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4.0 RODENT ACUTE ORAL LD_{50} REFERENCE VALUES USED TO ASSESS THE ACCURACY OF THE 3T3 AND NHK NRU TEST METHODS

The procedures and analyses presented in this section were designed to identify the most accurate rodent acute oral LD_{50} values for the 72 reference substances used in the validation study. These values were needed to ensure that the reference substances were correctly placed within the different GHS toxicity categories and to provide a data set against which to compare the predicted LD_{50} values estimated using the IC_{50} data obtained from the 3T3 and NHK NRU test methods (see **Section 6**). The predicted LD_{50} values are used to determine the starting dose for rodent acute oral toxicity tests and the more accurate the prediction, the fewer the number of rodents that would be used in an acute oral toxicity test (see **Sections 1.0** and **1.2.2**).

4.1 Methods Used to Obtain Rodent Acute Oral LD_{50} Reference Values

4.1.1 Identification of Candidate Rodent Acute Oral LD_{50} Reference Data

No animal testing was performed to obtain the rodent oral acute LD_{50} reference data for this validation study. To identify reference data for the 72 substances, rat acute oral LD_{50} studies were located using literature searches, secondary references, and electronic database searches. Literature searches were conducted in PubMed (U.S. NLM) and the Institute of Scientific Information (ISI) Web of Science® (Thomson Scientific, Philadelphia, PA) using each chemical name and "lethal dose 50" as search terms. Secondary references included NTP technical reports, Toxicological Profiles from the Agency for Toxic Substances and Disease Registry (ATSDR), Cosmetic Ingredient Reviews by the Cosmetics Industry Council, pesticide handbooks, the Merck Index, and various other summary sources. **Table 4-1** lists the electronic databases searched to locate references for rat oral LD_{50} values. Rat LD_{50} data were preferred because:

- The current acute oral toxicity test guidelines recommend using rats (OECD 2001a, c, d; EPA 2002a)
- The majority of LD_{50} data used in the RC millimole regression were from studies using rats (282 rat data points and 65 mouse data points) (Halle 1998, 2003)
- The majority of acute oral systemic toxicity testing is performed with rats

Table 4-1 Internet-Accessible Databases Searched for LD_{50} Information

Database/Source[1]	Sponsor(s)
Agency for Toxic Substances and Disease Registry (ATSDR)	U.S. Department of Health and Human Services (DHHS)
Center for Drug Evaluation and Research (CDER)	U.S. Food and Drug Administration (FDA)
CHEMFINDER	CambridgeSoft Corporation
Chemical Carcinogenesis Research Information System (CCRIS); National Cancer Institute (NCI) Website	NCI; National Institutes of Health (NIH); DHHS
Chemical Evaluation Search and Retrieval System (CESARS)	Michigan Department of Natural Resources; Ontario Ministry of the Environment; Canadian Centre for Occupational Health and Safety (CCOHS) CHEMpendium™
Chemical Hazard Response (CHRIS)	U.S. Coast Guard

Table 4-1 Internet-Accessible Databases Searched for LD_{50} Information

Database/Source[1]	Sponsor(s)
Chemical Ingredients Database	U.S. Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP); California EPA Department of Pesticide Regulation
CHEMINDEX; CHEMINFO	(CCOHS) CHEMpendium™
ChemRTK High Production Volume (HPV) Challenge Program; OPPT Chemical Fact Sheets; Chemical Information Collection and Data Development	EPA Office of Pollution Prevention and Toxics (OPPT)
CIS Chemical Information	World Health Organization (WHO) International Programme on Chemical Safety (IPCS); CCOHS; International Labour Organisation (ILO) Occupational Safety and Health Information Centre (CIS)
Concise International Chemical Assessment Documents (CICADS)	WHO IPCS; CCOHS; ILO; United Nations Environment Programme (UNEP)
Consumer Product Safety Commission Website	U.S. Consumer Product Safety Commission (CPSC)
Deutsches Institut fur Medizinische Dokumentation und Information (DIMDI) [The German Institute for Medical Documentation and Information]; Registry of Cytotoxicity (RC)	Zentralstelle zur Erfassung und Bewertungvon Ersatz- und Erganzungsmethoden zum Tierversuch (ZEBET) [German Centre for the Documentation and Validation of Alternative Methods]
Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART®/ETIC)	EPA; The National Library of Medicine (NLM); The National Institute of Environmental Health Sciences (NIEHS); National Center for Toxicological Research (NCTR)
Emergency Response Guidebook (ERG 2000)	Transport Canada; U.S. Department of Transportation (DOT); Secretariat of Communications and Transportation of Mexico
Environmental Health Criteria (EHC) monographs; Health and Safety Guides (HSG); International Agency for Research on Cancer (IARC)	WHO IPCS; CCOHS
European Centre for the Validation of Alternative Methods (ECVAM) Scientific Information Service (ECVAM SIS)	European Commission Joint Research Centre
HAZARDTEXT®; MEDITEXT®; INFOTEXT®; SARATEXT®; REPROTEXT®; REPROTOX®	TOMES Plus®, MICROMEDEX, Greenwood Village, CO
Integrated Risk Information System (IRIS)	EPA Office of Research and Development (ORD)
International Chemical Safety Cards (ICSC) IPCS/EC Evaluation of Antidotes Series	WHO IPCS; CCOHS; Commission of the European Union (EU)
International Uniform Chemical Information Database (IUCLID)	European Chemicals Bureau
Joint Expert Committee on Food Additives (JECFA); Joint Meeting on Pesticide Residues (JMPR); Pesticide Data Sheets (PDS)	WHO IPCS; CCOHS; Food and Agriculture Organization (FAO) of the United Nations
Material Safety Data Sheets (MSDS)	Interactive Learning Paradigms, Incorporated
Multicentre Evaluation of In Vitro Cytotoxicity (MEIC)	Scandinavian Society for Cell Toxicology
The National MSDS Repository	MSDSSEARCH, Inc.
National Toxicology Program (NTP) Chemical Health and Safety Database	NIEHS
National Transportation Library	DOT
New Jersey Hazardous Substance Fact Sheets	New Jersey Department of Health and Senior Services
Oil and Hazardous Materials/Technical Assistance	EPA Office of Waste and Water Management

Table 4-1 Internet-Accessible Databases Searched for LD_{50} Information

Database/Source[1]	Sponsor(s)
Data System (OHM/TADS)	
Organisation for Economic Co-operation and Development (OECD) Screening Information Data Sets (SIDS)	IPCS; CCOHS; International Register of Potentially Toxic Chemicals (IRPTC); UNEP
Pesticide Action Network Pesticide Database	Pesticide Action Network North America
Pesticide Product Information System (PPIS)	EPA Office of Pesticide Programs (OPP)
Poisons Information Monographs (PIMs)	IPCS; CCOHS
Registry of Toxic Effects of Chemical Substances (RTECS®);NIOSH Pocket Guide to Chemical Hazards	National Institute for Occupational Safety and Health (NIOSH)
SCORECARD	Environmental Defense
The EXtension TOXicology NETwork (EXTOXNET)	University of California, Davis; Oregon State University; Michigan State University; Cornell University; University of Idaho
The Right-to-Know Network (RTK NET)	Office of Management and Budget Watch; Center for Public Data access
Toxic Chemical Release Inventory (TRI); GENE-TOX	The National Library of Medicine (NLM)
Toxic Substances Control Act Test Submissions (TSCATS)	EPA OPPT
TOXLINE®; Hazardous Substances Data Bank (HSDB); ChemIDplus	NLM (TOXNET)

Abbreviations: LD_{50}=Dose lethal to 50% of the animals tested
[1]Includes public and proprietary databases

A total of 195 references containing LD_{50} data retrieved through these searches were reviewed and evaluated. Information regarding the materials, animals, and methods used to derive the 491 LD_{50} values reported by these references were compiled and are provided in **Appendix H1**. **Appendix H2** provides a narrative characterization and evaluation of the LD_{50} values.

4.1.2 Criteria Used to Select Candidate Rodent Acute Oral Data for Determination of LD_{50} Reference Values

This effort was to designed to derive a set of high quality reference oral LD_{50} values from data that were collected using standardized protocols, accompanied by documentation showing that established testing procedures were followed in compliance with national and international GLP guidelines (OECD 1998; FDA 2003; EPA 2003a,b). After a review of the collected data, the SMT determined that a requirement for GLP compliance would eliminate 99% (452 of the 459 values remaining after exclusion of 30 duplicate values and two erroneous values) of the oral LD_{50} values.

The SMT then considered limiting the selection of LD_{50} values to those from studies that used the specifications for animals recommended by the current acute oral toxicity test guidelines. The current guidelines recommend using young adult rats, 8 to 12 weeks of age, of a common laboratory strain (e.g., Sprague-Dawley) and the most sensitive sex (OECD 2001a, c, d; EPA 2002a). Female animals are recommended if there is no information from which to determine the most sensitive sex. A limited number of LD_{50} values were available

from animals that fit this description; only 3% (14/459) of the oral LD_{50} values were determined using 8 to 12 week old female laboratory rats. An additional 15 LD_{50} values were obtained from female rats in an appropriate weight range (age not provided in the reference) for that age range (~ 176-250 g according to Charles River [http://www.criver.com], Harlan [http://www.harlan.com/us/index.htm], and Taconic Farms [http://www.taconic.com/anmodels/spragued.htm] websites). Thus, only 6% (29/459) of the acute oral LD_{50} values in the database, covering 21 of the 72 reference substances (29%), were from studies that used the strain, sex, and age of rats recommended by current test guidelines (OECD 2001a; EPA 2002a).

4.1.2.1 *Final Exclusion Criteria*

Because so few studies met the initial criteria (i.e., GLP compliance and use of animals recommended by current acute oral toxicity test guidelines), the database was reviewed and evaluated to derive alternative criteria for the development of reference LD_{50} values. For this evaluation, the SMT looked for commonalities among the data records that, when selected, provided a comparable data set for each chemical. Review of the available data indicated that the majority of acute oral toxicity tests were conducted by gavage to unanesthetized, young adult laboratory rats of both genders. Thus, the selection process was revised to exclude studies that reflected the following, less typical, materials, animals, and methods in order to compile a homogenous set of reference LD_{50} values for each chemical. The studies excluded were those with:

- Feral rats
- Rats <4 weeks of age
- Anesthetized rats
- Test chemical administered in food or capsule
- LD_{50} reported as a range or inequality

Data from feral rats were excluded because the health status and age of these animals was uncertain. All laboratory rat strains/stocks were deemed acceptable on the assumption that they were healthy and provided with adequate care and housing during testing. Data from neonates and weanlings were excluded because their sensitivity to chemical toxicity may differ from that of adults. Four weeks was considered the minimum acceptable age because rats are typically weaned at approximately three weeks of age (Barrow 2000). Data from feeding experiments or experiments that involved administration of the chemical in capsules were also excluded because gavage is the most common mode of administration for acute oral studies and the rate of gastrointestinal absorption for these other methods is likely to be different (Nebendahl 2000). Because LD_{50} point estimates are required for the prediction model, LD_{50} values reported as ranges or inequalities were unacceptable.

4.1.2.2 *Assumptions Regarding Materials, Animals, and Methods*

The level of detail for describing the materials, animals, and methods for the LD_{50} studies varied greatly. For example, some studies reported only that white rats were used, while others provided complete information on stock/strain, gender, and age of animals. Details on other protocol components such as the number of animals tested per dose group, method of administration, doses administered, clinical signs, and times of death varied as well. In order to use as much of the available data as possible, the following assumptions were made if a study report did not state otherwise:

- Rats were young adults of a common laboratory strain
- Rats were not anesthetized
- Oral route of administration was by gavage

4.1.2.3 *Calculation of Reference LD_{50} Values*

If a substance had multiple LD_{50} values after the application of the exclusion criteria, the outliers at the 99% level (Dixon and Massey 1981) were excluded. A geometric mean and 95% confidence limits were calculated from the remaining values, and used as the reference LD_{50}. A geometric mean was used because it is the antilog of the mean of the logarithm of the values and is less affected than the arithmetic mean by extreme values. The use of a geometric mean also corresponds with the approach used for the RC millimole regression to derive a single IC_{50} value from multiple IC_{50} values (Halle 1998, 2003), and with the approach used to derive the IC_{50} value for each chemical for the *in vitro - in vivo* regressions evaluated in the NICEATM/ECVAM validation study (see **Section 6**).

In addition to the statistical evaluation of outliers, an extreme value, which was not a statistical outlier but was based on biological plausibility, was identified for trichloroacetic acid. This chemical had five reported LD_{50} values ranging from 400-8900 mg/kg after applying the exclusionary criteria. The lowest value (400 mg/kg) was rejected as biologically implausible because up to 1000 mg/kg/day had been used in an oral chronic rodent carcinogenicity study with no, or only minimal, toxicity (EPA 1996).

4.1.2.4 *Use of Rat and Mouse Data*

If no rat oral LD_{50} values could be found for a reference substance, mouse acute oral LD_{50} values were evaluated using the same approach as was used for rat values. Because an IC_{50}-LD_{50} regression model using only rat data was preferable, the three reference substances (i.e., epinephrine bitartrate, colchicine, and propylparaben) for which mouse values only were available were not used for the evaluations of accuracy (**Section 6**) or animal reduction (**Section 10**).

4.2 Final Rodent Acute Oral LD_{50} Reference Values

After the application of the exclusionary criteria, there were 385 acceptable rodent acute oral LD_{50} values from which to calculate reference LD_{50} values. **Table 4-2** shows the reference LD_{50} value for each substance in descending order of toxicity, presented both as mg/kg and as mmol/kg. Data are presented as mmol/kg in order to be consistent with the RC approach. The RC millimole regression used units of mmol/kg for the LD_{50} and mM for the IC_{50} (see **Section 1.1.3**). Also shown for each substance are the 95% confidence limits around the geometric mean, the ratio of the maximum to the minimum acceptable value, the number of LD_{50} values used to calculate the reference value, the number of LD_{50} values available (not including duplicate values or erroneous values), and the LD_{50} value initially used for hazard classification of the reference substance (see **Table 3-2**).

Table 4-2 lists the reference substances grouped by GHS acute oral toxicity category (UN 2005) using the reference LD_{50} values that were derived as described above. The initial categorization for this study, which used the LD_{50} values in the far right column of **Table 4-2** (i.e., values reported in **Table 3-2**, which come from the RC unless otherwise specified), placed 12 substances in each toxicity category. **Table 4-3** compares the number of substances in each GHS toxicity category based on their reference LD_{50} values with the number in each

category based on the initial LD_{50} values. The initial and reference LD_{50} values placed 53 (74%) of the substances in the same GHS category. Nineteen substances (26%) were reclassified based on the reference LD_{50} values (this value is the sum of the numbers in the discordant cells in **Table 4-3)**. Compared with the initial LD_{50} value, the reference LD_{50} value was higher for 18 (25%) and lower for only one (1%) of the substances.

Of the 19 reference substances that were reclassified because of the reference LD_{50} values, five substances originally assigned to the most toxic, $LD_{50} \leq 5$ mg/kg, category (i.e., aminopterin, mercury chloride, busulfan, parathion, and strychnine) were moved to the next, less toxic, category ($5 < LD_{50} \leq 50$ mg/kg). In the $5 < LD_{50} \leq 50$ mg/kg category, four substances (dichlorvos, fenpropathrin, sodium dichromate dihydrate, and nicotine) moved to the less toxic $50 < LD_{50} \leq 300$ mg/kg category, and one (triphenyltin hydroxide) moved two categories to $300 < LD_{50} \leq 2000$ mg/kg. In the $50 < LD_{50} \leq 300$ category, four substances (haloperidol, caffeine, copper sulfate pentahydrate, and sodium oxalate) moved to a lower toxicity category ($300 < LD_{50} \leq 2000$ mg/kg). Only carbamazepine moved from the $300 < LD_{50} \leq 2000$ mg/kg category to the $2000 < LD_{50} \leq 5000$ mg/kg category. In the $2000 < LD_{50} \leq 5000$ mg/kg category, citric acid, trichloroacetic acid and dimethylformamide moved to the next lower toxicity category ($LD_{50} > 5000$ mg/kg). In the $LD_{50} > 5000$ mg/kg category, 5-aminosalicylic acid moved to the higher toxicity, $2000 < LD_{50} \leq 5000$ mg/kg category. This was the only substance that moved to a more toxic category

4.3 Relevant Toxicity Information for Humans

The relevance of rodent acute oral LD_{50} data to human LC values was assessed by the MEIC program (Ekwall et al. 1998b), which used mouse and rat oral LD_{50} data from RTECS® (Ekwall et al. 1998a). Mean lethal doses in humans were collected primarily from handbooks containing human clinical toxicity information (Ekwall et al. 1998a) supplemented, when necessary, by an in-house compendium from the Swedish Poisons Information Centre. Ekwall et al. (1998b) calculated least squares linear regressions for the prediction of the mean human LC values by rat and/or mouse oral LD_{50} data for the 50 MEIC substances using units of log mol/kg. They reported a correlation of $R^2 = 0.607$ for the rat oral LD_{50} prediction of mean human LC values and $R^2 = 0.653$ for the mouse oral LD_{50} prediction of mean human LC values. It is important for comparisons of MEIC data with rodent LD_{50} values to note that the MEIC human values are not lethal doses, and therefore not equivalent to LD_{50} values. Many of the values (if not the majority) are blood concentrations that were associated with morbidity or mortality, and usually do not reflect the actual dose consumed by the patient. These are not necessarily the peak blood concentrations, but only the concentrations at the time of ascertainment, which could have ranged from immediately after onset of medical treatment to post-mortem. The MEIC organizers readily admitted that they could not relate the blood concentrations to the administered dose.

The relevance of the NRU data collected in the NICEATM/ECVAM validation study to the prediction of human acute toxicity will be addressed elsewhere by ECVAM in a separate evaluation.

Table 4-2 Rodent Acute Oral Reference LD_{50} Values Listed by GHS Category[1]

GHS Category[1]/ Reference Substance	Reference Acute Oral LD_{50}[2,3] (mg/kg)	95% Confidence Interval[4] (mg/kg)	Reference Acute Oral LD_{50} Range[5] (mg/kg)	Reference Acute Oral LD_{50}[2] (mmol/kg)	95% Confidence Interval[4] (mmol/kg)	Maximum: Minimum Value[6]	N	Initial Rodent Acute Oral LD_{50}[3,7] (mg/kg)
$LD_{50} \leq 5$ mg/kg (N =7)								
Cycloheximide	2	NC	1-2.5	0.00711	NC	2.5	3	2
Phenylthiourea	3	NC	3	0.0197	NC	NC	1	3
Sodium selenate	3	NC	1.6-5.98	0.0159	NC	3.7	2	2[8]
Epinephrine bitartrate	4 (mouse)	NC	4	0.0196	NC	NC	1	4 (mouse)
Triethylenemelamine	4	1-25	1-13	0.0120	0.0037-0.12	13.0	4	1
Physostigmine	5	NC	5	0.0182	NC	NC	1	5[8]
Disulfoton	5	2-10	2.3-12.6	0.0182	0.009-0.036	5.5	6	2
$5 < LD_{50} \leq 50$ mg/kg (N =12)								
Parathion	6	3-12	1.8-30	0.0209	0.010-0.041	16.7	10	2
Strychnine	6	NC	2.35-16.2	0.0188	NC	6.9	3	2[8]
Aminopterin	7	NC	7	0.016	NC	NC	1	3 (mouse)
Potassium cyanide	7	5-10	5-10	0.111	0.077-0.15	2.0	7	10
Busulfan	12	NC	1.9-29	0.049	0.008-0.38	15.3	4	2
Colchicine	15 (mouse)	NC	5.886-29	0.0375	NC	4.9	3	6 (mouse)
Thallium I sulfate	25	NC	25	0.0495	NC	NC	1	29 (mouse)
Arsenic III trioxide	25	10-64	13-81.5	0.127	0.050-0.32	6.3	5	20
Endosulfan	28	NC	18-43	0.068	NC	2.4	2	18[8]
Digoxin	28	NC	28	0.0362	NC	NC	1	18 (mouse)
Mercury II chloride	40	27-60	12-92	0.148	0.010-0.22	7.7	10	1
Sodium arsenite	44	36-53	36-53	0.336	0.28-0.40	1.5	5	41[8]
$50 < LD_{50} \leq 300$ mg/kg (N =12)								
Sodium dichromate dihydrate	51	44-58	34.17-64.5	0.193	0.17-0.22	1.9	11	50
Dichlorvos	59	40-88	17-97.5	0.266	0.18-0.40	5.7	9	17[8]
Nicotine	70	68-72	68-71	0.430	0.42-0.44	1.0	4	50
Fenpropathrin	76	57-100	48.5-164	0.217	0.16-0.29	3.4	9	18[8]
Hexachlorophene	82	68-98	56-215	0.202	0.17-0.24	3.8	19	61
Paraquat	93	65-132	57-115	0.498	0.35-0.71	2.0	5	58
Lindane	100	78-129	88-125	0.344	0.27-0.44	1.4	4	76
Verapamil HCl	111	NC	108-114	0.226	NC	1.1	2	108

Table 4-2 Rodent Acute Oral Reference LD_{50} Values Listed by GHS Category[1]

GHS Category[1]/ Reference Substance	Reference Acute Oral LD_{50}[2,3] (mg/kg)	95% Confidence Interval[4] (mg/kg)	Reference Acute Oral LD_{50} Range[5] (mg/kg)	Reference Acute Oral LD_{50}[2] (mmol/kg)	95% Confidence Interval[4] (mmol/kg)	Maximum: Minimum Value[6]	N	Initial Rodent Acute Oral LD_{50}[3,7] (mg/kg)
300 < LD_{50} ≤2000 mg/kg (N =16)								
Caffeine	310	256-374	192-483	1.59	1.32-1.93	2.5	10	192
Triphenyltin hydroxide	329	208-520	46.4-1200	0.896	0.57-1.42	25.9	15	44
Haloperidol	330	NC	128-850	0.877	NC	6.6	2	128[8]
Amitriptyline HCl	348	NC	320-380	1.18	NC	1.2	2	319
Propranolol HCl	466	NC	466	1.575	NC	NC	1	470 (mouse)
Cupric sulfate • 5 H_2O	474	269-836	236.2-960	1.90	1.08-3.35	4.1	6	300
Phenol	548	434-692	317-1500	5.82	4.82-7.68	4.7	14	414
Lithium carbonate	590	479-728	525-710	7.98	6.5-9.9	1.4	4	1187 (mouse; sulfate salt)
Glutethimide	600	NC	600	2.76	NC	NC	1	600
Sodium oxalate	633	NC	558-707	4.724	NC	1.3	2[11]	155 (mouse)[9]
Chloral hydrate	638	391-1040	479-863	3.86	2.36-6.29	1.8	4	479
Atropine sulfate	819	641-1045	600-1136	1.21	0.95-1.54	1.9	7	623
Valproic acid	995	NC	670-1480	6.91	NC	2.2	2	1695 (mouse)
Meprobamate	1387	1291-1489	1286-1522	6.35	5.92-6.82	1.2	6	794[8]
Acetylsalicylic acid	1506	1224-1854	616-2840	8.36	6.8-10.3	4.6	14[11]	1000
Procainamide HCl	1950	NC	1950	8.286	NC	NC	1	1950[8]
2000 < LD_{50} ≤5000 mg/kg (N =11)								
Acetaminophen	2163	NC	1944-2404	14.3	NC	1.2	2	2404
Potassium I chloride	2799	NC	2600-3020	37.6	NC	1.2	2	2602
Carbamazepine	2805	NC	1957-4025	11.9	NC	2.1	2	1957[8]
Boric aid	3426	2617-4486	2660-5140	55.4	42.3-72.6	1.9	6	2660[8]
5-Aminosalicylic acid	3429	NC	2800-4200	22.4	NC	1.5	2	7749 (mouse)
Chloramphenicol	3491	NC	2500-5000	10.8	NC	2.0	3	3393
Acetonitrile	3598	2951-4375	1320-8120	87.6	71.9-107	6.2	26	3798
Lactic acid	3639	NC	3543-3730	40.3	NC	1.1	2	3730

Table 4-2 Rodent Acute Oral Reference LD_{50} Values Listed by GHS Category[1]

GHS Category[1]/ Reference Substance	Reference Acute Oral LD_{50}[2,3] (mg/kg)	95% Confidence Interval[4] (mg/kg)	Reference Acute Oral LD_{50} Range[5] (mg/kg)	Reference Acute Oral LD_{50}[2] (mmol/kg)	95% Confidence Interval[4] (mmol/kg)	Maximum: Minimum Value[6]	N	Initial Rodent Acute Oral LD_{50}[3,7] (mg/kg)
Carbon tetrachloride	3783	3024-4732	2350-10054	24.6	20-31	4.3	15	2799
Sodium chloride	4046	2917-5623	3000-6140	69.3	50-96	2.0	5	2998
Xylene	4667	1294-16827	1537-8620	43.9	12-158	5.6	4	4300
LD_{50} >5000 mg/kg (N =14)								
2-Propanol	5105	4624-5636	4500-5840	84.9	77-94	1.3	6	5843
Trichloroacetic acid	5229	2745-9961	3320-8900	32.0	16.8-61.0	2.7	4	4999
Dimethylformamide	5309	3548-7925	2800-7182	72.6	49-108	2.6	6	2800
Citric Acid	5929	NC	3000-11700	30.9	NC	3.9	2	3000[8]
Gibberellic acid	6040	NC	5780-6300	17.4	NC	1.1	2	6305
Propylparaben	6332 (mouse)	NC	6332	35.1	NC	NC	1	6326 (mouse)
Ethylene glycol	7161	6266-8204	4000-9900	115.4	101-132	2.5	16	8567
Methanol	8710	6223-12218	5628-12880	272	194-381	2.3	6	13012
Dibutyl phthalate	8892	6180-12794	7499-12436	31.9	22-46	1.7	4	11998
Diethyl phthalate	9311	NC	8600-10100	41.9	NC	1.2	2	8602
Sodium hypochlorite	10328	NC	8200-13000	62.8	NC	1.6	2	8910[10]
Ethanol	11324	8610-14894	7060-17775	245.7	187-323	2.5	8	14008
1,1,1-Trichloroethane	12078	10000-14588	9600-16000	90.5	75-109	1.7	6	10298
Glycerol	19770	10495-37154	12600-27650	215	114-403	2.2	4	12691

Abbreviations: LD_{50}=dose lethal to 50% of the animals tested; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); N=Number of acceptable values used for geometric mean; NC=Not calculated.

[1]Categorized using the reference oral LD_{50}.

[2]Based on a geometric mean of acceptable LD_{50} values from adult laboratory rats unless otherwise specified.

[3]Values rounded to the nearest whole number.

[4]For the geometric mean of the acceptable LD_{50} values, NC is used for substances with three acceptable values or less, which was considered too few for calculation of a valid confidence interval.

[5]Range of acceptable oral LD_{50} values.

[6]Ratio of minimum acceptable LD_{50} to maximum acceptable LD_{50}.

[7]Values rounded to the nearest whole number. Values are from the RC unless otherwise specified; rat data unless otherwise specified.

[8]RTECS® (MDL Information Systems 2002).

[9]RC reference for rat oral LD_{50} of 155 mg/kg is Shrivastava et al. (1992), which references Klinger and Kersten (1961). Klinger and Kersten (1961) indicate the value was determined by intraperitoneal administration to mice.

[10]HSDB (NLM 2002).

[11]An erroneous value obtained from the literature was not included.

Table 4-3 GHS Category Matches for the Rodent Acute Oral LD_{50} Initial and Reference Values

Initial LD_{50} (mg/kg[1])	Reference LD_{50} (mg/kg)						Total	Category Match	Reference LD_{50} Lower	Reference LD_{50} Higher
	$LD_{50} \leq 5$	$5 < LD_{50} \leq 50$	$50 < LD_{50} \leq 300$	$300 < LD_{50} \leq 2000$	$2000 < LD_{50} \leq 5000$	$LD_{50} > 5000$				
$LD_{50} \leq 5$	7	5	0	0	0	0	12	58%	0%	42% (5)
$5 < LD_{50} \leq 50$	0	7	4	1	0	0	12	58%	0%	42% (5)
$50 < LD_{50} \leq 300$	0	0	8	4	0	0	12	67%	0%	33% (4)
$300 < LD_{50} \leq 2000$	0	0	0	11	1	0	12	92%	0%	8% (1)
$2000 < LD_{50} \leq 5000$	0	0	0	0	9	3	12	75%	0%	25% (3)
$LD_{50} > 5000$	0	0	0	0	1	11	12	92%	8%	0% (0)
Total	7	12	12	16	11	14	72	74%	1%	25% (18)

Abbreviations: GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); LD_{50}=Dose lethal to 50% of animals tested.
Note: Shaded cells show the number of chemicals for which both LD_{50} categories agree.
[1]Initial LD_{50} values were used for reference substance selection and were obtained from the RC (Halle 1998, 2003), RTECS® (MDL Information Systems 2002), and HSDB (NLM 2002) (see **Table 3-2**).

4.4 Accuracy and Reliability of the Rodent Acute Oral LD_{50} Reference Values

Accuracy (concordance) is the closeness of agreement between a test method result and an accepted reference value (in this case to the rodent acute oral LD_{50} measurement) (ICCVAM 2003). Because there are insufficient data to permit a comparison between rodent and human lethal doses, the accuracy of rodent acute oral LD_{50} values for predicting the oral LD_{50} in humans cannot be determined. Acute toxicity testing in rodents leads to a relative ranking of the toxicity of chemicals for regulatory purposes, with the default assumption that the rodent values and ranking are predictive of the human values and ranking.

The among laboratory reproducibility of the reference LD_{50} values determined in this section may be judged by evaluating the range of acceptable LD_{50} values for each reference substance and by comparing the values (and their variability) with the variability of LD_{50} values derived from controlled acute oral toxicity studies.

4.4.1 Variability Among the Acceptable LD_{50} Values

The variability among the acceptable rodent acute oral LD_{50} values used to calculate the reference LD_{50} value for each reference substance was assessed by calculating the ratio of the maximum to the minimum value (see **Table 4-2**). For the 62 reference substances with more than one acceptable LD_{50} value, the maximum:minimum ratio ranged from 1.1 to 25.9, with a mean of 4.3 and a median of 2.2. The maximum:minimum ratios were greater than 10 for four substances: triethylenemelamine, parathion, busulfan, and triphenyltin hydroxide. The low LD_{50} values for triethylenemelamine, busulfan, and parathion may have contributed to the high maximum:minimum ratios. The four LD_{50} values for triethylenemelamine ranged from 1 to 13 mg/kg, the four values for busulfan ranged from 1.9 to 29 mg/kg, and the 10 values for parathion ranged from 1.8 to 30 mg/kg.

Table 4-4 shows the maximum:minimum LD_{50} ratios by toxicity category. The more toxic substances (i.e., $LD_{50} \leq 50$ mg/kg) tended to have higher maximum:minimum ratios than substances with lower toxicity (i.e., $LD_{50} > 50$ mg/kg). This is anticipated because small day-to-day, or laboratory-to-laboratory variations in weighing and dosing the lower concentrations would have a higher impact on the chemicals being administered in low doses than those being administered in the high dose range.

Table 4-4 Maximum:Minimum LD_{50} Ratios by GHS Toxicity Category

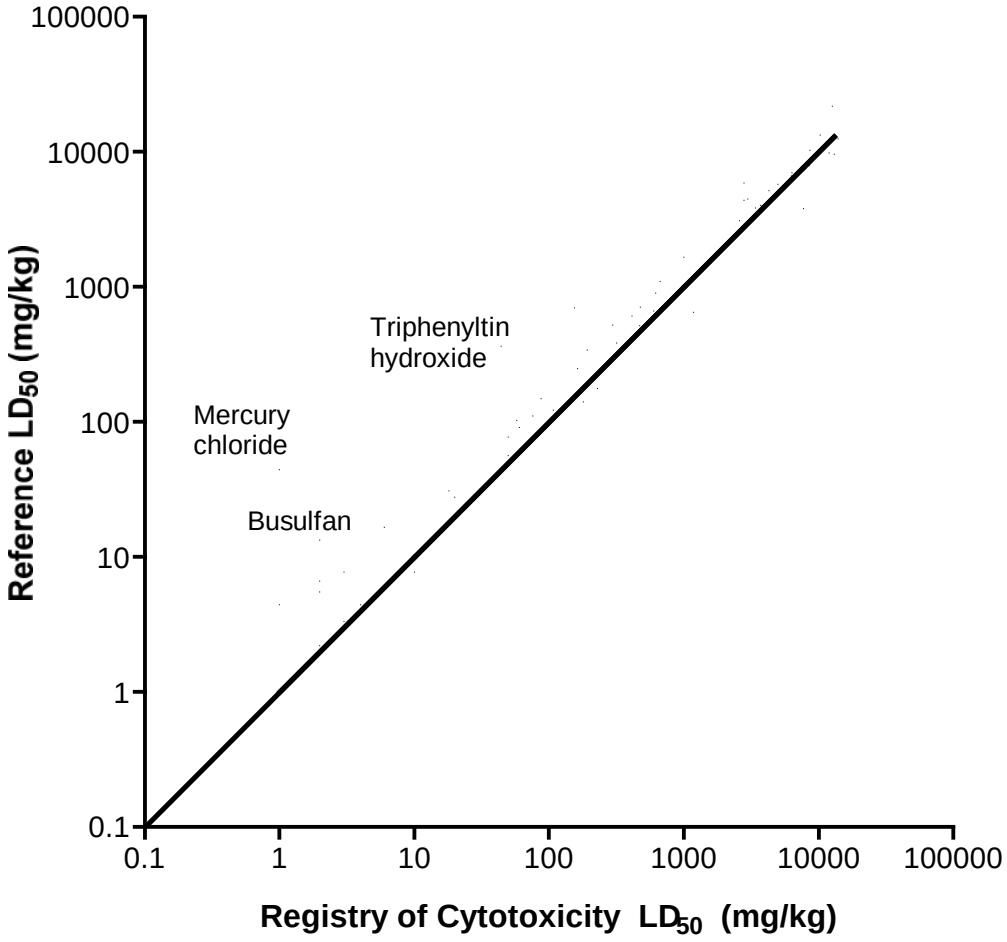
GHS Category (LD_{50} in mg/kg)	Mean Maximum:Minimum LD_{50} Ratio	Median Maximum:Minimum LD_{50} Ratio	Range of Maximum:Minimum LD_{50} Ratio	N
$LD_{50} \leq 5$	6.2	4.6	2.5 – 13.0	4
$5 < LD_{50} \leq 50$	7.1	6.3	2.0 - 16.7	9
$50 < LD_{50} \leq 300$	2.4	1.9	1.1 - 5.7	12
$300 < LD_{50} \leq 2000$	4.6	2.2	1.2 - 25.9	13
$2000 < LD_{50} \leq 5000$	2.6	2.0	1.2- 22.3	11
$LD_{50} > 5000$	2.3	2.3	1.1 - 3.9	13

Abbreviations: LD_{50}=Dose lethal to 50% of animals tested; GHS-Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); N=Number of chemicals with more than one acceptable LD_{50} value after application of the exclusion criteria described in **Section 4.1.2**.

4.4.2 Comparison of Rodent Acute Oral LD_{50} Reference Values with the Corresponding RC LD_{50} Values

The correspondence of the rodent acute oral LD_{50} reference values with the RC LD_{50} values for the 58 reference substances in common with the RC are shown on a log scale in **Figure 4-1**. Not surprisingly, a Spearman correlation analysis for the two sets of log transformed values yielded a significant correlation ($p < 0.0001$) with a correlation coefficient, r_s, of 0.97. **Figure 4-1** shows that the LD_{50} reference values tended to be higher than the RC LD_{50} values. One factor in this difference is that the majority of LD_{50} values used in the RC were from the 1983/84 RTECS®, which contains the lowest LD_{50} value found for a particular chemical without regard to the available methodological information, without consideration of whether it is an outlier with respect to the other available values, and without scientific review before publication. Thus, because the reference LD_{50} values are based on the geometric mean from multiple studies, it is not surprising that these values tended to be higher than the single values in the RC database.

Figure 4-1 Correlation of LD_{50} Values With the Reference LD_{50} Values for the 58 RC Chemicals



Abbreviations: LD_{50}=Dose lethal to 50% of animals tested; RC=Registry of Cytotoxicity.
The diagonal line shows the 1:1 relationship.

When comparing the reference LD_{50} values to the RC values, the substances with the largest differences were busulfan, triphenyltin hydroxide, and mercury chloride **(**see **Figure 4-1)**.

- The LD_{50} reference value for busulfan was six times that of the RC value (12 mg/kg vs. 1.9 mg/kg). The RC value (from 1983/84 RTECS®) was from a paper by Schmahl and Osswald (1970) in which they cited a rat oral LD_{50} of 1.86 mg/kg. The literature also contained rat oral LD_{50} values of 28 and 29 mg/kg for male and female Sprague-Dawley rats, respectively (Matsuno et al. 1971).
- The LD_{50} reference value for triphenyltin hydroxide was 7.5 times the RC LD_{50} (329 mg/kg vs. 44 mg/kg). The 15 LD_{50} values used to determine the reference value included the RC value, and had a wide range, 44-1200 mg/kg. Because of the large variation in the data, which was evenly distributed throughout the range neither the highest nor the lowest values were outliers.
- The LD_{50} reference value for mercury chloride was 40 mg/kg, while the RC value was 1 mg/kg. The RC value was from a summary document that reported the rat oral LD_{50} as a range of 1-5 mg/kg (Worthing and Walker 1991). Because it was reported as a range, it was excluded from the calculation of the reference value (see **Section 4.1.2.1**). The remaining 11 values ranged from 12 to 160 mg/kg. The highest value (160 mg/kg) was considered an outlier when compared to the other 10 values and therefore excluded from the reference value calculation.

4.4.3 Comparison of the Variability Among Acceptable LD_{50} Values to Those Obtained in Other Studies

The variation seen here for 62 reference substances is not atypical, considering the results of other studies that examined the variation among rodent acute oral LD_{50} values derived for the same substance. For example, Weil and Wright (1967) showed that LD_{50} values varied by as much as five-fold for the 10 substances tested in eight laboratories using exactly the same protocol. Another international study involving 65 participating laboratories in eight countries that did not control the LD_{50} protocols among laboratories, reported maximum:minimum ratios from 3.6 to 11.3 (with LD_{50} values ranging from 44 to 5420 mg/kg) for five substances (Hunter et al. 1979). The chemicals tested, and the LD_{50} ranges were:

- PCP[1] 44-523 mg/kg
- Sodium salicylate 800-4150 mg/kg
- Aniline 350-1280 mg/kg
- Acetanilide 805-5420 mg/kg
- Cadmium chloride 70-513 mg/kg

The results of a follow-on study in which the same substances were tested by 100 laboratories in 13 countries showed that adherence to a specific protocol reduced the range of maximum:minimum LD_{50} ratios from 3.6 to 11.3 to 2.4 to 8.4 (Zbinden and Flury-Roversi 1981).

[1] Compound undefined in the publication.

Although the LD_{50} data collected from the literature for the NICEATM/ECVAM validation study used various rat strains, sexes, observation durations, and calculation methods for estimating the LD_{50}, the variation in LD_{50} values for individual substances was similar to the data of the earlier cited studies. The current study found four of the 62 substances with multiple LD_{50} values had maximum:minimum LD_{50} values higher than that reported by Hunter et al. (1979) (i.e., >11.3), and three of those were in the highest toxicity category. Hunter et al. (1979) also observed that the largest variation was associated with the more highly toxic substances.

4.5 Summary

To enable the comparison of *in vitro* NRU data with rodent acute oral toxicity data, LD_{50} reference values for the 72 reference substances were calculated using data obtained from the literature, database searches, and secondary references. Rat acute oral LD_{50} values were preferred, but mouse acute oral LD_{50} values were collected for three substances with no available or acceptable rat data. The 491 LD_{50} values that were retrieved comprised 485 rat LD_{50} values and six mouse values. It was not possible to identify a high quality data set produced under GLP guidelines because only 3% of the data records were in GLP compliance. Instead, as described in **Section 4.1.2.1**, a homogenous set of LD_{50} values for each substance was identified by applying specific exclusion criteria related to the materials, animals, and methods used for each study.

After analysis of the acceptable values for outliers, the remaining 385 values were used to derive rodent acute oral LD_{50} reference values by calculation of a geometric mean of the values for each substance. As a result of this procedure, the LD_{50} reference values for 19 of the 72 reference substances were sufficiently different from the values that were used in the RC and other summary sources, so that they were reclassified into different GHS oral toxicity categories.

Because there is no reference standard against which to evaluate the accuracy of the rodent acute oral toxicity test, the reliability of the LD_{50} reference values was assessed by comparison to other evaluations of the performance of this test method. The maximum:minimum ratio of the acceptable values for the 62 reference substances that had more than one LD_{50} value ranged from 1.1 to 25.9, and the ratios for four of the substances were greater than one order of magnitude.