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8.0 3T3 AND NHK NRU TEST METHOD DATA QUALITY

This section of the BRD presents the extent of adherence to GLP regulations for generation of the validation study data. Data quality is described, along with deviations from the regulations and their effect (if any) on the quality of the data. Statistical analyses are provided to compare the data generation, collection, and reporting by the two GLP compliant laboratories and the one non-GLP compliant laboratory, as well as for the GLP-compliant laboratory that distributed the reference substances and performed solubility studies. Discussions of various quality assurance aspects of the study are included.

8.1 Compliance With Good Laboratory Practice Regulations

8.1.1 Guidelines Followed for Cytotoxicity Testing

8.1.1.1 *Good Laboratory Practices*

The SOW provided the following definition of U.S. Regulatory agency GLPs to each laboratory:

> "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, and conduct (U.S. Food and Drug Administration, Title 21 CFR Part 58; U.S. Environmental Protection Agency, Title 40 CFR Part 160)."

IIVS, ECBC, and BioReliance performed testing under GLP guidelines. The details of GLP compliance and training are addressed in **Section 11.2**.

8.1.1.2 *Spirit of GLP*

The SMT determined a definition for "spirit of GLP" and provided the following to the laboratories:

> "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."

FAL performed testing in the "spirit of GLP" (see **Section 11.2.2.1**) by following the international GLP standards referenced in the ECVAM Workshop 37 Report (Cooper-Hannan 1999) and the OECD Principles of GLP (OECD 1998). The laboratory did not have their data and test procedures reviewed by an independent, quality assurance (QA) auditor. The SOW directed FAL to, at a minimum, routinely document their equipment monitoring and record keeping (see **Table 8-1**), and to archive all documents. The FAL already had most of the requested procedures and guidelines in place for routine laboratory procedures before initiation of this study. The various general laboratory-related activities were documented in workbooks and logbooks, and the information was made available to the SMT.

Table 8-1 SMT-Recommended Documentation for FAL

Daily	Per Use	Periodic
Temperatures Laboratory (ambient), incubators, water baths, refrigerators, freezers	Cryogenic Storage Unit Liquid N_2 volume	Laboratory Supplies[1] Lot numbers and expiration dates for stock media formulations and components, NRU reagents, tissue culture plasticware
Humidity/CO_2 Cell culture incubators	Equipment Calibration Balances, pH meters, cell counters	Cells Quantity, and cryogenic storage conditions, for 3T3 and NHK cells
Visual Observations Cell Culture Growth	Reagents Lot numbers and expiration dates of medium/supplements	Equipment Calibration Incubators, laminar flow hoods, autoclaves, micropipettors, spectrophotometer plate readers, computers (software)

Abbreviations: SMT=Study Management Team; FAL=Fund for the Replacement of Animals in Medical Experiments Alternatives Laboratory; NRU=Neutral red uptake; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes.
[1]Documentation for laboratory supplies begins when supplies are purchased and received by the laboratory

8.1.1.3 *Good Cell Culture Practices (GCCP)*

The SMT provided guidance in the SOW for implementing GLPs in a cell culture laboratory environment. The initial assumption by the SMT was that each laboratory had the basic cell culture skills and knowledge (e.g., as described in Freshney 2000) to reliably perform the *in vitro* NRU cytotoxicity test methods. Reviews of historical laboratory documents, and scientific and professional exchanges with the laboratory personnel, assured the SMT that each laboratory had demonstrated, through previous validation studies and other experience, that the personnel were capable of providing quality scientific data through the use of good cell culture practices. A comparison of the SOW and the *in vitro* NRU cytotoxicity protocols showed that the guidelines developed for the NICEATM/ECVAM study were harmonious with the guidelines in the ECVAM Good Cell Culture Practices Reports (Hartung 2002; Coecke et al. 2005), and the OECD document on GLPs and *in vitro* studies (OECD 2004a).

8.1.2 Quality Assurance (QA) for NRU Cytotoxicity Test Data

8.1.2.1 *Coded Reference Substances*

BioReliance acquired 73 high purity chemicals (72 reference substances and one positive control substance) from reputable commercial sources. Sixty-four of the reference substances were ≥99% pure, and seven were between 90 and 99% pure. Lactic acid had the lowest purity, 89% (See **Appendix F1**). The substances were coded with unique identification numbers and provided to the testing laboratories in a blinded fashion. Procurement of chemicals and their preparation for distribution was performed under GLP guidelines and the SOW provided by the SMT (see **Appendix G**). **Section 3.4** provides detailed information on the acquisition and distribution of reference substances.

8.1.2.2 *Solubility Testing and Data Review*

All laboratories performed solubility tests on all reference substances using the solvents and procedures specified in the protocols provided by the SMT, and submitted solubility data to the SMT in the form of hard copy printouts and electronic worksheets. The Study Directors reviewed all laboratory procedures and all data produced at their respective laboratories, and the QA designee in each GLP-compliant laboratory reviewed all data in their laboratory. The SMT Project Coordinators served as informal QA reviewers for FAL (i.e., reviewed all the raw data sheets). The errors and omissions detected were reported to FAL, and corrections were requested. The SMT reviewed all solubility data and NRU assay data produced by all of the laboratories.

The SMT reviews of the submitted data in Phases Ia and Ib revealed that, even after data review by the Study Directors, the data files contained an unacceptably high frequency of errors (see **Section 2.6.2.5**). The laboratories were alerted to the problem and personnel from all laboratories attended a weeklong training session at the IIVS laboratories in Gaithersburg, Maryland to enhance harmonization among the laboratories. Errors continued to be found in data files submitted for Phase III after the training, albeit less frequently; however, such errors generally resulted from the rush to rapidly complete the data files for submission to the SMT shortly after the conclusion of each test. The formal QA reviews of the files occurred later in each phase of the study.

The most common errors included typographical mistakes, transcriptional and data entry errors in the Microsoft® EXCEL® and the GraphPad PRISM® 3.0 templates, and incorrect labeling of files. The SMT reviewed every electronic file and hard copy printout throughout the study and alerted the Study Directors of the affected laboratories when errors were found. All data files were checked for consistency within the documents, and for compliance with the protocols. The SMT also documented errors on the hard copy printouts in the form of handwritten notations to the files (at NICEATM) and added these notations to the electronic data summary files compiled for data management. Files that were revised and/or corrected by the Study Director were resubmitted to the SMT and identified as corrected files.

8.1.2.3 *NRU Cytotoxicity Test Tallies*

The Study Directors periodically received individualized test tallies specific to their laboratories from NICEATM that detailed:

- The number of range finder tests performed by the laboratory
- The number of definitive tests performed, and the pass/fail status of each
- The number of PC tests performed, and the pass/fail status of each
- The number of acceptable tests completed
- The test completion status for each chemical (i.e., whether one range finder test had been completed, and the number of acceptable definitive tests had been completed)

The laboratories compared the NICEATM tallies to their own records to verify their consistency and accuracy. Discrepancies were resolved through direct communication between the Study Director and the SMT.

8.1.3 Guidelines Followed for Rodent Acute Oral LD_{50} Data Collection

For the purposes of this validation study, the *in vitro* NRU test methods were proposed for predicting starting doses for rodent acute oral toxicity test methods, rather than as replacement tests for the *in vivo* test method. No *in vivo* tests were performed for this validation study. All *in vivo* data (i.e., rat and mouse LD_{50} values) were collected by NICEATM through reviews of the literature and from publicly available databases. All relevant data and pertinent information were gathered and stored in an Excel® spreadsheet.

8.1.3.1 *Rodent Acute Oral LD_{50} Values Used in the Registry of Cytotoxicity (RC)*

The RC is a database of acute oral LD_{50} values for rats and mice obtained primarily from the 1983/84 RTECS® database compiled by NIOSH, and IC_{50} values from *in vitro* cytotoxicity assays using multiple cell lines and cytotoxicity endpoints for chemicals with known molecular weights (Halle 1998, 2003). Collection and reporting methods used for generating the data in RTECS® were not a part of the data collection hierarchy employed by NIOSH, and the data in this database were not evaluated for quality and accuracy. Many of the values come from secondary sources with no citation to the original report. GLP guidelines were not used to determine acceptable data for the database. The only criterion used by NIOSH for reporting acute oral toxicity data in RTECS® was that the LD_{50} value was the most toxic LD_{50} value for a chemical that could be found in the literature, regardless of the number of other values available, or their distribution.

8.1.3.2 *Rodent Acute Oral LD_{50} Values Collected by NICEATM from Other Sources*

One critical aspect of the validation study design was the establishment of a rat acute oral LD_{50} reference value for each of the 72 reference substances (see **Section 4**). These reference values were used to evaluate the extent to which the two *in vitro* NRU test methods could predict rat acute oral LD_{50} values. Primary rat acute oral LD_{50} studies were located through searching electronic databases, published articles, and secondary references. Rat data were not available for three of the reference substances and mouse acute oral LD_{50} values were used. Only seven of the 455 LD_{50} values collected from the literature were produced under GLP guidelines.

8.2 Results of Data Quality Audits

The QA unit or designee in each GLP laboratory provided a systematic and critical comparison of the data provided in the laboratory's study reports to the raw data in the laboratory records. The SOW provided to each laboratory contained the following guidance regarding QA statements:

> "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."

8.2.1 QA Statements

The QA statements from the GLP-compliant laboratories addressed the reviews of:

- Protocols
- Laboratory standard operating procedures (SOPs)

- Laboratory operations, in general
- 3T3 and NHK NRU experiment data
- The report submitted to the SMT

The QA statements from the GLP laboratories affirm that the methods described in the protocols are the methods that the laboratory personnel used, and that the data reported to the SMT accurately reflect the raw data obtained by the laboratory. See **Section 8.2.2** for information about the QA statements for the non-GLP laboratory.

8.2.2 QA Statements from the Laboratories

8.2.2.1 *BioReliance QA Statements*

The Study Director/Laboratory Director provided the following statement in all of the final reports:

> "The solubility studies, acquisition, preparation, and distribution of the test chemicals were conducted in compliance with GLP. Although not audited (per SOW), the work described in this report for Phase X (i.e., Ia, Ib, and II) fully and accurately reflects to the best of my knowledge the raw data generated in the study."

8.2.2.2 *FAL QA Statements*

The Study Director for FAL performed the final review of all data and reports before sending them to the SMT, and provided the following two statements in the final reports provided to the SMT.

- "The laboratory worked under the principles of GLP whilst not being a GLP-compliant laboratory."
- "The report accurately reflects the work undertaken and the results obtained at the FRAME Alternatives Laboratory."

Formal QA statements were not provided to FAL because the SMT performed informal QA reviews.

8.2.2.3 *ECBC QA Statements*

The QA statements reported the particular study phase and laboratory procedures that were examined for GLP compliance. In addition, the laboratory's statement noted that the scope of work, associated protocols, and quality control (QC) acceptance criteria were updated or changed during the study, which made the assessment of the procedures and data for conformance to the SOPs more difficult. However, compliance with the requirements and intent of GLP guidelines was continually assessed during the review of the SOPs and the observance of operations. The QA reviews found the ECBC protocols to be in compliance with the NICEATM/ECVAM study protocols. The aspects of the studies inspected by the QA designee were:

- Review of protocols and laboratory SOPs
- Review of waste handling procedures
- Review of laboratory operations
- Certification of new personnel
- Review of data
- Review of the final report for each testing phase

The QA designee also observed the preparation of reference substances, 96-well plate configuration, application of reference substance, annotation to the workbook, and appropriate sterile technique while performing the testing. The number of inspections of laboratory operations was reduced in the latter phases of the study because the same personnel conducted the testing throughout the entire study.

ECBC Review Dates of the Study Phases

- Phase Ia: July 2002 through May 2003
- Phase Ib: July 2002 through January 2003
- Phase II: May 2003 through February 2004
- Phase III: November 2003 through March 2005

8.2.2.4 *IIVS QA Statements*

Because the IIVS QA unit is small, it carried out reviews of different aspects of the procedures at different times. The IIVS QA Statement reads:

> "This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice regulations (21 CFR 58), the U.S. EPA GLP Standards (40 CFR 792 and 40 CFR 160) and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures."

The aspects of the studies inspected by the QA designee were as follows:

- Protocol and initial paperwork
- Reading of the plates (definitive test)
- Dilution of the test articles (definitive test)
- Treatment of the cells
- Termination of treatment and addition of the NR dye (definitive test)
- Cell concentration determination and seeding of the plates (third definitive test)
- Termination of treatment and addition of the NR dye
- Washing the cells
- Draft report and data
- Final report

IIVS Review Dates of Various Aspects of the Test Phases

- Phase Ia: August 2002 Final Report Review: October 2005
- Phase Ib: January 2003 Final Report Review: October 2005
- Phase II: July-August 2003 Final Report Review: October 2005
- Phase III: January-November 2004 Final Report Review: October 2005

8.2.2.5 *Other QA Information*

Data generated by the laboratories and reviewed by their respective Study Directors were submitted to the SMT. Often, the data were provided electronically within days of the end of testing. The SMT was active as a secondary QA reviewer of all information provided by the

Study Directors. If the SMT found discrepancies, the Project Coordinators corresponded with the appropriate Study Director to identify and rectify the error. The Study Director made corrections/adjustments to the discrepancies in data reporting and presented the changes to the SMT. The SMT did not initiate any external data quality audits.

The quality of the reference substances was assured in the form of certificates of analysis provided by the chemical manufacturer to BioReliance at the time of purchase. The SMT and the laboratories obtained certificates of analysis from CAMBREX for Clonetics® NHK culture medium and supplements. In addition, the SMT obtained QC data directly from CAMBREX technical departments concerning the NHK medium's ability to support keratinocyte growth.

8.3 Effect of Deviations or Non-compliance with GLPs

Rates for several types of errors (i.e., documentation, testing methods, and data management) were determined by the SMT. Many of the errors (particularly in Phases Ia and Ib) were the result of minor mistakes (e.g., typographical, mislabeling) and did not affect the quality of the data.

8.3.1 Laboratory Error Rates

The SMT was concerned about the number of errors that were seen in documentation and testing methods during Phases Ia and Ib, and compiled the detected errors from each laboratory. The types of errors found included errors in documentation (e.g., reference substance identification did not match on all associated data sheets; IC_{20} and IC_{80} values were transposed in the EXCEL® template; a test acceptance criterion flag in a data sheet was incorrect) and in testing (e.g., wrong dilution scheme was used for the PC; wrong SLS IC_{50} was used as the PC IC_{50}). Error rates were compiled as the number of tests with errors per total number of tests. As shown in **Table 2-3**, FAL had the highest error rates: 93% for the 3T3 NRU test method and 41% for the NHK NRU test method. The highest error rates in the other laboratories were 10% for the 3T3 NRU test method and 23% for the NHK NRU test method (both ECBC).

There were relatively few errors detected in the Phase III data files. The SMT did not compile the typographical and transcriptional errors found, but reported them directly to the appropriate Study Director so that the data sheets could be immediately corrected. The SMT did not detect errors in the raw optical density data from the 96-well plates provided in each data file. The laboratories and the SMT corrected typographical and transcriptional errors (e.g., incorrect $logIC_{50}$ value entered) in the EXCEL® templates. The EXCEL® template formulas were used for the statistical analyses.

An assessment of error rates was performed specifically for Phase III for one particular clerical error – the transfer of the final results (e.g., ICx values) from the GraphPad PRISM® 3.0 template to the Microsoft® EXCEL® template. It was often necessary for the SMT to revise the EXCEL® data files provided by the laboratories because the incorrect values had been transferred to EXCEL®. **Table 8-2** summarizes the Phase III error rates resulting from the transfer of data from PRISM® to EXCEL®.

Table 8-2 Phase III Error Rates in the Transfer of Data to the EXCEL® Template

Laboratory	Number of Errors Detected	Number of Definitive Tests	Percentage of Tests with Detected Errors
ECBC	49	402	12
FAL	171	513	33
IIVS	25	419	6

Abbreviations: ECBC=Edgewood Chemical Biological Center; FAL=Fund for the Replacement of Animals in Medical Experiments Alternatives Laboratory; IIVS=Institute for *In Vitro* Sciences.

8.3.2 Failure Rates for Definitive and PC Tests

Table 8-3 presents the test failure (i.e., did not meet test acceptance criteria) rates experienced in Phase III. Approximately 25% of all 3T3 definitive tests and 18% of all NHK definitive tests failed. If a definitive test (see **Section 2.3.2.2** for the definition of a definitive test) failed, the laboratory repeated the test and attempted to obtain three acceptable definitive tests for each reference substance in each cell type (see **Section 2.5** for criteria for repeating tests). The PC tests failed 0 to 18% of the time with a combined average failure rate of 8% for both cell types. FAL had the highest individual laboratory test failure rates for 3T3 definitive tests (30%), NHK definitive tests (32%), and NHK PC tests (18%). ECBC had the highest failure rate for 3T3 PC tests (11%). IIVS had no PC test failures.

Table 8-3 Definitive Test and Positive Control (PC) Test Failure Rates in Phase III

Test Type	3T3 NRU Test Method				NHK NRU Test Method				Total
	ECBC	FAL	IIVS	Total	ECBC	FAL	IIVS	Total	
Definitive Tests - Acceptable	169	177	176	522	173	175	174	522	1044
Definitive Tests - Total	215	257	225	697	187	256	194	637	1334
% Failed Definitive Tests	21	30	22	25	8	32	10	18	22
PC Tests - Acceptable	66	40	16	122	58	37	20	115	237
PC Tests - Total	74	42	17	133	59	45	20	124	257
% Failed PC Tests	11	5	6	8	2	18	0	7	8
Definitive Tests Failed Only Because PC Tests Failed	14	6	14	34	0	22	0	22	56
% Definitive Tests Failed Only Because PC Tests Failed	7	2	6	5	0	9	0	4	4

Abbreviations: ECBC=Edgewood Chemical Biological Center; FAL=Fund for the Replacement of Animals in Medical Experiments Alternatives Laboratory; IIVS=Institute for *In Vitro* Sciences; NRU=Neutral red uptake; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes.

The Phase III guidelines required each laboratory to provide three acceptable definitive tests for each substance for both cell types (3 x 60 x 2 = 360 definitive tests). PC tests were run concurrently with the definitive tests, and more than one reference substance was usually tested in conjunction with each PC test. Because of test failures, each laboratory performed additional testing to obtain the three acceptable definitive tests required for each substance.

Table 8-4 presents the success rates for each laboratory for Phase III testing and a total for all the laboratories combined.

Table 8-4 Combined Definitive and Positive Control (PC) Test Success Rates for the 3T3 and NHK Methods in Phase III

Test Type	ECBC	FAL	IIVS	Total
Acceptable Definitive Tests/ Total Definitive Tests	342/402	352/513	350/419	1044/1334
% Acceptable Definitive Tests	85%	69%	84%	78%
Acceptable PC Tests/Total PC Tests	124/133	77/87	36/37	237/257
% Acceptable PC Tests	93%	89%	97%	92%

Abbreviations: ECBC=Edgewood Chemical Biological Center; FAL=Fund for the Replacement of Animals in Medical Experiments Alternatives Laboratory; IIVS=Institute for *In Vitro* Sciences; NRU=Neutral red uptake; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes.

8.3.3 Intralaboratory Reproducibility

CV values for each method were determined for each reference substance in each laboratory using the IC_{50} values from the acceptable definitive tests, as described in **Section 5.5.2**. **Table 8-5** presents the average CV values for the substances tested in each of the study phases, and for the entire study.

Table 8-5 CV Values for Definitive Tests

Cell Type	Labs	Phases I & II		Phase III		All Phases	
		Number of Reference Substances	Average % CV	Number of Reference Substances	Average % CV	Number of Reference Substances	Average % CV
3T3	ECBC	12	17	57	24	69	23
	FAL	11	28	55	33	66	33
	IIVS	11	20	56	22	68	21
NHK	ECBC	12	24	57	22	69	23
	FAL	12	31	57	45	69	42
	IIVS	12	14	58	14	70	14

Abbreviations: ECBC=Edgewood Chemical Biological Center; FAL=Fund for the Replacement of Animals in Medical Experiments Alternatives Laboratory; IIVS=Institute for *In Vitro* Sciences; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; CV=Coefficient of variation.

8.3.4 Prediction of GHS Acute Oral Toxicity Categories

Predicted LD_{50} values were determined using the *in vitro* NRU IC_{50} values in the IC_{50}-LD_{50} regressions presented in **Table 6-5.** The predicted LD_{50} values were used to assign each substance to a predicted GHS acute oral toxicity category (UN 2005). The accuracy of the 3T3 and NHK NRU test methods for predicting GHS categories was determined by comparison with categorization based on *in vivo* rat oral LD_{50} values (in mg/kg) in **Table 4-2**. Using the RC rat-only millimole regression, the accuracy of the predictions and the extent of underprediction or overprediction are shown for each laboratory in **Table 8-6**. The laboratories generally agreed with each other in their predictions. Although FAL had the highest error rates and CV values, their predictions of GHS categories were consistent with the other laboratories. The laboratories determined category matches for 25 to 30% of the reference substances for the 3T3 NRU test method and 29 to 31% of the reference substances for the NHK NRU test method. For the 3T3 NRU test method, toxicity was overpredicted for 38% of the reference substances and underpredicted for 33 to 38% of them. For the NHK NRU test method, toxicity was overpredicted for 35 to 38% of the reference substances and underpredicted for 32 to 34% of them. (See **Appendix J** for additional laboratory comparisons for the other *in vitro – in vivo* regressions evaluated in **Section 6**.)

8.4 Availability of Laboratory Notebooks

All laboratories maintained laboratory notebooks using a template provided by IIVS, and provided copies of the notebooks to the SMT (archived at NICEATM) after completion of each testing phase. The notebooks contained information from all aspects of testing including, but not limited to:

- Environmental conditions
- Reagent identification
- Preparation of 96-well plates
- Preparation of reference substances
- Treatment of cell cultures
- Visual observations of cell cultures
- NRU assays
- Data analysis

Table 8-6 GHS Acute Oral Toxicity Category Predictions by Laboratory[1]

	Labs	Total Reference Substances	Category Match	Toxicity Overpredicted	Toxicity Underpredicted
3T3	ECBC	64	30%	38%	33%
	FAL	64	25%	38%	38%
	IIVS	64	27%	38%	36%
NHK	ECBC	68	31%	35%	34%
	FAL	68	29%	38%	32%
	IIVS	68	31%	37%	32%

Abbreviations: ECBC=Edgewood Chemical Biological Center; FAL=Fund for the Replacement of Animals in Medical Experiments Alternatives Laboratory; IIVS=Institute for *In Vitro* Sciences; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; GHS=Globally Harmonized System for Classification and Labelling of Chemicals (UN 2005).

[1]3T3 and NHK NRU test method IC_{50} data (geometric mean of within laboratory replicates) used with the RC rat-only millimole regression, log LD_{50} (mmol/kg) = 0.439 x log IC_{50} (mM) + 0.621, to assign GHS category. *In vivo* category was based on reference rodent oral LD_{50} values (mg/kg) in **Table 4-2**. For each method, the reference substances evaluated were those for which all three laboratories obtained IC_{50} values.

8.5 Summary

- The determinations of test method and data collection errors showed that FAL consistently had the highest error levels; however, the laboratory's GHS acute oral toxicity category predictions were comparable to the other laboratories' results.
- The laboratories reported no significant deviations from the protocols, and deviations that did occur during the testing phases were generally quickly acknowledged and addressed by the Study Directors. If a deviation occurred that would affect the data (e.g., improper concentration of DMSO solvent), the Study Director would reject the test, notify the SMT, and perform an additional test. Improper transfer of data to either the EXCEL® or PRISM® templates, which would affect the data summaries and analyses, were recognized, documented, and rectified by the Study Director and/or the SMT.
- The SMT reviewed all data sheets to ensure that data were not inadvertently attributed to the incorrect data summary files, and that the correct data were used in all statistical analyses.
- An electronic copy of all data for this validation study can be obtained from NICEATM upon request by mail, fax, or e-mail to Dr. William S. Stokes, 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.

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