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4.0 *IN VIVO* RODENT TOXICITY REFERENCE VALUES USED TO ASSESS THE ACCURACY OF THE 3T3 AND NHK NRU TEST METHODS

The aim of the procedures and analyses presented in this section is to identify the most appropriate *in vivo* rodent toxicity data with which to compare the *in vitro* cytotoxicity data. The *in vitro* NRU cytotoxicity test methods are intended to be used in a weight of evidence approach to determine the starting dose for the *in vivo* acute oral systemic toxicity test methods using rodents. Thus, rodent LD_{50} values from acute oral systemic toxicity tests are the most appropriate reference data for the *in vitro* NRU IC_{50} values. This section describes the methods for identifying and evaluating the most appropriate rodent LD_{50} data to use for determining reference LD_{50} values for the 72 reference substances tested in the NICEATM/ECVAM validation study. These *in vivo* rodent toxicity reference values will be used in **Section 6** to establish the accuracy of the *in vitro* IC_{50} data from the 3T3 and NHK NRU test methods for predicting LD_{50} values from rodent acute oral systemic toxicity tests.

4.1 Methods Used to Determine *In Vivo* Rodent Toxicity Reference Values

4.1.1 Identification of Candidate *In Vivo* Rodent Toxicity Reference Data

No animal experiments were performed to obtain *in vivo* reference data for acute oral systemic toxicity. To identify LD_{50} reference data for the 72 reference substances, rat oral LD_{50} data were located through literature searches, secondary references, and electronic database searches. PubMed and ISI Web of Science® searches were conducted using each chemical name and "lethal dose 50." Secondary sources 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, Merck Index, and various other summary sources. **Table 4-1** lists databases searched via the Internet to locate references for rat oral LD_{50} values. Rat LD_{50} data were preferred because the current oral acute toxicity test guidelines recommend using rats (OECD 2001a, c, d; EPA 2002a). Taking the same approach used for the Registry of Cytotoxicity (RC), mouse LD_{50} data were sought for a particular chemical if rat LD_{50} values could not be located. [The RC is a database of acute oral LD_{50} values for rats and mice, obtained from

63 RTECS® and IC_{50} values from *in vitro* cytotoxicity assays using multiple cell lines and
64 cytotoxicity endpoints for chemicals with known molecular weights (Halle 1998).]
65

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

Database	Sponsor
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; CCOHS CHEMpendium™
Chemical Hazard Response (CHRIS)	U.S. Coast Guard
Chemical Ingredients Database	U.S. Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP); California EPA Department of Pesticide Regulation
CHEMINDEX CHEMINFO	Canadian Centre for Occupational Health and Safety (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

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

Database	Sponsor
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
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 (PDSs)	WHO IPCS; CCOHS; Food and Agriculture Organization (FAO) of the United Nations
Material Safety Data Sheets (MSDS)	Interactive Living Paradigms, Incorporated
Multicentre Evaluation of In Vitro Cytotoxicity (MEIC)	Scandinavian Society for Cell Toxicology
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 Data System (OHM/TADS)	EPA Office of Waste and Water Management
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)

A total of 195 LD_{50} references retrieved through these searches were reviewed and evaluated. Information regarding the materials and methods used to derive the 491 LD_{50} values reported by these references were compiled and are provided in **Appendix H-1** in a spreadsheet format. **Appendix H-2** provides a narrative characterization and evaluation of the values.

4.1.2 Criteria Used to Select Candidate *In Vivo* Rodent Toxicity Data for Determination of Reference Values

From the data retrieved, the goal was to derive a set of high quality reference LD_{50} values (i.e., 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, since only seven had been obtained in compliance with GLP guidelines. GLP-compliant studies were found for only four of the 72 (6%) reference substances.

The SMT then considered limiting the selection of LD_{50} values to those from studies that used the type of animals recommended by the current oral acute toxicity test guidelines, since these guidelines will be used for future acute systemic toxicity testing. The current guidelines recommend using young adult rats, 8-12 weeks of age, of a common laboratory strain and the most sensitive sex (OECD 2001a, c, d; EPA 2002a). Female animals are suggested if there is no information on which to determine the most sensitive sex. Selecting LD_{50} values from animals that fit this description yielded a limited number of values. Only 3% (14/459) of the oral LD_{50} values were determined using 8-12 week old female laboratory rats. Another 15 LD_{50} values were obtained with female rats in an appropriate weight 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) for that age. Thus, only 6% (29/459) of the LD_{50} values in the database, covering 21 of the 72 reference substances (29 %), were obtained from studies that used the strain, sex, and age of rats recommended by current test guidelines (OECD 2001a; EPA 2002a).

Final Exclusion Criteria

Since 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 with unanesthetized young adult laboratory rats of both genders, to which chemicals were administered by gavage. Thus, to compile a homogenous set of reference LD_{50} values for each chemical, the selection process was revised to exclude studies that reflected the following, less typical, materials and methods:

- 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, since the health status of these animals was uncertain. All laboratory rat strains/stocks were deemed acceptable, since they were expected to be healthy and provided with adequate care and housing during testing. Data from neonates or weanlings were excluded since their sensitivity to chemical toxicity may differ from that of adults. Four weeks was considered the minimum acceptable age, since rats are weaned at about 3 weeks of age (Barrow 2000). Data from feeding experiments or experiments that involved administration of the chemical in capsules were also excluded, since gavage is the most common mode of administration for acute oral studies and the rate of gastrointestinal absorption for these methods is likely to be different (Nebendahl 2000). Since LD_{50} point estimates are required for the prediction model, LD_{50} values reported as ranges or inequalities were considered unacceptable.

Assumptions

The level of detail for materials and methods for the LD_{50} studies varied greatly. Some studies reported only the use of white rats while other acute oral toxicity studies provided complete information on stock/strain, gender, and age of animals; the number of animals tested per dosing group, method of administration, doses administered, clinical signs, and

times of death. To use as much of the available data as possible, the following assumptions were made if a study report did not declare otherwise.

- The rats were assumed to have been young adults of a common laboratory strain.
- The rats were assumed to have been unanesthetized.
- The oral route of administration was by gavage.

Calculation of Reference Values

If there were multiple acceptable LD_{50} values for a chemical after the application of the exclusionary criteria, outliers at the 99% level (Dixon and Massey 1981) were excluded. A geometric mean and 95% confidence limits were calculated from the remaining values to serve as the reference LD_{50}. A geometric mean is the antilog of the mean of the logarithm of the values and it is less affected by extreme values than the arithmetic mean. Use of a geometric mean corresponds with the approach used for the RC regression to obtain a single IC_{50} value from multiple IC_{50} values (Halle 1998) and with the approach used to derive the IC_{50} value for each chemical for the *in vitro* - *in vivo* regressions for 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, for trichloroacetic acid was also evaluated based on biological plausibility. There were five LD_{50} values that ranged from 400-8900 mg/kg after applying the exclusionary criteria for trichloroacetic acid. The lowest value of 400 mg/kg was rejected as biologically implausible since up to 1000 mg/kg/day has been used in chronic rodent carcinogenicity studies (EPA 1996).

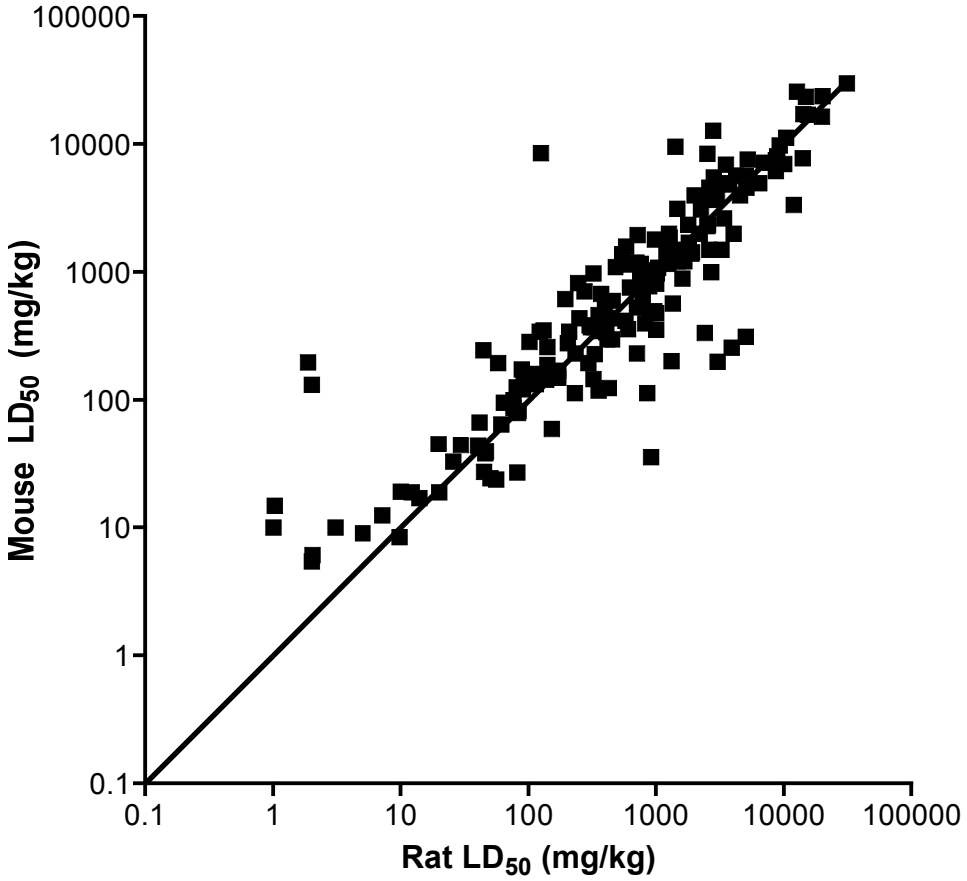
Use of Rat and Mouse Data

If no rat oral LD_{50} values could be found for a chemical, mouse oral LD_{50} values were located, retrieved, and evaluated by the same method as that used for rat oral values. Although a model using entirely rat data or entirely mouse data would be preferable, the use of mouse values was considered to be justified by a significant correlation of rat and mouse oral LD_{50} values reported by Ekwall et al. (1998a) for the 50 chemicals tested in the MEIC

study. Using values from RTECS®, Ekwall et al. (1998a) reported a coefficient of determination, R^2, of 0.85 for a linear regression analysis of rat LD_{50} - mouse LD_{50}. Furthermore, Halle (1998) compared IC_{50} - LD_{50} linear regressions with 285 rat values and 242 mice values and found no significant difference in the intercepts or slopes.

A correlation of the 173 chemicals in the RC that had both rat and mouse LD_{50} values is shown in **Figure 4-1**. A Spearman correlation analysis of the log transformed rat and mouse data yielded a significant correlation ($p < 0.0001$) with $r_s = 0.88$.

Figure 4-1 Correlation of Rat and Mouse LD_{50} Values for 173 RC Chemicals



The diagonal line shows the 1:1 relationship.

4.2 Final *In Vivo* Rodent Toxicity Reference Values

After the application of the exclusionary criteria, there were 385 acceptable LD_{50} values with which to calculate reference values. **Table 4-2** shows the reference LD_{50} value for each reference substance in ascending order. The reference values are the geometric means of the acceptable LD_{50} values. Also shown for each substance are the 95% confidence limits around the 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 the erroneous values for acetylsalicylic acid and sodium oxalate), and the LD_{50} initially used for hazard category (often referred to as "toxicity" or "LD_{50}" category) classification of the substance (see **Table 3-2**). Ratios for the maximum to minimum LD_{50} values ranged from 1.0 to 25.9. The average ratio was 4.1. Six of the 62 reference substances for which ratios were calculated had ratios greater than one order of magnitude: triethylenemelamine, parathion, busulfan, triphenyltin hydroxide, phenol, and trichloroacetic acid. Three of these substances, triethylenemelamine, parathion, and busulfan, were in the two highest toxicity categories (i.e., $LD_{50} \leq 50$ mg/kg).

Table 4-2 shows the reference substances grouped by GHS acute oral toxicity category (UN 2005) using the reference LD_{50} values. 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 the reference LD_{50} values with the number of substances in each category based on the initial LD_{50} values. **Table 4-3** shows that the initial and reference LD_{50} values placed 74% of the substances in the same GHS category. Compared with the initial LD_{50}, the reference LD_{50} was higher for 25% of the substances and lower for 1% of the substances.

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

GHS Category[1]/Chemical	Reference Oral LD_{50}[2] (mg/kg)	95% Confidence Interval[3] (mg/kg)	Reference Oral LD_{50}[2] (mmol/kg)	95% Confidence Interval[3] (mmol/kg)	Maximum: Minimum Value[3]	N Averaged[5]	Initial Rodent Oral LD_{50}[6] (mg/kg)
$LD_{50} \leq 5$ mg/kg (N = 7)							
Cycloheximide	2	NC	0.00711	NC	2.5	3	2
Phenylthiourea	3	NC	0.0197	NC	NC	1	3
Sodium selenate	3	NC	0.0159	NC	3.7	2	2[7]
Epinephrine bitartrate	4 (mouse)	NC	0.0196	NC	NC	1	4 (mouse)
Triethylenemelamine	4	1-25	0.0120	0.0037-0.12	13.0	4	1
Physostigmine	5	NC	0.0182	NC	NC	1	5[7]
Disulfoton	5	2-10	0.0182	0.009-0.036	5.5	6	2
$5 < LD_{50} \leq 50$ mg/kg (N = 12)							
Parathion	6	3-12	0.0209	0.010-0.041	16.7	10	2
Strychnine	6	NC	0.0188	NC	6.9	3	2[7]
Aminopterin	7	NC	0.016	NC	NC	1	3 (mouse)
Potassium cyanide	7	5-10	0.111	0.077-0.15	2.0	7	10
Busulfan	12	NC	0.049	0.008-0.38	15.3	4	2
Colchicine	15 (mouse)	NC	0.0375	NC	4.9	3	6 (mouse)
Thallium I sulfate	25	NC	0.0495	NC	NC	1	29 (mouse)
Arsenic III trioxide	25	10-64	0.127	0.050-0.32	6.3	5	20
Endosulfan	28	NC	0.068	NC	2.4	2	18[7]
Digoxin	28	NC	0.0362	NC	NC	1	18 (mouse)
Mercury II chloride	40	27-60	0.148	0.010-0.22	7.7	10	1
Sodium arsenite	44	36-53	0.336	0.28-0.40	1.5	5	41[7]
$50 < LD_{50} \leq 300$ mg/kg (N =12)							
Sodium dichromate dihydrate	51	44-58	0.193	0.17-0.22	1.9	11	50
Dichlorvos	59	40-88	0.266	0.18-0.40	5.7	9	17[7]
Nicotine	70	68-72	0.430	0.42-0.44	1.0	4	50
Fenpropathrin	76	57-100	0.217	0.16-0.29	3.4	9	18[7]
Hexachlorophene	82	68-98	0.202	0.17-0.24	3.8	19	61
Paraquat	93	65-132	0.498	0.35-0.71	2.0	5	58
Lindane	100	78-129	0.344	0.27-0.44	1.4	4	76
Verapamil HCl	111	NC	0.226	NC	1.1	2	108
Sodium I fluoride	127	92-175	3.020	2.19-4.16	4.4	12	180

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

GHS Category[1]/Chemical	Reference Oral LD_{50}[2] (mg/kg)	95% Confidence Interval[3] (mg/kg)	Reference Oral LD_{50}[2] (mmol/kg)	95% Confidence Interval[3] (mmol/kg)	Maximum: Minimum Value[3]	N Averaged[5]	Initial Rodent Oral LD_{50}[6] (mg/kg)
Cadmium II chloride	135	88-208	0.738	0.48-1.14	2.4	5	88
Diquat dibromide	160	NC	0.466	NC	1.9	3	231
Phenobarbital	224	NC	0.966	NC	2.0	3	163
300 < LD_{50} ≤ 2000 mg/kg (N = 16)							
Caffeine	310	256-374	1.59	1.32-1.93	2.5	10	192
Triphenyltin hydroxide	329	208-520	0.896	0.57-1.42	25.9	15	44
Haloperidol	330	NC	0.877	NC	6.6	2	128[7]
Amitriptyline HCl	348	NC	1.18	NC	1.2	2	319
Propranolol HCl	466	NC	1.575	NC	NC	1	470 (mouse)
Cupric sulfate * 5 H2O	474	269-836	1.90	1.08-3.35	4.1	6	300
Phenol	548	434-692	5.82	4.82-7.68	4.7	14	414
Lithium carbonate	590	479-728	7.98	6.5-9.9	1.4	4	1187 (mouse; sulfate salt)
Glutethimide	600	NC	2.76	NC	NC	1	600
Sodium oxalate	633	NC	4.724	NC	1.3	2	155 (mouse)[8]
Chloral hydrate	638	391-1040	3.86	2.36-6.29	1.8	4	479
Atropine sulfate	819	641-1045	1.21	0.95-1.54	1.9	7	623
Valproic acid	995	NC	6.91	NC	2.2	2	670[7]
Meprobamate	1387	1291-1489	6.35	5.92-6.82	1.2	6	794[7]
Acetylsalicylic acid	1506	1224-1854	8.36	6.8-10.3	4.6	14	1000
Procainamide HCl	1950	NC	8.286	NC	NC	1	1950[7]
2000 < LD_{50} ≤ 5000 mg/kg (N = 11)							
Acetaminophen	2163	NC	14.3	NC	1.2	2	2404
Potassium I chloride	2799	NC	37.6	NC	1.2	2	2602
Carbamazepine	2805	NC	11.9	NC	2.1	2	1957[7]
Boric aid	3426	2617-4486	55.4	42.3-72.6	1.9	6	2660[7]
5-Aminosalicylic acid	3429	NC	22.4	NC	1.5	2	7749 (mouse)
Chloramphenicol	3491	NC	10.8	NC	2.0	3	3393
Acetonitrile	3598	2951-4375	87.6	71.9-107	6.2	26	3798
Lactic acid	3639	NC	40.3	NC	1.1	2	3730
Carbon tetrachloride	3783	3024-4732	24.6	20-31	4.3	15	2799
Sodium chloride	4046	2917-5623	69.3	50-96	2.0	5	2998

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

GHS Category[1]/Chemical	Reference Oral LD_{50}[2] (mg/kg)	95% Confidence Interval[3] (mg/kg)	Reference Oral LD_{50}[2] (mmol/kg)	95% Confidence Interval[3] (mmol/kg)	Maximum: Minimum Value[3]	N Averaged[5]	Initial Rodent Oral LD_{50}[6] (mg/kg)
Xylene	4667	1294-16827	43.9	12-158	5.6	4	4300
LD_{50} > 5000 mg/kg (N = 14)							
2-Propanol	5105	4624-5636	84.9	77-94	1.3	6	5843
Trichloroacetic acid	5229	2745-9961	32.0	16.8-61.0	2.7	4	4999
Dimethylformamide	5309	3548-7925	72.6	49-108	2.6	6	2800
Citric Acid	5929	NC	30.9	NC	3.9	2	3000[7]
Gibberellic acid	6040	NC	17.4	NC	1.1	2	6305
Propylparaben	6332 (mouse)	NC	35.1	NC	NC	1	6326 (mouse)
Ethylene glycol	7161	6266-8204	115.4	101-132	2.5	16	8567
Methanol	8710	6223-12218	272	194-381	2.3	6	13012
Dibutylphthalate	8892	6180-12794	31.9	22-46	1.7	4	11998
Diethylphthalate	9311	NC	41.9	NC	1.2	2	8602
Sodium hypochlorite	10328	NC	62.8	NC	1.6	2	8910[9]
Ethanol	11324	8610-14894	245.7	187-323	2.5	8	14008
1,1,1-Trichloroethane	12078	10000-14588	90.5	75-109	1.7	6	10298
Glycerol	19770	10495-37154	215	114-403	2.2	4	12691

[1]GHS- Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005). Chemicals categorized using reference oral LD_{50}.
[2]Based on a geometric mean of acceptable LD_{50} values from laboratory rats unless otherwise specified.
[3]For the geometric mean of the acceptable LD_{50} values.
[4]Ratio of minimum acceptable LD_{50} to maximum acceptable LD_{50}
[5]Number of values used for geometric mean.
[6]Values rounded to the nearest one; from the RC unless otherwise specified; rat data unless otherwise specified.
[7]RTECS® (MDL Information Systems 2002).
[8]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) indicates the value was determined by intraperitoneal administration to mice.
[9]NLM (2002).
Abbreviations: NC – Not calculated. N was three or less and considered too small for a meaningful result.

The reference LD_{50} values caused the reclassification of 19 reference substances (i.e., the sum of the numbers in the mismatching cells in **Table 4-3)**. Seven substances remain in the lowest LD_{50} category (i.e., $LD_{50} \leq 5$ mg/kg). Five substances originally in this category (aminopterin, mercury chloride, busulfan, parathion, and strychnine) moved to the next higher category ($5 < LD_{50} \leq 50$ mg/kg) due the change in the reference LD_{50} values. In the $5 < LD_{50} \leq 50$ mg/kg category, four substances (dichlorvos, fenpropathrin, sodium dichromate dihydrate, and nicotine) moved to the next higher LD_{50} category ($50 < LD_{50} \leq 300$ mg/kg) and one substance (triphenyltin hydroxide) moved up 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 up to the next toxicity category ($300 < LD_{50} \leq 2000$ mg/kg). In the $300 < LD_{50} \leq 2000$ mg/kg category, only carbamazepine moved up to the next toxicity category ($2000 < LD_{50} \leq 5000$ mg/kg). In the $2000 < LD_{50} \leq 5000$ mg/kg category, citric acid, trichloroacetic acid and dimethylformamide moved up to the next higher LD_{50} category ($LD_{50} > 5000$ mg/kg). In the $LD_{50} > 5000$ mg/kg category, 5-aminosalicylic acid moved down into the $2000 < LD_{50} \leq 5000$ mg/kg category. 5-Aminosalicylic acid was the only substance that moved to a lower LD_{50} (i.e., more toxic) category.

238 **Table 4-3 GHS[1] Toxicity Category Matches for the Initial and Reference LD_{50} Values[2]**

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

239 [1]Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005):
240 ≤ 5: $LD_{50} \leq 5$ mg/kg
241 5 - 50: $5 < LD_{50} \leq 50$ mg/kg
242 50 - 300: $50 < LD_{50} \leq 300$ mg/kg
243 300 - 2000: $300 < LD_{50} \leq 2000$ mg/kg
244 2000 - 5000: $2000 < LD_{50} \leq 5000$ mg/kg
245 > 5000: $LD_{50} > 5000$ mg/kg
246 [2]Number of chemicals. Darkened cells show the number of chemicals for which the categories match.
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4.3 Relevant Toxicity Information for Humans

The relevance of rodent acute systemic toxicity data to human lethality was assessed by the MEIC program as a comparison to the evaluation of *in vitro* predictions of human acute toxicity (Ekwall et al. 1998b). The MEIC program collected mouse and rat oral LD_{50} data from RTECS® (Ekwall et al. 1998a). Mean lethal doses in humans were collected mainly from handbooks containing human clinical toxicity information (Ekwall et al. 1998a). Data from the handbooks were 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 lethal doses by rat oral LD_{50} data and by mouse oral LD_{50} data for the 50 MEIC chemicals using units of log mol/kg. Ekwall et al. (1998b) reported $R^2 = 0.607$ for the rat LD_{50} prediction of mean human lethal doses and $R^2 = 0.653$ for the mouse LD_{50} prediction of mean human lethal doses.

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

4.4 Accuracy and Reliability of the *In Vivo* Rodent Toxicity Reference Values

Accuracy is the closeness of agreement between the results of an alternative test method and an accepted reference test method (ICCVAM 2003). Since there is no accepted reference test for the rodent acute oral toxicity test, the accuracy of the reference 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. The reliability of the reference LD_{50} values determined in this section may be judged by evaluating the range of acceptable LD_{50} values for each chemical and by comparing the values (and their variability) with other LD_{50} values.

Variability Among the Acceptable LD_{50} Values

The variability of the acceptable LD_{50} values used to calculate the reference 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 average 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 for these substances, since the range of values did not seem to be extremely wide. The four LD_{50} values for triethylenemelamine ranged from 1 to 13 mg/kg, the four LD_{50} values for busulfan ranged from 1.9 to 29 mg/kg, and the 10 LD_{50} values for parathion ranged from 1.8 to 30 mg/kg. **Table 4-4** shows the maximum:minimum ratios by toxicity category. The substances in the higher toxicity categories (i.e., $LD_{50} \leq 50$ mg/kg) tended to have higher maximum:minimum LD_{50} ratios than substances in the lower toxicity categories (i.e., $LD_{50} > 50$ mg/kg); however, there were also fewer substances in the higher toxicity categories.

Table 4-4 Maximum:Minimum LD_{50} Ratios by GHS[1] Toxicity Category

GHS Category[1] (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

[1]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 in **Section 4.1.2**.

Comparison of Reference Values with RC Values

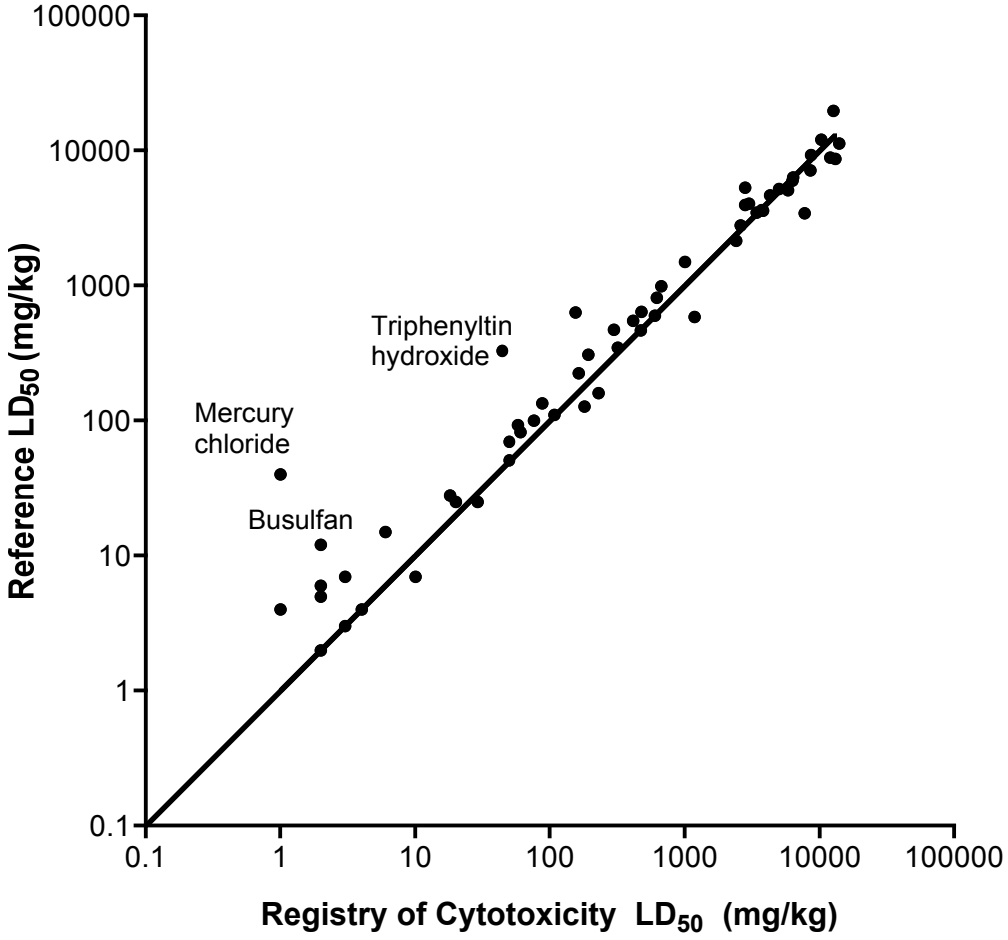
The correspondence of the reference LD_{50} values with the LD_{50} values for the 58 validation study reference substances in common with the RC are shown on a log scale in **Figure 4-2**. A Spearman correlation analysis for the two sets of log transformed values yielded a significant correlation ($p < 0.0001$) with a correlation coefficient, r_s, of 0.97. **Figure 4-2** shows that the reference values tended to be higher than the RC LD_{50} values. The LD_{50}

values used in the RC were largely from the 1983/84 RTECS®, which publishes the lowest LD_{50} value found for a particular chemical without regard to the source (i.e., from a primary publication or a review) and without scientific review before publication. Thus, since 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 those included in the RC database.

When comparing the reference LD_{50} values to the RC values, the substances with the largest differences in LD_{50} were busulfan, triphenyltin hydroxide, and mercury chloride **(**see **Figure 4-2)**.

- The reference LD_{50} for busulfan was six times that of the RC value (12 mg/kg vs. 1.9 mg/kg). The RC value (i.e., the 1983/84 RTECS® value) was from a paper by Schmahl and Osswald (1970) in which they cited a rat oral LD_{50} of 1.86 mg/kg. We also found rat oral LD_{50} values of 28 and 29 mg/kg for male and female Sprague-Dawley rats, respectively (Matsuno et al. 1971).
- The reference LD_{50} 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. Due to the relatively large variation in the data, neither the highest nor the lowest values were statistical outliers.
- The reference LD_{50} 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). Since it was reported as a range, it was excluded from the calculation of the reference value. The remaining 11 LD_{50} values ranged from 12 to 160 mg/kg. As previously stated, 160 mg/kg was an outlier compared to the other 10 values and therefore excluded from the calculation of the reference value.

Figure 4-2 Correlation of LD_{50} Values for the 58 RC Chemicals



The diagonal line shows the 1:1 relationship.

Comparison of the Variability Among Acceptable LD_{50} Values to Other Studies

When compared to other studies on the variation of acute oral LD_{50} values, the variation determined for 61 reference substances with multiple LD_{50} values was not unusual. Weil and Wright (1967) showed that even LD_{50} values from multiple laboratories using exactly the same protocol varied by as much as five-fold for the 10 substances they tested in eight laboratories. In addition, they showed that allowing the laboratories to use their own protocols for LD_{50} determination produced data somewhat more variable, but the observed differences were not reported. Another multicenter study that did not control the LD_{50} protocols reported maximum:minimum ratios from 3.6 to 11.3 for five substances (Hunter et

al. 1979). The 65 participating laboratories in eight countries reported LD_{50} values ranging from 44 to 5420 mg/kg for the five substances tested:

- Compound I/PCP 44 – 523 mg/kg
- Compound II/Sodium salicylate 800 - 4150 mg/kg
- Compound III/Aniline 350 – 1280 mg/kg
- Compound IV/Acetanilide 805 – 5420 mg/kg
- Compound V/Cadmium chloride 70 – 513 mg/kg

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

Although the LD_{50} data collected from the literature for the NICEATM/ECVAM validation study used various 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 by Hunter et al. (1979). The current study found six of the 61 substances with multiple LD_{50} values had maximum:minimum LD_{50} values higher than that reported by Hunter et al. (1979). Three of the reference substances: triethylenemelamine, parathion, and busulfan, were in the lowest LD_{50} (i.e., highest toxicity categories). Hunter et al. (1979) also observed that the largest variation was associated with the most toxic substances.

4.5 Summary

In vivo reference data for comparison with the *in vitro* NRU cytotoxicity data for the 72 substances were determined by analyzing rodent LD_{50} values identified by literature searches and secondary references. Rat LD_{50} values were preferred, but when rat data could not be located for three substances, mouse LD_{50} values were used. The 491 LD_{50} values located consisted of 485 rat oral LD_{50} values and six mouse oral LD_{50} values. Identifying a high quality data set determined under GLP guidelines was not possible since only 3% of the data

records were in compliance. Instead, a homogenous set of LD_{50} values for each substance was identified by excluding studies that employed the following materials and methods:

- feral rats
- rats < 4 weeks of age
- anesthetized rats
- test chemical administered in food or capsule
- LD_{50} reported as a range or inequality

After analyzing the remaining acceptable data for outliers, the remaining 385 values were used to determine *in vivo* reference values by calculating a geometric mean of the values for each reference substance. The reference LD_{50} values for 20 substances varied enough from the initial LD_{50} values, which came from the RC and other summary sources, that the substances were classified into different GHS oral toxicity categories.

Since there is no reference test for the rodent oral LD_{50}, the accuracy of the reference values for predicting the oral LD_{50} in humans could not be determined. The reliability of the reference values was assessed by comparison to other evaluations of the performance of the *in vivo* acute oral toxicity tests. Although the correlation of the reference values for the 58 RC chemicals with the RC LD_{50} was high ($r_s = 0.97$), the reference LD_{50} values tended to be higher than the RC values. 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. The maximum:minimum ratios for four chemicals were greater than one order of magnitude.

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