# ESTABLISHMENT OF RAT $LD_{50}$ REFERENCE VALUES FOR CHEMICALS TESTED IN A VALIDATION STUDY OF IN VITRO CYTOTOXICITY ASSAYS

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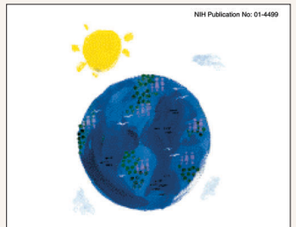
## Introduction




In October, 2000, the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity was convened to evaluate the validation status of *in vitro* methods for predicting acute systemic toxicity. Workshop participants recommended further evaluation of the usefulness of *in vitro* methods for predicting rodent and human acute toxicity (ICCVAM 2001), and developed recommendations for selection of chemicals that could be used in the validation of individual tests or prediction models. NICEATM and the European Centre for the Validation of Alternative Methods (ECVAM) subsequently designed a multi-laboratory validation study to evaluate the utility of two *in vitro* cytotoxicity tests for predicting rodent and human acute toxicity[1].

One critical aspect of the study design is the establishment of a rat $LD_{50}$ reference value for each of the 72 chemicals selected for the validation effort. These reference values will be used to evaluate the extent to which the two *in vitro* test methods can predict rat oral $LD_{50}$ values. Primary rat oral $LD_{50}$ studies were located through searching electronic databases, published literature, and secondary references. The primary study reports were reviewed to evaluate the suitability of the study for estimating the $LD_{50}$. Standard criteria were developed to exclude $LD_{50}$ data considered inappropriate for inclusion.

[1]See poster #761 entitled "Design of a Validation Study to Evaluate *In Vitro* Cytotoxicity Assays for Predicting Rodent and Human Acute Systemic Toxicity" by Strickland et al. for more information on the study design and the use of these values.

**Table 1. Internet-Accessible Databases with $LD_{50}$ Information**

| Database | Sponsor |
|---|---|
| • Registry of Toxic Effects of Chemical Substances (RTECS®)<br>• NIOSH Pocket Guide to Chemical Hazards | National Institute for Occupational Safety and Health (NIOSH) |
| • Integrated Risk Information System (IRIS) | U.S. Environmental Protection Agency (U.S. EPA); Office of Research and Development (ORD) |
| • Toxic Chemical Release Inventory (TRI)<br>• GENE-TOX | The National Library of Medicine (NLM); U.S. EPA |
| • Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART®/ETIC) | U.S. EPA; NLM; The National Institute of Environmental Health Sciences (NIEHS); National Center for Toxicological Research (NCTR) |
| • Oil and Hazardous Materials/Technical Assistance Data System (OHM/TADS) | U.S. EPA Office of Waste and Water Management |
| • ChemRTK High Production Volume (HPV) Challenge Program<br>• OPPT Chemical Fact Sheets<br>• Chemical Information Collection and Data Development | U.S. EPA Office of Pollution Prevention and Toxics (OPPT) |
| • Pesticide Product Information System (PPIS) | U.S. EPA Office of Pesticide Programs (OPP) |
| • Toxic Substances Control Act Test Submissions (TSCATS) | U.S. EPA OPPT |
| • Chemical Ingredients Database | U.S. EPA Office of Pesticide Programs (OPP); California EPA Department of Pesticide Regulation |
| • TOXLINE®<br>• Hazardous Substances Data Bank (HSDB)<br>• ChemIDplus | NLM [TOXNET] |
| • Chemical Carcinogenesis Research Information System (CCRIS)<br>• National Cancer Institute Website<br>• Chemical Hazard Response (CHRIS) | National Cancer Institute (NCI); National Institutes of Health (NIH); U.S. Department of Health and Human Services (U.S. DHHS)<br>U.S. Coast Guard |
| • Emergency Response Guidebook (ERG 2000) | Transport Canada; U.S. Department of Transportation (U.S. DOT); Secretariat of Communications and Transportation of Mexico |
| • Agency for Toxic Substances and Disease Registry (ATSDR)<br>• National Toxicology Program (NTP)<br>• Chemical Health and Safety Database<br>• Center for Drug Evaluation and Research (CDER) | U.S. Department of Health and Human Services (U.S. HHS)<br>NIEHS<br>U.S. Food and Drug Administration (U.S. FDA) |
| • National Transportation Library | U.S. DOT |
| • Consumer Product Safety Commission Website | U.S. Consumer Product Safety Commission (U.S. CPSC) |
| • The EXtension TOXicology NETwork (EXTOXNET) | University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho |
| • The Right-to-Know Network (RTK NET) | Office of Management and Budget Watch; Center for Public Data access |
| • CHEMINDEX<br>• CHEMINFO | Canadian Centre for Occupational Health and Safety (CCOHS) CHEMpendium™ |
| • Chemical Evaluation Search and Retrieval System (CESARS) | Michigan Department of Natural Resources; Ontario Ministry of the Environment; (CCOHS) CHEMpendium™ |
| • CIS Chemical Information (ILO/CIS) | The International Programme on Chemical Safety (IPCS); CCOHS; Labour Organisation (ILO) Occupational Safety and Health Information Centre (CIS) |
| • Concise International Chemical Assessment Documents (CICADS) | IPCS; CCOHS; World Health Organization (WHO), the International Labour Organisation (ILO), and the United Nations Environment Programme (UNEP) |
| • Environmental Health Criteria (EHC) monographs<br>• Health and Safety Guides (HSG)<br>• International Agency for Research on Cancer (IARC) | IPCS, CCOHS; WHO |
| • International Chemical Safety Cards (ICSC) | IPCS; CCOHS; Commission of the European Union |
| • IPCS/EC Evaluation of Antidotes Series<br>• Joint Expert Committee on Food Additives (JECFA)<br>• Joint Meeting on Pesticide Residues (JMPR)<br>• Pesticide Data Sheets (PDSs) | IPCS; CCOHS; WHO; Food and Agriculture Organization (FAO) of the United Nations |
| • Poisons Information Monographs (PIMs) | IPCS; CCOHS |
| • Organisation for Economic Co-operation and Development (OECD) Screening Information Data Sets (SIDS) | IPCS; CCOHS; International Register of Potentially Toxic Chemicals (IRPTC); United Nations Environmental Programme (UNEP) |
| • Deutsches Institut für Medizinische Dokumentation und Information (DIMDI) [The German Institute for Medical Documentation and Information]<br>• Registry of Cytotoxicity (RC) | Zentralstelle zur Erfassung und Bewertungvon Ersatz- und Ergänzungsmethoden zum Tierversuch (ZEBET) [German Centre for the Documentation and Validation of Alternative Methods] |
| • European Chemical Substances Information System (ESIS) | European Chemicals Bureau |
| • European Centre for the Validation of Alternative Methods Scientific Information Service (ECVAM SIS) | European Commission Joint Research Centre |
| • Multicentre Evaluation of In Vitro Cytotoxicity (MEIC) | Scandinavian Society for Cell Toxicology |
| • New Jersey Hazardous Substance Fact Sheets | New Jersey Department of Health and Senior Services |
| • HAZARDTEX®; MEDITEX®; INFOTEX®; SARATEX®; REPROTEX®; REPROTOX® | TOMES Plus®; MICROMEDEX, Greenwood Village, CO |
| • CHEMFINDER | CambridgeSoft Corporation |
| • Pesticide Action Network Pesticide Database | Pesticide Action Network North America |
| • SCORECARD | Environmental Defense |
| • Material Safety Data Sheets (MSDS) | Interactive Living Paradigms Incorporated |

### Determination of Rat Oral $LD_{50}$ Reference Values

Review and consideration of the available rat oral $LD_{50}$ data indicated that the majority of tests were conducted using unanesthetized young adult laboratory rats with chemicals administered by gavage. To derive comparable reference $LD_{50}$ values for each chemical, a relatively homogenous dataset was identified by excluding studies that reported less typical materials and methods:

- rats < 4 weeks of age
- feral rats
- anesthetized rats
- test chemical administered in food or capsule

In addition, studies that reported the $LD_{50}$ value as a range or inequality were excluded since point estimates are required for use in the prediction model.

If there were multiple acceptable $LD_{50}$ values for a particular chemical, statistical outliers at the 99% level were identified (Dixon and Massey 1981) and excluded. From the remaining acceptable data points (if >1), a geometric mean was calculated to serve as the proposed reference $LD_{50}$.

**Example: Selection of Rat Oral $LD_{50}$ Value from Primary References**

**Arsenic (III) Trioxide**

- Eight primary $LD_{50}$ references, reporting nine values, were identified (see **Table 2**).
- $LD_{50}$ values ranged from 13 to 385 mg/kg.
- Excluded values were from four studies that used feral or anesthetized rats or administered arsenic trioxide in food or gel capsule.
  - Harrison et al. (1958) excluded because arsenic trioxide was administered in food
  - Done and Peart (1971) excluded because arsenic trioxide was administered in gel capsules to anesthetized rats
  - Dieke and Richter (1946) and Peardon et al. (1972) were excluded because they used feral Norway rats.
- The geometric mean $LD_{50}$ of the five values (first five values in **Table 2**) which met the selection criteria = 25.1 mg/kg (95% confidence limits = 10-64 mg/kg) (see **Table 3**).

**Table 2. Available Rat Oral $LD_{50}$ Data for Arsenic Trioxide**

| Rat Oral $LD_{50}$ (mg/kg) | $LD_{50}$ Range (mg/kg) | $LD_{50}$ Calculation Method | Animal Information (species/strain/ stock, sex, age, weight) | Route/ Method of Exposure | Dose | Observations | Notes | Primary Reference |
|---|---|---|---|---|---|---|---|---|
| 19.9 (reported as 15.1 mg As/kg) | +/- 2.4 (reported as +/- 1.8 mg As/kg; what do these # stand for | de Beer, E.J. 1945. J. Pharmacol. & Exper. Therap. 85:1. | Rat; Sprague-Dawley; sex NR; 5 wk, weight NR | Intraesoph-ageal via feeding needles | 10 - 50 mg As/kg as pure arsenic trioxide dissolved in distilled water; 0.03 mL/g body weight; max volume 8 mL | $LD_{50}$ at 96 h; Rats observed for 2 wk; no difference in mortality between sexes; 95 deaths at 24 h<br>Deaths/dose at 96 h:<br>10 mg/kg 9/30<br>20 mg/kg 20/30<br>30 mg/kg 27/30<br>40 mg/kg 28/30<br>50 mg/kg 30/30 | Fasted 24 h before dosing; 5 groups of 30 rats each; male and female rats tested but only male results reported. | Harrison et al.1958 |
| 81.5 | 70.5 - 94.3 what do these # stand for | Bliss-Probit method | Rat, Sprague-Dawley; sex NR; 5 wk, weight NR | Oral gavage | Dissolved in saline; doses (mg/kg): 51.2, 66.5, 86.5, 112.5, 146.2 | Observed 6 h after dosing and daily for 1 - 2 wk; most rats died within 3 d; 27/30 died; Deaths/dose at 14 d:<br>51.2 mg/kg 0/10<br>66.5 mg/kg 3/10<br>86.5 mg/kg 6/10<br>112.5 mg/kg 9/10<br>146.2 mg/kg 10/10 | Animals acclimated to environment 1 wk before testing; 5 groups of 10 rats each; fasted 16 h before dosing; 100% lethal dose = 146.2 mg/kg; 0% lethal dose = 51.2 mg/kg | Kitagawa et al. 1982 |
| 32.6 | 28.4 - 38.7 (95% confidence limits) | Finney, D.J. (1971). Statistical Methods in Biological Assay, 3rd ed. London:Griffin Press. Probit Analysis. | Rat; strain, sex, age, weight NR | Intubated; single dose | Dissolved in distilled water; gavaged in 2 mL/kg | 8-10 rats dosed with one of 5 or 6 doses; not sure what 1 of 5 or 6 means; deaths recorded daily for 7 d | Animals acclimated to environment 2 wk before testing; used only healthy rats | Pryor et al. 1983 |
| 14.6 | NR | NR | Rat; strain, sex, age, weight NR | Oral | NR | NR | Russian reference is untranslated. | Tulukino and Novikov 1987 |
| 13 | NR | NR | Rat; strain, sex, age, weight NR | Oral; stomach tube | NR | Violent gastroenteritis, diarrhea, rice water stools. | Fasted animals; information from U.S. FDA laboratories. | Lehman 1951 |
| 191.8 (reported as 145.2 mg As/kg) | +/- 11.5 (reported as +/- 8.7 mg As/kg; what do these # stand for | de Beer, E.J. 1945. J. Pharmacol. & Exper. Therap. 85:1. | Rat; Sprague-Dawley Albino; male, age NR; 125 - 200 g | Oral; **feed**; 3 g Purina rat chow consumed in 1 h | Dose range 30.1 - 338 mg As/kg | $LD_{50}$ at 96 h; Rats observed for 2 wk; no difference in response between sexes; most died within 24 h.<br>Deaths/dose at 96 h (male):<br>30.1 mg/kg 0/20<br>91 mg/kg 2/20<br>126.1 mg/kg 6/20<br>207.6 mg/kg 18/20<br>269 mg/kg 20/20<br>28 mg/kg 20/20 | Fasted 24 h before dosing; 7 groups of 20 rats each; male and female tested but only male results reported. | Harrison et al. 1958 |
| 385 | 350 - 424 (95% confidence | Litchfield and Wilcoxon method Is there a date | Rat; Holtzman; sex NR; 13 - 41 wk; 300 - 500 g | Oral **gelatin capsules under light ether anesthesia**; Oral gavage via metal needle | 20, 50, 100, 250, 500, 750, 1000 mg/kg | Death occurred within 4 d | < 70 rats used; 24 h fasting before dosing. | Done and Peart 1971 |
| 138 | +/- 13 (standard error) | Litchfield, J.T.,Jr., and Fattig, J.W. 1941. On a graphic solution of the dosage-effect curve. Bull. Johns Hopkins Hosp., 69: 276 - 286. | Rat; **feral** Norway; (trapped in Baltimore, MD); males and females; adult; 148 - 493 g (ave. = 253 g) | Oral gavage via metal needle | Suspended in 10% acacia; 1mL/100g body weight | Rats survived 6- 72 h | 41 rats used (= equal number of male and females); fasted overnight before dosing; $LD_{50}$ from assays performed in winter and repeated in summer. | Dieke and Richter 1946 |
| 140 | NR | Statistical formula based on mortality rates | Rat, **feral** Norway; sex, age, and weight NR | Oral, stomach tube; single dose | Gave several sublethal doses to an equal number of rats; each dose at a different concentration. | Enteritis and neuritis | NR | Peardon et al. 1972 |

Notes: NR = not reported. Bold type indicates reason for exclusion.

**Table 3. Preliminary Reference $LD_{50}$ Values for Chemicals to be Tested**

| GHS[a] Category/Chemical | Rodent Oral $LD_{50}$[b] (mg/kg) | Proposed Reference $LD_{50}$[c] (mg/kg) | 95% Confidence Interval (mg/kg) | N Averaged[d] | Total N[e] | Product/Use |
|---|---|---|---|---|---|---|
| ***LD 50 ≤ 5 mg/kg*** | | | | | | |
| Mercury II chloride | 1 | 40.2 | 27-60 | 10 | 13 | Pesticide |
| Triethylenemelamine | 1.0 | 7.1 | 2-31 | 3 | 3 | Manufacturing |
| Sodium selenate | 1.6[f] | 3.1 | NC | 2 | 2 | Feed additive |
| Busulphan | 2 | 28.5 | NC | 2 | 2 | Pharmaceutical |
| Cycloheximide | 2 | 1.6 | 0.5-5 | 3 | 3 | Pesticide |
| Disulfoton | 2.0 | 4.8 | 2-10 | 6 | 6 | Insecticide |
| Parathion | 2.0 | 6.1 | 3-12 | 10 | 10 | Insecticide |
| Strychnine | 2.4[f] | 6.3 | 0.57-69 | 3 | 4 | Pesticide |
| Aminopterin | 3.0 (mouse) | | No rat oral data available | | | Medicinal |
| Phenylthiourea | 3.0 | 3.1 | NC | 1 | 2 | Pesticide |
| Epinephrine bitartrate | 4.0 (mouse) | | No rat oral data available | | | Pharmaceutical |
| Physostigmine | 4.5[f] | 4.5 | NC | 1 | 1 | Pharmaceutical |
| ***LD 50 > 5 - ≤ 50 mg/kg*** | | | | | | |
| Colchicine | 6 (mouse) | | No rat oral data available | | | Pharmaceutical |
| Potassium cyanide | 10 | 7.2 | 5-10 | 7 | 7 | Electroplating |
| Dichlorvos (DDVP) | 17[f] | 58.7 | 41-83 | 9 | 9 | Pesticide |
| Digoxin | 18 (mouse) | 28.27 | NC | 1 | 1 | Pharmaceutical |
| Fenpropathrin | 18[f] | 75.7 | 57-100 | 9 | 13 | Pesticide |
| Endosulfan | 18[f] | 27.8 | NC | 2 | 2 | Pesticide |
| Arsenic III trioxide | 20 | 25.1 | 10-64 | 5 | 9 | Pesticide |
| Thallium I sulfate | 29 (mouse) | 25 | NC | 1 | 3 | Pesticide |
| Sodium arsenite | 41[f] | 43.6 | 36-52 | 5 | 6 | Pesticide |
| Triphenyltin hydroxide | 44 | 328.8 | 207-520 | 15 | 15 | Pesticide |
| Sodium dichromate dihydrate | 50 | 50.5 | 44-58 | 11 | 11 | Oxidizing agent |
| Nicotine | 50 | 69.7 | 68-72 | 4 | 5 | Pharmaceutical |
| ***LD 50 > 50 - ≤ 300 mg/kg*** | | | | | | |
| Paraquat | 58 | 92.7 | 65-132 | 5 | 8 | Pesticide |
| Hexachlorophene | 61 | 83.6 | 66-106 | 14 | 14 | Disinfectant |
| Lindane | 76 | 100.0 | 78-128 | 4 | 5 | Pesticide |
| Cadmium II chloride | 88 | | | | | Veterinary pharmaceutical |
| Verapamil HCl | 108 | 135.2 | 88-208 | 5 | 5 | Pharmaceutical |
| Haloperidol | 128[f] | 110.9 | NC | 2 | 2 | Pharmaceutical |
| Sodium oxalate | 155 | 329.6 | NC | 2 | 3 | Pharmaceutical |
| Phenobarbital | 163 | 11,160 | NC | 1 | 1 | Paints, cleaners |
| Sodium I fluoride | 180 | 224.4 | 97-520 | 3 | 3 | Pharmaceutical |
| Caffeine | 192 | 126.8 | 92-175 | 12 | 14 | Electroplating, fluoridation |
| Diquat dibromide | 231 | 309.7 | 256-374 | 10 | 11 | Pharmaceutical, food additive |
| Cupric sulfate * 5 H2O | 300 | 160.3 | 70-365 | 3 | 3 | Pesticide |
| | | 474.2 | 269-836 | 6 | 11 | Pesticide |
| ***LD 50 > 300 - ≤ 2000 mg/kg*** | | | | | | |
| Amitriptyline | 319 | 348.3 | NC | 2 | 2 | Pharmaceutical |
| Phenol | 414 | 762.1 | 449-1294 | 14 | 15 | Disinfectant |
| Propranolol HCl | 470 (mouse) | 466 | NC | 1 | 1 | Pharmaceutical |
| Chloral hydrate | 479 | 638.3 | 391-1040 | 4 | 5 | Pharmaceutical |
| Glutethimide | 600 | 600 | NC | 1 | 1 | Pharmaceutical |
| Atropine sulfate | 623 | 818.5 | 641-1045 | 7 | 7 | Pharmaceutical |
| Valproic acid | 670[f] | 995.4 | NC | 2 | 2 | Pharmaceutical |
| Meprobamate | 794[f] | 1386.8 | 1291-1489 | 6 | 9 | Pharmaceutical |
| Acetylsalicylic acid | 1000 | 1513.6 | 1230-1862 | 14 | 15 | Pharmaceutical |
| Lithium sulfate | 1187 (mouse) | 710 (carbonate salt) | NC | 1 | 1 | Pharmaceutical |
| Procainamide | 1950[f] | 1950 | NC | 1 | 2 | Pharmaceutical |
| Carbamazepine | 1957[f] | 2805.4 | NC | 2 | 2 | Pharmaceutical |
| ***LD 50 > 2000 - ≤ 5000 mg/kg*** | | | | | | |
| Acetaminophen | 2404 | 2162.7 | NC | 2 | 2 | Pharmaceutical |
| Potassium I chloride | 2602 | 2799.0 | NC | 2 | 2 | Pharmaceutical, manufacturing |
| Sodium chloride | 2998 | 4045.8 | 2917-5623 | 5 | 5 | Pharmaceutical, food additive |
| Chloramphenicol | 3393 | 3491.4 | 1472-8260 | 3 | 6 | Pharmaceutical |
| Boric aid | 2660[f] | 3311.3 | 1288-8531 | 3 | 3 | Pesticide |
| Lactic acid | 3730 | 3639.2 | NC | 2 | 2 | Food additive |
| Citric Acid | 3000[f] | 3000 | NC | 1 | 1 | Food additive |
| Dimethylformamide | 2800 | 5023.4 | 3055-8241 | 5 | 7 | Solvent |
| Xylene | 4300 | 4666.6 | 1294-16827 | 4 | 4 | Solvent |
| Trichloroacetic acid | 4999 | 2280.3 | 44-116950 | 3 | 3 | Fixative |
| Acetonitrile | 3798 | 3589.2 | 2924-4406 | 25 | 28 | Solvent |
| Carbon tetrachloride | 2799 | 3953.7 | 3148-4954 | 16 | 20 | Solvent |
| ***LD 50 > 5000 mg/kg*** | | | | | | |
| 2-Propanol | 5843 | 4965.9 | 4529-5445 | 5 | 8 | Disinfectant |
| Ethylene glycol | 8567 | 7161 | 6266-8203 | 16 | 19 | Antifreeze |
| Ethanol | 14008 | 11324 | 8610-14894 | 8 | 9 | Solvent |
| 1,1,1-Trichloroethane | 10298 | 12078.1 | 10000-14588 | 6 | 6 | Solvent |
| Methanol | 13012 | 8709.6 | 6223-12218 | 6 | 7 | Solvent |
| Propylparaben | 6326 (mouse) | | No rat oral data available | | | Food additive |
| 5-Aminosalicylic acid | 7749 (mouse) | 3427.7 | NC | 2 | 3 | Pharmaceutical |
| Sodium hypochlorite | 8910[f] | 10327.6 | NC | 2 | 4 | Disinfectant |
| Dibutyl phthalate | 11998 | 7943.3 | 6918-9141 | 3 | 3 | Plasticizer |
| Glycerol | 12691 | 21232.4 | 6902-65313 | 3 | 3 | Solvent |
| Gibberellic acid | 6305 | 6039.5 | NC | 2 | 9 | Plant growth regulator |
| Diethyl phthalate | 8602 | 9311.0 | NC | 2 | 3 | Plasticizer |

[a]GHS-Globally Harmonised System categories of acute oral toxicity (OECD 2001).

[b]Values used to classify chemicals into GHS toxicity categories before primary $LD_{50}$ references were collected. Data are from Registry of Cytotoxicity (mostly Registry of Toxic Effects of Chemical Substances [RTECS]) (1983/84) unless otherwise specified. Rat data unless otherwise specified. The Registry of Cytotoxicity is a database of chemical specific $IC_{50}$ s and $LD_{50}$ s (Halle 1998)

[c]Based on available values in adult laboratory rats. If more than one value was available the reference value represents a geometric mean of the acceptable values.

[d]Number of values used for geometric mean.

[e]Total number of values available.

NC: Not calculated. N was too small for a meaningful result.

## Findings/Results

- A number of studies reporting rat oral $LD_{50}$ values exist for most of the 72 validation chemicals. The highest number of values for any one chemical was 28 for acetonitrile.
- A rat oral $LD_{50}$ value has yet to be identified for four chemicals (epinephrine bitartrate, aminopterin, colchicine, and propylparaben), although mouse data are available.
- Reported rat oral $LD_{50}$ values for individual chemicals may vary greatly, as evidenced by some of the larger confidence limits in **Table 3** (see busulfan, endosulfan, haloperidol, valproic acid, carbamazepine, trichloroacetic acid, and sodium hypochlorite).
- Some $LD_{50}$ references reported by databases are secondary references and provide totally unsupported $LD_{50}$ values.
- The level of detail reported for acute lethality studies varies greatly. Some studies report few experimental details and other studies provide complete information on animals, administration, doses, clinical signs, and times of death. For example, compare Peardon et al. (1972) with Kitagawa et al. (1982) in **Table 2**.
- Very few studies report the use of Good Laboratory Practices.

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