. ATLA, 18, 103–116.

Shrivastava, R., Delomenie, C., Chevalier, A., John, G., Ekwall, B., Walum, E., and Massingham, R. (1992) Comparison of *in vivo* acute lethal potency and *in vitro* cytotoxicity of 48 chemicals. Cell Biol. Toxicol., 8(2), 157–170.

Spielmann, H., Genschow, E., Liebsch, M., and Halle, W. (1999) Determination of the starting dose for acute oral toxicity (LD50) testing in the up and down procedure (UDP) from cytotoxicity data. ATLA, 27(6), 957–966.

Walum, E, Nilsson, M, Clemedson, C. and Ekwall, B. (1995) The MEIC program and its implications for the prediction of acute human systemic toxicity, pp 275–282 In Proceedings of the World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing. Alternative Methods in Toxicology and the Life Sciences, Vol. 11. Mary Ann Liebert, New York, 1995.

Dated: June 6, 2000.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 00–14968 Filed 6–13–00; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

[Docket No. FR–4564–N–03]

Notice of Proposed Information Collection: Lead Hazard Control Grant Program Data Collection—Progress Reporting

AGENCY: Office of Lead Hazard Control.

ACTION: Notice.

SUMMARY: The revised information collection requirement described below will be submitted to the Office of Management and Budget (OMB) for review, as required by the Paperwork Reduction Act. The Department is soliciting public comments on the subject proposal.

DATES: Comments Due Date: August 14, 2000.

ADDRESSES: Interested persons are invited to submit comments regarding this proposal. Comments should refer to the proposal by name and/or OMB Control Number and should be sent to: Gail Ward, Reports Liaison Officer, Department of Housing and Urban Development, 451 7th Street, SW, Room P–3206, Washington, DC 20410.

FOR FURTHER INFORMATION CONTACT: Matthew Ammon at (202) 755–1785, ext. 158 (this is not a toll-free number) for copies of the proposed forms and other available documents.

SUPPLEMENTARY INFORMATION: The Department is submitting the revised information collection to OMB for review, as required by the Paperwork Reduction Act of 1995 (44 U.S.C. Chapter 35, as amended).

This Notice is soliciting comments from members of the public and affected agencies concerning the proposed collection of information to: (1) Evaluate whether the revised collection of information is necessary for the proper performance of the functions of the agency, including whether the

Appendix P

In Vitro Cytotoxicity Test Methods and the High Production Volume (HPV) Challenge Program

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Appendix P1

Supplemental Acute Toxicity Protocol

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U.S. EPA/OPPTS/OPPT/High Production Volume (HPV) Challenge Program

[NOTE: This statement was extracted from the EPA web site. The original can be visited at: http://www.epa.gov/chemrtk/toxprtcl.htm]

Supplemental Acute Toxicity Protocol

The EPA, along with the National Toxicology Program and the National Institute of Environmental Health Sciences (NIEHS), sponsored an International Workshop on *In Vitro* Methods held on October 17-20, 2000, to review the validation status of available *in vitro* methods for predicting acute oral toxicity, among other goals.

The October 2000 Workshop concluded that *in vitro* cytotoxicity data could be useful in estimating starting doses for *in vivo* acute toxicity testing, and in this way could also reduce the number of animals used in subsequent *in vivo* tests. The two candidate cytotoxicity tests recommended for use with the regression model for estimating starting doses from *in vitro* cytotoxicity data are neutral red uptake assays using BALB/c 3T3 mouse fibroblasts and normal human keratinocytes. Other cell lines/cells could also be used with the regression model to estimate starting doses, but first the correlation between the *in vitro* test and the *in vivo* test must be established quantitatively. Guidance on these *in vitro* tests, protocols for use of recommended tests, and a reporting template for results of *in vitro* tests are all contained in the **ICCVAM Guidance Document** (2001), which is one of the products of the October Workshop. Further background on the October workshop can be found in the **ICCVAM Workshop Report** (2001).

While the formal request to EPA from NIH that would ask the Agency to accept or reject these protocols has not yet been received (nor have these methods been incorporated in OECD or the EPA acute toxicity test guidelines), the findings of this workshop included a recommendation to all Agencies participating in ICCVAM to consider the use of these *in vitro* cytotoxicity tests as supplements to the current acute oral *in vivo* acute toxicity protocols. These *in vitro* cytotoxicity protocols were recognized earlier in Steven Johnson's letter of October 30, 2001. The *in vitro* tests are supplements to, not replacements for, the

OECD acute toxicity test guideline 425 (known as the Up-and-Down Procedure) which is currently recommended for use in the HPV Challenge Program. The new *in vitro* tests are intended to better estimate the starting doses for new *in vivo* acute oral toxicity studies conducted under the HPV Challenge.

We encourage those participating in the HPV Challenge Program to consider using the recommended *in vitro* tests noted here as a supplemental component in conducting any new *in vivo* acute oral toxicity studies under the HPV Challenge Program, to note the intention to use these protocols in HPV Challenge test plans submitted to EPA, and to summarize the results using the recommended reporting template. This information on the *in vitro* template should accompany results from the *in vivo* acute oral tests, and be provided to EPA as part of the HPV Challenge Program. The October workshop documents and the recommended reporting template for the *in vitro* tests can be found below. The ICCVAM website - *In Vitro* methods page - should be consulted for any future updates to the *in vitro* guidance methodologies prior to proceeding with testing.

In order to gain more familiarity with these methods, technical experts from industry and other organizations were invited to a workshop sponsored by EPA, NIEHS, and others on these *in vitro* methods. The workshop was held February 19-21, 2001 (see the ICCVAM website at http://iccvam.niehs.nih.gov/meetings/schedule.htm for more details).

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods)
Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity. 2001. NIH Publication No. 01-4499. National Institute of Environmental Health Sciences (NIEHS)

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods)

Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity. 2001. NIH Publication No. 01-4500. National Institute of Environmental Health Sciences (NIEHS)

Standard Test Reporting Template

Any updates to this methodology can be found under ***In Vitro* Methods on the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) web site.**

Last updated on September 16, 2002

Visit the ICCVAM Home Page

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Appendix P2

Office of Pollution Prevention and Toxics (OPPT)
Letters to Manufacturers/Importers

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Office of Pollution Prevention
And Toxics

Letters to Manufacturers/Importers

[High Production Volume Voluntary Challenge Program]

October 14, 1999
Company name
Street #
City, State, Zip

Dear Company Contact:

On behalf of the Environmental Protection Agency (EPA), I would like to thank you for your commitment to participate in the voluntary High Production Volume Challenge (HPV) program. We look forward to working with you over the coming years as we achieve our goals for this important program.

As you may be aware, a number of animal protection organizations and the public have raised concerns that the HPV Challenge program may lead to the excessive use of animals in tests and to inadequate attention to existing information and alternative testing methods that do not require animals as test subjects. As a general matter, animal experiments should not be performed if another validated method -- not involving the use of animals -- is reasonably and practically available for use in the HPV Challenge program. To respond to these concerns, and after consultation with the organizations involved in developing the framework for this initiative, I am asking you and your fellow HPV Challenge participants to observe the following principles as we proceed with the program:

1. In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested.

2. Participants shall maximize the use of existing and scientifically adequate data to minimize further testing. To reinforce this approach, EPA will consider information contained in the databases identified in the enclosure, or in databases maintained by the organizations identified in the enclosure, to have been known to the Agency within the meaning of Section 8(e) of the Toxic Substances Control Act (TSCA), 42 U.S.C. 2607(e). This policy is limited to information reported by participants under the HPV Challenge program and generated for or contained in these databases as of the date of this letter. In addition, any other potential liability under TSCA Section 8(e) for existing data on HPV Challenge program chemicals will be limited according to the terms of the “Registration

Agreement for TSCA Section 8(e) Compliance Audit Program (56 Fed. Reg. 4128, Feb. 1, 1991)." This policy does not affect prior 8(e) enforcement actions.

3. Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships.

4. Consistent with the Screening Information Data Set (SIDS) program of the Organization for Economic Cooperation and Development (OECD), participants shall not conduct any terrestrial toxicity testing.

5. Participants are encouraged to use in vitro genetic toxicity testing to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use.

6. Consistent with the OECD/SIDS program, participants generally should not develop any new dermal toxicity data.

7. Participants shall not develop sub-chronic or reproductive toxicity data for the HPV chemicals that are solely closed system intermediates, as defined by the OECD/SIDS guidelines.

8. In analyzing the adequacy of screening data for chemicals that are substances Generally Recognized as Safe (GRAS) for a particular use by the Food and Drug Administration (FDA), participants should consider all relevant and available information supporting the FDA's conclusions. Participants reviewing the adequacy of existing data for these chemicals should specifically consider whether the information available makes it unnecessary to proceed with further testing involving animals. As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be useful or relevant.

9. Because validated non-animal tests for some SIDS endpoints may be available soon, participants shall make the following revisions to the sequence of testing:

(a) Testing of closed system intermediates, which present less risk of exposure, shall be deferred until 2003;

(b) Individual chemicals (i.e., those HPV chemicals not proposed for testing in a category) that require further testing on animals shall be deferred until November 2001.

These revisions should not be construed to suggest that delay or deferral is appropriate with respect to testing of scientifically appropriate categories of related chemicals.

10. Companies shall allow 120 days between the posting of test plans and the implementation of any testing plans.

To promote the availability and use of alternatives to tests involving animals, the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program

(NTP) will commit at least $1.5 million in FY 2000, and $3 Million in FY 2001, and any further funds appropriated by Congress, to the development and validation of non-animal alternative test methods and protocols. EPA will provide an additional $250,000 this year and will seek to provide a similar amount next year to these efforts. The Multicenter Evaluation of In Vitro Cytotoxicity (MEIC), on the agenda for the October 14 meeting of NTP's Advisory Committee on Alternative Toxicological Methods, will be given priority attention. EPA will promptly incorporate, as appropriate, the work of NIEHS and NTP into the HPV Challenge program.

EPA recognizes that the HPV Challenge is a voluntary program that includes substantial public review and involvement. The successful implementation of the changes described in this letter will depend upon the good faith effort and cooperation of all parties. We appreciate the spirit of cooperation and commitment that has characterized this initiative to date. The changes to the HPV Challenge program outlined above present the opportunity to advance our shared goals of expanding the basic health data available to the public, while incorporating certain animal welfare concerns and scientific principles. It is the intention of the Agency that the HPV Challenge program, including the test rule(s), should proceed in a manner that is consistent with these principles and concerns.

Again, I thank you for your commitment to participate in the HPV Challenge program. If you need further clarification or assistance with this program, please contact Barbara Leczynski at 202-260-3749 or visit the website at www.epa.gov/chemrtk.

Sincerely,
/s/
Susan H. Wayland
Deputy Assistant Administrator

Enclosure

ENCLOSURE A

The IUCLID database administered by the European Union's Existing Chemicals Bureau
Aquatic Information Retrieval (AQUIRE)
Catalog of Teratogenic Agents (CTA)
Chemical Carcinogenesis Research Information System (CCRIS)
Chemical Information System (CIS)
The ChemID database of the National Library of Medicine (NLM)
Datalog
Developmental and Reproductive Technology (DART)
Envirofate Environmental Mutagen Information Center (EMIC)
Environmental Teratology Information Center (ETIC/ETICBACK)
GENE-TOX
Hazardous Substances Data Bank (HSDB)
Integrated Risk Management System (IRIS)
Merck Index National Institute for Occupational Safety and Health (NIOSH)
National Library of Medicine TOXLINE and TOXNET
National Toxicology Program (NTP) Testing Information and Study Results
NTP Technical Reports
NTP Chemical Health and Safety Data
Phytotox Registry of Toxic Effects of Chemical Substances
Structure and Nomenclature Search System (SANSS)
Toxics Substances Control Act Test Submissions (TSCATS)
WHO/IPCS Documents (CICADS and Environmental Health Criteria Documents) BIODEG
BIOLOG
CANCERLIT
CHEMFATE
CHRIS
FIFRA Database/MRID
IRAC Documents
MEDLINE
National Cancer Institute Journal
POISINDEX
Shepard's Catalog
STN (Chemical Abstracts Service)

Document Source: http://www.epa.gov/opptintr/chemrtk/ceoltr.htm

Appendix Q

Additional UDP Simulation Modeling Results

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Appendix Q1

UDP Simulation Results for the RC Rat-Only Millimole Regression Starting at the LD_{50} Predicted by the 3T3 and NHK NRU IC_{50} - 5000 mg/kg Upper Limit Dose

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Summary of Animals Used and Animals Dead for UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
			Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3T3	0.12	Cyto	0.2099	7.35	0.63	0.0017	0.2270	3.62	-0.16	0.1577	7.9%	-4.7%
		Default	0.1750	7.97			0.1999	3.45				
	0.25	Cyto	0.2053	8.06	0.63	0.0036	0.2257	3.97	-0.19	0.1615	7.2%	-4.9%
		Default	0.1746	8.69			0.1955	3.78				
	0.50	Cyto	0.1904	8.72	0.63	0.0044	0.2166	4.31	-0.19	0.2406	6.8%	-4.6%
		Default	0.1614	9.35			0.1821	4.12				
	1.25	Cyto	0.1649	9.27	0.67	0.0022	0.1917	4.67	-0.12	0.8288	6.7%	-2.7%
		Default	0.1310	9.94			0.1491	4.55				
	2.00	Cyto	0.1421	9.41	0.60	0.0011	0.1678	4.76	-0.08	0.8530	6.0%	-1.8%
		Default	0.0956	10.02			0.1265	4.68				
			Average Difference		0.63		Average Difference		-0.15			
NHK	0.12	Cyto	0.2225	7.44	0.50	0.0060	0.2357	3.58	-0.18	0.1299	6.3%	-5.3%
		Default	0.1741	7.94			0.2023	3.40				
	0.25	Cyto	0.2124	8.12	0.54	0.0050	0.2317	3.91	-0.19	0.1848	6.3%	-5.0%
		Default	0.1697	8.67			0.1967	3.73				
	0.50	Cyto	0.1919	8.79	0.56	0.0045	0.2191	4.28	-0.20	0.1974	5.9%	-4.9%
		Default	0.1543	9.35			0.1812	4.08				
	1.25	Cyto	0.1633	9.34	0.62	0.0010	0.1931	4.66	-0.13	0.7671	6.2%	-2.8%
		Default	0.1241	9.96			0.1478	4.53				
	2.00	Cyto	0.1405	9.47	0.56	0.0005	0.1696	4.75	-0.09	0.7533	5.6%	-1.9%
		Default	0.0921	10.03			0.1249	4.66				
			Average Difference		0.56		Average Difference		-0.16			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean number of animals used for the default starting dose and mean number of animals used for the NRU-based starting dose.

[4]P-value is from one-sided Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

Summary of Stopping Rules Used for the UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	3 Animals at Limit Dose[2]	5 Reversals[2]	Likelihood Ratio[2]	Maximum Number of Animals Used[2]
3T3	0.12	Cyto	15.8%	58.7%	24.3%	1.1%
		Default	15.4%	57.3%	24.9%	2.4%
	0.25	Cyto	15.2%	33.9%	48.3%	2.7%
		Default	14.6%	34.3%	45.9%	5.2%
	0.5	Cyto	13.8%	19.7%	60.4%	6.1%
		Default	13.0%	20.0%	57.5%	9.6%
	1.25	Cyto	10.5%	13.2%	64.7%	11.6%
		Default	9.1%	13.6%	60.9%	16.3%
	2	Cyto	9.4%	12.1%	65.4%	13.2%
		Default	7.4%	12.5%	62.5%	17.6%
NHK	0.12	Cyto	17.0%	54.8%	26.7%	1.5%
		Default	16.6%	56.3%	24.8%	2.3%
	0.25	Cyto	16.3%	32.7%	48.0%	3.0%
		Default	15.8%	33.7%	45.5%	5.1%
	0.5	Cyto	14.4%	19.3%	59.6%	6.6%
		Default	13.8%	19.9%	56.9%	9.5%
	1.25	Cyto	10.5%	13.3%	64.2%	11.9%
		Default	9.5%	13.5%	60.5%	16.4%
	2	Cyto	9.2%	12.0%	65.2%	13.6%
		Default	7.6%	12.5%	62.1%	17.7%

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 175 mg/kg; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Percentage of the 10,000 test simulations that satisfied the specified condition for completion of testing (see OECD [2001a]; EPA [2002a]).

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
1	3T3	0.12	Cyto	0.650	9.65	0.01	0.8750	0.586	6.31	0.06	0.8750	0.1%	1.0%
			Default	0.273	9.65			0.170	6.37				
		0.25	Cyto	0.642	10.24	0.23	0.6250	0.579	6.69	0.15	0.8750	2.2%	2.2%
			Default	0.192	10.47			0.162	6.84				
		0.50	Cyto	0.646	10.87	0.35	0.6250	0.596	7.14	0.18	0.6250	3.1%	2.4%
			Default	0.201	11.22			0.198	7.31				
		1.25	Cyto	0.624	11.27	0.40	0.6250	0.587	7.30	0.22	0.6250	3.4%	2.9%
			Default	0.141	11.67			0.161	7.52				
		2.00	Cyto	0.563	11.16	0.25	0.6250	0.532	7.05	0.17	0.6250	2.2%	2.3%
			Default	0.123	11.41			0.140	7.21				
				Average Difference		0.25		Average Difference		0.15			
	NHK	0.12	Cyto	0.815	9.91	-0.30	0.8750	0.682	6.46	-0.11	1.0000	-3.1%	-1.7%
			Default	0.292	9.61			0.178	6.35				
		0.25	Cyto	0.693	10.44	-0.05	1.0000	0.603	6.84	-0.03	1.0000	-0.5%	-0.4%
			Default	0.267	10.39			0.195	6.81				
		0.50	Cyto	0.629	11.05	0.09	0.8750	0.578	7.28	0.00	1.0000	0.8%	0.0%
			Default	0.257	11.14			0.232	7.28				
		1.25	Cyto	0.583	11.43	0.22	0.8750	0.565	7.44	0.09	0.8750	1.9%	1.1%
			Default	0.176	11.65			0.188	7.53				
		2.00	Cyto	0.561	11.26	0.15	0.8750	0.532	7.15	0.05	0.8750	1.3%	0.7%
			Default	0.155	11.40			0.153	7.20				
				Average Difference		0.02		Average Difference		0.00			
2	3T3	0.12	Cyto	0.433	9.04	-0.60	0.1272	0.417	5.64	-0.53	0.0942	-7.1%	-10.4%
			Default	0.284	8.44			0.241	5.11				
		0.25	Cyto	0.460	9.66	-0.68	0.1099	0.428	5.99	-0.56	0.1099	-7.6%	-10.4%
			Default	0.208	8.98			0.201	5.43				
		0.50	Cyto	0.491	10.24	-0.71	0.1272	0.447	6.31	-0.61	0.0942	-7.4%	-10.6%
			Default	0.227	9.53			0.209	5.71				
		1.25	Cyto	0.449	10.71	-0.65	0.0942	0.425	6.52	-0.61	0.0942	-6.5%	-10.2%
			Default	0.236	10.06			0.216	5.92				
		2.00	Cyto	0.364	10.70	-0.58	0.0942	0.361	6.41	-0.53	0.1099	-5.7%	-9.1%
			Default	0.178	10.12			0.177	5.87				
				Average Difference		-0.64		Average Difference		-0.67			
	NHK	0.12	Cyto	0.494	9.17	-0.76	0.0942	0.486	5.66	-0.57	0.1677	-9.1%	-11.1%
			Default	0.263	8.41			0.231	5.09				
		0.25	Cyto	0.473	9.79	-0.83	0.0803	0.478	6.02	-0.60	0.0942	-9.2%	-11.1%
			Default	0.160	8.96			0.183	5.41				
		0.50	Cyto	0.498	10.33	-0.81	0.0942	0.495	6.33	-0.63	0.0942	-8.5%	-11.1%
			Default	0.153	9.52			0.179	5.70				
		1.25	Cyto	0.471	10.77	-0.71	0.0803	0.480	6.53	-0.62	0.0681	-7.0%	-10.4%
			Default	0.179	10.07			0.192	5.91				
		2.00	Cyto	0.392	10.77	-0.63	0.0574	0.417	6.42	-0.55	0.0803	-6.2%	-9.4%
			Default	0.147	10.14			0.164	5.87				
				Average Difference		-0.75		Average Difference		-0.59			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3	3T3	0.12	Cyto	0.255	7.32	-0.61	0.0522	0.213	4.02	-0.60	0.0161	-9.1%	-17.5%
			Default	0.212	6.71			0.133	3.42				
		0.25	Cyto	0.269	8.07	-0.78	0.0093	0.221	4.42	-0.66	0.0068	-10.8%	-17.4%
			Default	0.138	7.28			0.094	3.76				
		0.50	Cyto	0.274	8.71	-0.94	0.0093	0.220	4.75	-0.70	0.0068	-12.2%	-17.3%
			Default	0.094	7.76			0.077	4.05				
		1.25	Cyto	0.193	9.35	-0.79	0.0049	0.170	5.04	-0.58	0.0068	-9.2%	-13.1%
			Default	0.059	8.56			0.056	4.45				
		2.00	Cyto	0.120	9.54	-0.48	0.0068	0.128	5.10	-0.42	0.0122	-5.3%	-8.9%
			Default	0.038	9.07			0.047	4.69				
				Average Difference		-0.72		Average Difference		-0.59			
	NHK	0.12	Cyto	0.258	7.11	-0.44	0.0923	0.196	3.80	-0.40	0.0269	-6.6%	-11.8%
			Default	0.222	6.67			0.139	3.40				
		0.25	Cyto	0.297	7.78	-0.56	0.0269	0.222	4.17	-0.44	0.0640	-7.7%	-11.8%
			Default	0.173	7.23			0.112	3.73				
		0.50	Cyto	0.271	8.45	-0.68	0.0269	0.210	4.51	-0.47	0.1294	-8.8%	-11.7%
			Default	0.107	7.77			0.083	4.04				
		1.25	Cyto	0.168	9.13	-0.52	0.0093	0.154	4.83	-0.36	0.0923	-6.0%	-8.1%
			Default	0.061	8.61			0.059	4.47				
		2.00	Cyto	0.104	9.42	-0.33	0.0425	0.118	4.95	-0.26	0.0923	-3.7%	-5.6%
			Default	0.037	9.09			0.048	4.69				
				Average Difference		-0.51		Average Difference		-0.39			
4	3T3	0.12	Cyto	0.156	6.76	0.78	0.0092	0.053	3.31	0.13	0.1754	10.3%	3.9%
			Default	0.259	7.54			0.078	3.45				
		0.25	Cyto	0.181	7.33	0.71	0.0089	0.050	3.58	0.09	0.0386	8.8%	2.5%
			Default	0.231	8.04			0.060	3.67				
		0.50	Cyto	0.197	7.85	0.79	0.0092	0.053	3.81	0.13	0.0443	9.2%	3.2%
			Default	0.237	8.64			0.059	3.93				
		1.25	Cyto	0.162	8.61	0.63	0.0092	0.051	4.17	0.02	0.1754	6.8%	0.5%
			Default	0.154	9.24			0.022	4.19				
		2.00	Cyto	0.121	9.01	0.43	0.0052	0.045	4.35	-0.06	0.0577	4.6%	-1.4%
			Default	0.089	9.44			0.018	4.29				
				Average Difference		0.67		Average Difference		0.06			
	NHK	0.12	Cyto	0.202	6.95	0.59	0.0833	0.092	3.43	0.02	0.4637	7.8%	0.5%
			Default	0.257	7.54			0.077	3.45				
		0.25	Cyto	0.208	7.44	0.63	0.0386	0.087	3.66	0.03	0.0739	7.8%	0.8%
			Default	0.219	8.07			0.057	3.69				
		0.50	Cyto	0.221	7.93	0.72	0.0290	0.087	3.88	0.06	0.1167	8.4%	1.5%
			Default	0.233	8.66			0.059	3.94				
		1.25	Cyto	0.188	8.68	0.57	0.0290	0.073	4.23	-0.04	0.3755	6.1%	-0.9%
			Default	0.150	9.24			0.022	4.19				
		2.00	Cyto	0.136	9.04	0.41	0.0155	0.056	4.39	-0.10	0.0443	4.3%	-2.4%
			Default	0.090	9.45			0.017	4.29				
				Average Difference		0.58		Average Difference		-0.01			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
5	3T3	0.12	Cyto	0.299	7.16	2.03	0.0020	0.035	3.18	0.14	0.0645	22.1%	4.2%
			Default	0.216	9.19			0.045	3.32				
		0.25	Cyto	0.233	8.10	2.29	0.0020	0.031	3.43	0.16	0.0645	22.1%	4.6%
			Default	0.141	10.39			0.075	3.59				
		0.50	Cyto	0.178	8.54	2.25	0.0020	0.050	3.53	0.14	0.0488	20.9%	3.8%
			Default	0.090	10.79			0.071	3.68				
		1.25	Cyto	0.141	8.60	2.15	0.0020	0.045	3.62	0.28	0.0020	20.0%	7.3%
			Default	0.062	10.75			0.034	3.91				
		2.00	Cyto	0.118	8.68	1.77	0.0020	0.040	3.74	0.26	0.0020	16.9%	6.5%
			Default	0.055	10.45			0.017	4.00				
				Average Difference		2.10		Average Difference		0.20			
	NHK	0.12	Cyto	0.358	7.38	1.81	0.0020	0.058	3.22	0.10	0.3750	19.7%	2.9%
			Default	0.218	9.19			0.056	3.32				
		0.25	Cyto	0.314	8.26	2.12	0.0020	0.049	3.44	0.16	0.1934	20.5%	4.4%
			Default	0.111	10.38			0.081	3.60				
		0.50	Cyto	0.240	8.75	2.02	0.0020	0.041	3.57	0.10	0.3750	18.7%	2.6%
			Default	0.062	10.77			0.079	3.66				
		1.25	Cyto	0.156	8.81	1.91	0.0020	0.035	3.67	0.22	0.0020	17.9%	5.7%
			Default	0.049	10.72			0.041	3.89				
		2.00	Cyto	0.123	8.86	1.56	0.0020	0.036	3.79	0.20	0.0020	15.0%	5.1%
			Default	0.038	10.42			0.024	3.99				
				Average Difference		1.89		Average Difference		0.15			
6	3T3	0.12	Cyto	0.561	5.71	2.03	0.0005	0.325	0.90	-0.06	0.1294	26.2%	-6.6%
			Default	0.576	7.74			0.300	0.85				
		0.25	Cyto	0.536	6.56	2.08	0.0005	0.326	1.37	-0.08	0.0049	24.1%	-6.2%
			Default	0.531	8.64			0.305	1.29				
		0.50	Cyto	0.399	7.65	2.19	0.0005	0.249	2.07	-0.05	0.0640	22.2%	-2.4%
			Default	0.337	9.84			0.254	2.02				
		1.25	Cyto	0.245	8.41	2.48	0.0005	0.120	2.97	0.23	0.0034	22.7%	7.1%
			Default	0.062	10.89			0.124	3.20				
		2.00	Cyto	0.196	8.45	2.34	0.0005	0.083	3.27	0.35	0.0005	21.7%	9.6%
			Default	0.022	10.78			0.070	3.62				
				Average Difference		2.22		Average Difference		0.08			
	NHK	0.12	Cyto	0.561	5.87	1.76	0.0002	0.309	0.85	-0.06	0.0500	23.0%	-8.0%
			Default	0.548	7.63			0.285	0.79				
		0.25	Cyto	0.534	6.80	1.79	0.0002	0.317	1.36	-0.11	0.0034	20.8%	-9.1%
			Default	0.486	8.59			0.283	1.25				
		0.50	Cyto	0.392	7.95	1.88	0.0002	0.245	2.12	-0.12	0.0024	19.1%	-5.9%
			Default	0.309	9.83			0.233	2.00				
		1.25	Cyto	0.226	8.67	2.20	0.0002	0.116	3.04	0.14	0.0134	20.3%	4.3%
			Default	0.059	10.87			0.115	3.18				
		2.00	Cyto	0.180	8.67	2.11	0.0002	0.080	3.35	0.27	0.0002	19.6%	7.5%
			Default	0.021	10.78			0.064	3.62				
				Average Difference		1.95		Average Difference		0.02			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); Toxcat=Category from Globally Harmonized System of Classification and Labeling of Chemicals[5] (GHS; UN 2005); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean animals used for the default starting dose and mean animals used for the NRU-based starting dose.

[4]P-value is from one-side Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

[5]GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

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Concordance of NRU-Based Starting Dose with Default Starting Dose for GHS Acute Oral Toxicity Category Outcome Based on Simulated UDP LD_{50}[1]

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with NHK NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	15	1	0	17	88%	6%	6%
5	0	0	0	0	22	0	22	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	15	23	0	68	96%	1%	3%

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with 3T3 NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	0	16	1	0	17	94%	6%	0%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	11	16	22	0	67	97%	1%	1%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; NA=Not applicable; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg. The NRU-based starting dose was the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621). The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[2]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

Discordant Substances for GHS Category Outcomes of UDP Simulations[1]

NRU Test Method	Substance	NRU-Based Starting Dose[2]		Default Starting Dose[3]		LD_{50} Difference
		LD_{50}	Toxcat[4]	LD_{50}	Toxcat[4]	
3T3	Acetaminophen	2046.78	5	1765.44	4	-281.34
	Sodium dichromate dihydrate	43.70	2	51.87	3	8.17
NHK	Acetaminophen	2173.95	5	1755.26	4	-418.69
	Caffeine	279.63	3	357.17	4	77.55
	Sodium dichromate dihydrate	45.09	2	51.77	3	6.69

Abbreviations: Toxcat=Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2005); UDP= Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes.

[1]Substances for which the simulated UDP outcome (in terms of GHS category) at the NRU-based starting dose did not match the simulated UDP outcome at the default starting dose. Simulations were performed with 10,000 runs at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]NRU-based starting dose was the LD_{50} predicted by the NRU IC_{50} in the RC rat-only millimole regression (log LD_{50} [mmol/kg] = 0.439 log IC_{50} [mM] + 0.621).

[3]The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[4]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
	$5 < LD_{50} \leq 50$ mg/kg
	$50 < LD_{50} \leq 300$ mg/kg
	$300 < LD_{50} \leq 2000$ mg/kg
2	$2000 < LD_{50} \leq 5000$ mg/kg
3	$LD_{50} > 5000$ mg/kg
4	
5	
6	

Appendix Q2

UDP Simulation Results for the RC Rat-Only Weight Regression Starting at the LD_{50} Predicted by the 3T3 and NHK NRU IC_{50} - 5000 mg/kg Upper Limit Dose

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Summary of Animals Used and Animals Dead for UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
			Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3T3	0.12	Cyto	0.210	7.33	0.66	0.0013	0.224	3.60	-0.15	0.2888	8.2%	-4.2%
		Default	0.177	7.98			0.201	3.46				
	0.25	Cyto	0.202	8.03	0.66	0.0015	0.221	3.94	-0.16	0.1284	7.6%	-4.3%
		Default	0.174	8.70			0.196	3.78				
	0.50	Cyto	0.184	8.67	0.68	0.0023	0.211	4.28	-0.16	0.2071	7.2%	-3.9%
		Default	0.160	9.35			0.182	4.12				
	1.25	Cyto	0.159	9.24	0.71	0.0009	0.187	4.65	-0.10	0.9458	7.1%	-2.2%
		Default	0.130	9.95			0.149	4.55				
	2.00	Cyto	0.137	9.39	0.63	0.0005	0.163	4.75	-0.07	0.8240	6.2%	-1.4%
		Default	0.095	10.02			0.127	4.68				
			Average Difference		0.66		Average Difference		-0.13			
NHK	0.12	Cyto	0.216	7.37	0.59	0.0021	0.230	3.55	-0.15	0.1185	7.4%	-4.3%
		Default	0.175	7.96			0.203	3.41				
	0.25	Cyto	0.209	8.07	0.61	0.0017	0.227	3.90	-0.16	0.2017	7.0%	-4.3%
		Default	0.169	8.68			0.197	3.74				
	0.50	Cyto	0.189	8.73	0.62	0.0019	0.215	4.26	-0.17	0.1974	6.6%	-4.2%
		Default	0.153	9.35			0.181	4.08				
	1.25	Cyto	0.161	9.28	0.68	0.0004	0.190	4.63	-0.10	0.8704	6.8%	-2.3%
		Default	0.124	9.96			0.148	4.53				
	2.00	Cyto	0.139	9.43	0.60	0.0004	0.167	4.74	-0.07	0.9230	6.0%	-1.5%
		Default	0.092	10.03			0.125	4.66				
			Average Difference		0.62		Average Difference		-0.13			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [mg/mL] + 2.024); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean number of animals for the default starting dose and mean number of animals for the NRU-based starting dose.

[4]P-value is from one-sided Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

Summary of Stopping Rules Used for the UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	3 Animals at Limit Dose[2]	5 Reversals[2]	Likelihood Ratio[2]	Maximum Number of Animals Used[2]
3T3	0.12	Cyto	15.8%	60.2%	22.9%	1.1%
		Default	15.4%	57.4%	24.8%	2.4%
	0.25	Cyto	15.1%	34.2%	48.1%	2.6%
		Default	14.6%	34.3%	45.9%	5.2%
	0.5	Cyto	13.7%	19.6%	60.8%	5.8%
		Default	12.9%	20.1%	57.5%	9.5%
	1.25	Cyto	10.4%	13.3%	65.1%	11.2%
		Default	9.1%	13.6%	61.0%	16.3%
	2	Cyto	9.3%	12.1%	65.7%	12.9%
		Default	7.4%	12.5%	62.5%	17.6%
NHK	0.12	Cyto	17.0%	56.2%	25.5%	1.2%
		Default	16.6%	56.4%	24.6%	2.3%
	0.25	Cyto	16.2%	33.1%	47.8%	2.8%
		Default	15.8%	33.8%	45.4%	5.1%
	0.5	Cyto	14.5%	19.3%	60.0%	6.2%
		Default	13.8%	19.9%	56.8%	9.5%
	1.25	Cyto	10.5%	13.2%	64.7%	11.6%
		Default	9.6%	13.6%	60.4%	16.4%
	2	Cyto	9.2%	12.0%	65.5%	13.2%
		Default	7.6%	12.5%	62.1%	17.7%

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024); Default=Default starting dose of 175 mg/kg; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Percentage of the 10,000 test simulations that satisfied the specified condition for completion of testing (see OECD [2001a]; EPA [2002a]).

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
1	3T3	0.12	Cyto	0.581	9.85	-0.21	0.6250	0.532	6.49	-0.12	0.6250	-2.2%	-1.9%
			Default	0.263	9.64			0.167	6.36				
		0.25	Cyto	0.560	10.45	-0.03	1.0000	0.515	6.87	-0.05	1.0000	-0.3%	-0.7%
			Default	0.188	10.42			0.163	6.82				
		0.50	Cyto	0.582	11.06	0.12	0.8750	0.541	7.30	-0.01	1.0000	1.1%	-0.1%
			Default	0.202	11.18			0.198	7.29				
		1.25	Cyto	0.559	11.45	0.20	0.6250	0.535	7.47	0.05	1.0000	1.7%	0.6%
			Default	0.141	11.65			0.161	7.51				
		2.00	Cyto	0.513	11.31	0.09	0.6250	0.488	7.19	0.02	1.0000	0.8%	0.3%
			Default	0.116	11.40			0.136	7.21				
				Average Difference		0.03		Average Difference		-0.02			
	NHK	0.12	Cyto	0.773	10.35	-0.80	0.6250	0.632	6.77	-0.44	0.6250	-8.3%	-7.0%
			Default	0.284	9.56			0.176	6.33				
		0.25	Cyto	0.614	10.66	-0.30	0.8750	0.538	7.02	-0.22	0.8750	-2.9%	-3.2%
			Default	0.259	10.36			0.190	6.80				
		0.50	Cyto	0.550	11.24	-0.13	0.8750	0.512	7.45	-0.18	0.8750	-1.2%	-2.5%
			Default	0.247	11.11			0.226	7.27				
		1.25	Cyto	0.510	11.60	0.03	0.8750	0.506	7.59	-0.08	0.8750	0.2%	-1.0%
			Default	0.174	11.62			0.189	7.51				
		2.00	Cyto	0.493	11.42	-0.02	0.8750	0.479	7.30	-0.09	0.8750	-0.2%	-1.3%
			Default	0.149	11.40			0.150	7.20				
				Average Difference		-0.24		Average Difference		-0.20			
2	3T3	0.12	Cyto	0.423	8.84	-0.35	0.3054	0.396	5.48	-0.36	0.1677	-4.1%	-6.9%
			Default	0.307	8.49			0.250	5.13				
		0.25	Cyto	0.422	9.54	-0.52	0.0942	0.390	5.88	-0.44	0.0942	-5.7%	-8.1%
			Default	0.214	9.02			0.204	5.44				
		0.50	Cyto	0.449	10.13	-0.58	0.1272	0.406	6.21	-0.49	0.1272	-6.1%	-8.6%
			Default	0.218	9.55			0.205	5.72				
		1.25	Cyto	0.416	10.60	-0.54	0.1099	0.390	6.42	-0.50	0.1099	-5.3%	-8.4%
			Default	0.227	10.07			0.213	5.92				
		2.00	Cyto	0.335	10.61	-0.47	0.1272	0.330	6.31	-0.44	0.1272	-4.7%	-7.4%
			Default	0.174	10.13			0.175	5.88				
				Average Difference		-0.49		Average Difference		-0.44			
	NHK	0.12	Cyto	0.423	8.74	-0.23	0.4548	0.434	5.40	-0.27	0.3054	-2.7%	-5.3%
			Default	0.287	8.51			0.239	5.13				
		0.25	Cyto	0.434	9.64	-0.62	0.0803	0.442	5.90	-0.47	0.1677	-6.9%	-8.6%
			Default	0.175	9.02			0.188	5.43				
		0.50	Cyto	0.465	10.25	-0.71	0.1099	0.460	6.25	-0.54	0.1465	-7.4%	-9.4%
			Default	0.158	9.54			0.183	5.71				
		1.25	Cyto	0.445	10.70	-0.61	0.1099	0.447	6.46	-0.53	0.1099	-6.1%	-9.0%
			Default	0.182	10.08			0.194	5.92				
		2.00	Cyto	0.364	10.70	-0.57	0.0681	0.385	6.35	-0.48	0.0803	-5.6%	-8.2%
			Default	0.147	10.13			0.164	5.87				
				Average Difference		-0.55		Average Difference		-0.46			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3	3T3	0.12	Cyto	0.255	7.32	-0.61	0.0522	0.213	4.02	-0.60	0.0161	-9.1%	-17.5%
			Default	0.212	6.71			0.133	3.42				
		0.25	Cyto	0.269	8.07	-0.78	0.0093	0.221	4.42	-0.66	0.0068	-10.8%	-17.4%
			Default	0.138	7.28			0.094	3.76				
		0.50	Cyto	0.274	8.71	-0.94	0.0093	0.220	4.75	-0.70	0.0068	-12.2%	-17.3%
			Default	0.094	7.76			0.077	4.05				
		1.25	Cyto	0.193	9.35	-0.79	0.0049	0.170	5.04	-0.58	0.0068	-9.2%	-13.1%
			Default	0.059	8.56			0.056	4.45				
		2.00	Cyto	0.120	9.54	-0.48	0.0068	0.128	5.10	-0.42	0.0122	-5.3%	-8.9%
			Default	0.038	9.07			0.047	4.69				
				Average Difference		-0.63		Average Difference		-0.53			
	NHK	0.12	Cyto	0.256	7.24	-0.54	0.1514	0.193	3.88	-0.46	0.0923	-8.0%	-13.6%
			Default	0.217	6.70			0.136	3.42				
		0.25	Cyto	0.260	7.77	-0.49	0.0425	0.193	4.16	-0.40	0.0771	-6.7%	-10.6%
			Default	0.165	7.29			0.107	3.76				
		0.50	Cyto	0.228	8.38	-0.58	0.0342	0.178	4.47	-0.41	0.0923	-7.5%	-10.1%
			Default	0.102	7.79			0.080	4.06				
		1.25	Cyto	0.136	9.07	-0.46	0.0342	0.130	4.80	-0.33	0.0771	-5.3%	-7.3%
			Default	0.056	8.62			0.058	4.48				
		2.00	Cyto	0.086	9.40	-0.31	0.0122	0.102	4.94	-0.25	0.1099	-3.4%	-5.3%
			Default	0.035	9.09			0.048	4.69				
				Average Difference		-0.47		Average Difference		-0.37			
4	3T3	0.12	Cyto	0.179	6.73	0.80	0.0092	0.053	3.30	0.15	0.0739	10.7%	4.3%
			Default	0.259	7.53			0.079	3.44				
		0.25	Cyto	0.173	7.34	0.69	0.0092	0.050	3.58	0.09	0.0386	8.6%	2.4%
			Default	0.224	8.03			0.057	3.66				
		0.50	Cyto	0.180	7.86	0.77	0.0092	0.052	3.80	0.12	0.0507	8.9%	3.1%
			Default	0.227	8.63			0.055	3.93				
		1.25	Cyto	0.144	8.64	0.59	0.0092	0.050	4.16	0.02	0.2744	6.4%	0.4%
			Default	0.147	9.23			0.020	4.18				
		2.00	Cyto	0.104	9.03	0.41	0.0052	0.043	4.34	-0.06	0.1167	4.3%	-1.4%
			Default	0.084	9.44			0.018	4.28				
				Average Difference		0.65		Average Difference		0.06			
	NHK	0.12	Cyto	0.202	6.92	0.61	0.0934	0.098	3.41	0.03	0.3484	8.2%	1.0%
			Default	0.256	7.53			0.077	3.44				
		0.25	Cyto	0.189	7.43	0.63	0.0443	0.076	3.64	0.04	0.0833	7.8%	1.0%
			Default	0.216	8.06			0.056	3.68				
		0.50	Cyto	0.201	7.92	0.73	0.0250	0.076	3.86	0.08	0.1046	8.4%	2.0%
			Default	0.226	8.65			0.056	3.94				
		1.25	Cyto	0.168	8.65	0.59	0.0155	0.067	4.20	-0.01	0.3755	6.4%	-0.3%
			Default	0.147	9.24			0.021	4.19				
		2.00	Cyto	0.123	9.02	0.43	0.0155	0.056	4.37	-0.08	0.0934	4.6%	-1.8%
			Default	0.087	9.45			0.017	4.29				
				Average Difference		0.60		Average Difference		0.01			

Summary of Animals Used and Animals Dead for UDP Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
5	3T3	0.12	Cyto	0.287	7.12	2.07	0.0020	0.039	3.19	0.13	0.0840	22.5%	4.0%
			Default	0.220	9.19			0.042	3.32				
		0.25	Cyto	0.228	8.01	2.39	0.0020	0.038	3.43	0.17	0.0488	23.0%	4.8%
			Default	0.145	10.40			0.074	3.60				
		0.50	Cyto	0.186	8.45	2.36	0.0020	0.047	3.52	0.16	0.0488	21.8%	4.4%
			Default	0.091	10.81			0.071	3.68				
		1.25	Cyto	0.133	8.55	2.21	0.0020	0.035	3.62	0.29	0.0020	20.6%	7.3%
			Default	0.061	10.76			0.034	3.91				
		2.00	Cyto	0.105	8.64	1.81	0.0020	0.027	3.75	0.26	0.0020	17.4%	6.5%
			Default	0.051	10.46			0.019	4.01				
				Average Difference		2.17		Average Difference		0.20			
	NHK	0.12	Cyto	0.335	7.31	1.90	0.0020	0.048	3.22	0.11	0.3223	20.6%	3.3%
			Default	0.219	9.21			0.057	3.33				
		0.25	Cyto	0.301	8.17	2.21	0.0020	0.047	3.44	0.16	0.2324	21.3%	4.4%
			Default	0.114	10.38			0.081	3.60				
		0.50	Cyto	0.224	8.62	2.16	0.0020	0.039	3.56	0.11	0.2754	20.1%	3.1%
			Default	0.065	10.79			0.077	3.67				
		1.25	Cyto	0.148	8.73	2.01	0.0020	0.038	3.67	0.22	0.0039	18.7%	5.6%
			Default	0.051	10.74			0.041	3.89				
		2.00	Cyto	0.114	8.78	1.66	0.0020	0.036	3.79	0.21	0.0020	15.9%	5.3%
			Default	0.039	10.44			0.023	4.00				
				Average Difference		1.99		Average Difference		0.16			
6	3T3	0.12	Cyto	0.596	5.75	1.99	0.0005	0.327	0.91	-0.06	0.0923	25.7%	-7.5%
			Default	0.575	7.74			0.300	0.84				
		0.25	Cyto	0.574	6.61	2.02	0.0005	0.335	1.40	-0.10	0.0015	23.4%	-8.1%
			Default	0.529	8.63			0.305	1.29				
		0.50	Cyto	0.411	7.69	2.15	0.0005	0.258	2.10	-0.08	0.0068	21.8%	-3.7%
			Default	0.335	9.83			0.253	2.02				
		1.25	Cyto	0.241	8.42	2.46	0.0005	0.125	2.98	0.21	0.0010	22.6%	6.6%
			Default	0.062	10.88			0.123	3.19				
		2.00	Cyto	0.194	8.47	2.31	0.0005	0.088	3.29	0.33	0.0005	21.4%	9.0%
			Default	0.021	10.78			0.069	3.62				
				Average Difference		2.19		Average Difference		0.06			
	NHK	0.12	Cyto	0.588	5.79	1.84	0.0002	0.310	0.85	-0.06	0.0327	24.1%	-7.7%
			Default	0.548	7.63			0.285	0.79				
		0.25	Cyto	0.561	6.72	1.87	0.0002	0.318	1.36	-0.11	0.0012	21.8%	-8.9%
			Default	0.486	8.59			0.283	1.25				
		0.50	Cyto	0.413	7.85	1.97	0.0002	0.247	2.11	-0.11	0.0046	20.1%	-5.4%
			Default	0.309	9.83			0.232	2.00				
		1.25	Cyto	0.240	8.56	2.31	0.0002	0.121	3.02	0.16	0.0061	21.2%	5.0%
			Default	0.059	10.87			0.115	3.18				
		2.00	Cyto	0.194	8.57	2.21	0.0002	0.085	3.33	0.30	0.0005	20.5%	8.2%
			Default	0.021	10.78			0.063	3.62				
				Average Difference		2.04		Average Difference		0.03			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); Toxcat=Category from Globally Harmonized System of Classification and Labeling of Chemicals[5] (GHS; UN 2005); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mmol/kg] = 0.372 log IC_{50} [mM] + 2.024); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]Mean number of animals for 10,000 simulations.

[3]Difference between mean animals used for the default starting dose and mean animals used for the NRU-based starting dose.

[4]P-value is from one-side Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

[5]GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

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Concordance of NRU-Based Starting Dose with Default Starting Dose for GHS Acute Oral Toxicity Category Outcome Based on Simulated UDP LD_{50}[1]

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with NHK NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	15	1	0	17	88%	6%	6%
5	0	0	0	0	22	0	22	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	15	23	0	68	96%	1%	3%

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with 3T3 NRU-Based Starting Dose									
	1	2	3	4	5	6	Total	Category Match	Higher NRU Category	Lower NRU Category
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	14	2	0	17	82%	12%	6%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	14	23	0	67	94%	3%	3%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; NA=Not applicable; RC=Registry of Cytotoxicity.

[1]For 10,000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg. The NRU-based starting dose was the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024). The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[2]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
	$LD_{50} > 5000$ mg/kg

Discordant Substances for GHS Category Outcomes of UDP Simulations[1]

NRU Test Method	Substance	NRU-Based Starting Dose[2]		Default Starting Dose[3]		LD_{50} Difference
		LD_{50}	Toxcat[4]	LD_{50}	Toxcat[4]	
3T3	Acetaminophen	2146.93	5	1768.39	4	-378.54
	Caffeine	297.82	3	342.76	4	44.95
	Procainamide HCl	2000.24	5	1529.98	4	-470.26
	Sodium dichromate dihydrate	44.48	2	52.17	3	7.69
NHK	Acetaminophen	2171.18	5	1755.21	4	-415.96
	Caffeine	292.06	3	353.96	4	61.91
	Sodium dichromate dihydrate	45.85	2	51.91	3	6.06

Abbreviations: Toxcat=Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2005); UDP= Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes.

[1]Substances for which the simulated UDP outcome (in terms of GHS category) at the NRU-based starting dose did not match the simulated UDP outcome at the default starting dose. Simulations were performed with 10,000 runs at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =5000 mg/kg.

[2]NRU-based starting dose was the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024).

[3]The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[4]

GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
	$5 < LD_{50} \leq 50$ mg/kg
	$50 < LD_{50} \leq 300$ mg/kg
	$300 < LD_{50} \leq 2000$ mg/kg
2	$2000 < LD_{50} \leq 5000$ mg/kg
3	$LD_{50} > 5000$ mg/kg
4	
5	
6	