APPENDIX E

NICEATM Summary of The Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC)

This document was provided in the Background Materials and Supplemental Information Notebook for the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity [Section I, TAB 6].

The following ATLA (Alternatives To Laboratory Animals) excerpts are reprinted with permission from Professor Michael Balls, editor of ATLA.

- Clemedson et al., 1998. MEIC Evaluation of Acute Systemic Toxicity, Part IV. ATLA 26: 131-183. **[Table 1]**

- Ekwall et al., 1998. MEIC Evaluation of Acute Systemic Toxicity, Part V. ATLA 26: 571-616. **[Tables II, III, IV, V, VI, IX]**

- Ekwall et al., 2000. MEIC Evaluation of Acute Systemic Toxicity, Part VIII, ATLA 28 Suppl 1, 201-234**. [Figures 1 and 10]**

- Ekwall et al., 1999. EDIT: A new international multicentre programme to develop and evaluate batteries of in vitro tests for acute chronic systemic toxicity. ATLA 27: 339-349. **[Table 1 and Figure 1]**

The following table was reproduced with permission from Dr. Gary Hook (NIEHS).

- Wallum, E. 1998. Acute Oral Toxicity. EHP 106: 497-503**. [reproduction of Table 1]**

The Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC)

Summary

September 2000

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

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1.0 Introduction

The Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) program was organized by the Scandinavian Society for Cell Toxicology in 1989. MEIC was started with two goals. The first was to investigate the relevance of results from *in vitro* tests for predicting the acute toxic action of chemicals in humans. The second was to establish batteries of existing *in vitro* toxicity tests as replacements for acute toxicity tests on animals (LD50). Achievement of the second goal, the practical and ethical one, was considered to be entirely dependent on a successful outcome of the first, scientific goal. At the same time, it was recognized that a demonstrated high relevance of *in vitro* toxicity tests for human acute toxicity did not mean that all problems of replacement of animal tests would be solved. MEIC was a voluntary effort involving 96 international laboratories that evaluated the relevance and reliability of *in vitro* cytotoxicity tests originally developed as alternatives to or supplements for animal tests for acute systemic toxicity, chronic systemic toxicity, organ toxicity, skin irritancy, or other forms of general toxicity. In establishing the framework for this program, a minimum of methodological directives was provided in order to maximize protocol diversity among the participating laboratories. The collection of test method data was completed in 1996. The multiple publications originating from these studies are provided in chronological order in Section 12. All *in vitro* toxicity test results collected during MEIC are available on the Cytotoxicology Laboratory, Uppsala (CTLU) website (www.ctlu.se) as a searchable database.

2.0 Test Chemicals

Fifty reference chemicals were selected for testing (**Appendix 1**). Selection was based on the availability of reasonably accurate human data on acute toxicity. Due to the anticipated five-year duration of MEIC, it was recognized that multiple samples (lots) of each chemical would be needed. However, it was decided that the chemicals would not be provided by a central supplier, but rather that each laboratory would purchase each chemical at the highest purity obtainable with the proviso that storage duration would be kept to a minimum. The decision to not have a central supplier was based on the rationale that most reference chemicals are drugs, which presents fewer impurity problems. It is also based on the recognition that the results would be evaluated against human poisonings, which involve chemicals of different origin and purity.

3.0 *In Vitro* Test Assays

By the end of the project in 1996, 39 laboratories had tested the first 30 reference chemicals in 82 *in vitro* assays, while the last 20 chemicals were tested in 67 *in vitro* assays (**Appendix 2**). Slight variants of four of the assays were also used to test some chemicals. The primary 82 assays included:

- Twenty human cell line assays utilizing Chang liver, HeLa, Hep 2, Hep G2, HFL1, HL-60, McCoy, NB-1, SQ-5, and WI-1003 cells;
- Seven human primary culture assays utilizing hepatocytes, keratinocytes, and polymorphonuclear leukocytes;
- Nineteen animal cell line assays utilizing 3T3, 3T3-L1, Balb 3T3, BP8, ELD, Hepa-1c1c7, HTC, L2, LLC-PK1, LS-292, MDBK, PC12h, and V79 cells;
- Eighteen animal primary culture assays utilizing bovine spermatozoa, chicken neurons, mouse erythrocytes, rat hepatocytes, and rat muscle cells; and
- Eighteen ecotoxicological tests utilizing bacteria (*Bacillus subtilis*, *Escherichia coli* B, *Photobacterium phosphoreum*, *Vibrio fisheri*), rotifer (*Brachionus calyciflorus*), crustacea (*Artemia salina*, *Daphnia magna*, *Streptocephalus proscideus*), plant (*Alium cepa* root, tobacco plant pollen tubes), and fish (trout hepatocytes, trout R1 fibroblast-like cells).

4.0 Assay Endpoints

The analyses conducted by the MEIC management team were based on *in vitro* toxicity data presented as IC50 values (i.e., the dose estimated to reduce the endpoint in question by 50%) (**Appendix 2**).

These values were generated by the participating laboratories and were not independently verified; original data were not presented in the MEIC publications. Thirty-eight of these assays were based on viability, 29 on growth, and the remaining assays involved more specific endpoints, such as locomotion, contractility, motility, velocity, bioluminescence, and immobilization. The endpoints assessed were based on exposure durations ranging from five minutes to six weeks, and included:

- Cell viability as measured by the metabolism of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H* tetrazolium bromide (MTT), neutral red uptake (NRU), lactate dehydrogenase (LDH) release, cell morphology, adenosine triphosphate (ATP) content or leakage, trypan blue exclusion, viable cell count, tritiated-proline uptake, 86Rb leakage, creatine kinase activity, and glucose consumption;
- Cell growth as measured by protein content, macromolecule content, cell number, pH change, and optical density;
- Colony formation as measured by plating efficiency;
- An organotypic cellular endpoint (i.e., contractility of rat skeletel muscle cells);
- Motility and velocity for bovine sperm;
- Bioluminescence; and
- Mortality in lower eukaryotic organisms.

5.0 Comparative Data

The types of comparative data used to evaluate the predictive accuracy of the *in vitro* IC50 toxicity data for human acute toxicity included:

- Oral rat and mouse LD50 values obtained from Registry of Toxic Effects of Chemical Substances (RTECS) (**Appendix 3,** which contains rat and mouse LD50 data and average human lethal dose data for the 50 MEIC chemicals, ranked in three consecutive tables according to potency for rat, then mouse, and finally human. It also contains an U.S. Environmental Protection Agency (EPA) classification scheme for the acute toxicity of chemicals in humans.);
- Acute oral lethal doses in humans obtained from nine reference handbooks (**Appendix 4**);
- Clinically measured acute lethal serum concentrations in humans obtained from ten reference handbooks (**Appendix 5**);
- Acute lethal blood concentrations in humans measured post-mortem obtained from one forensic handbook and six forensic tabulations (**Appendix 6**);
- Human pharmacokinetics following single doses, including absorption, peak time, distribution/elimination curves, plasma half-life, distribution volume, distribution to organs (notably brain), and blood protein binding (**Appendix 7**);
- Peaks from curves of an ~50% lethal blood/serum concentration over time after ingestion (LC50 curves derived from human acute poisoning case reports) (**Appendix 8**);
- Qualitative human acute toxicity data, including lethal symptoms, main causes of death, average time to death, target organs, presence of histopathological injury in target organs, presence of toxic metabolites, and known or hypothetical mechanisms for the lethal injury (**Appendix 9**).

Early in the MEIC project, the *in vitro* cytotoxicity results were compared with average lethal blood concentrations (LCs) from acute human poisoning. However, these LCs were of limited value because they were averages of data with a wide variation due to different time between exposure and sampling (clinical) or death (forensic medicine). Therefore, a project was started to collect published and unpublished (from poison information centers and medico-legal institutes) case reports from human poisonings for the 50 MEIC reference chemicals that had lethal or sublethal blood concentrations with known time between ingestion and sampling/death. The aim was to compile enough case reports to be able to construct time-related lethal concentration curves to be compared with the IC50 values for different incubation times *in vitro*. The results from the project were presented and analyzed in a series of 50 MEIC monographs. All monographs with sufficient case reports contain five tables presenting blood concentrations and two figures presenting LC curves. Three tables present (i) clinically measured, time-related sublethal blood concentrations, (ii) clinically measured, time-related lethal blood concentrations, and (iii) post-mortem, time-related blood concentrations. In these tables, blood concentration and the time interval between exposure and sampling for these concentrations are listed, as well as other important information on the cases. One table contains case reports with blood concentrations without a known time after ingestion and one table presents average blood concentrations calculated from the values presented in the other tables. The two figures presented in each of the monographs are scatter plots of sublethal and lethal blood concentrations. Based on these plots, concentration curves over time were drawn for the highest no lethal concentrations (NLC100); the lowest lethal concentrations (LC0); and the median curve between NLC100 and LC0, which is called the approximate LC50 even though it is not equivalent to a 50% mortality.

6.0 Statistical Analyses

The statistical analyses conducted by the MEIC management team involved:

- Principal components analysis (PCA);
- Analysis of Variance (ANOVA) and pairwise comparison of means using Tukey's method;
- Linear regression and ANOVA linear contrast analysis; and
- Multivariable partial least square (PLS) modeling with latent variables.

7.0 Results (based on IC50 response)

The MEIC management team, based on their analyses of the *in vitro* IC50 data, obtained the following results:

- The 1st PCA component described 80% of the variance of all the cytotoxicity data.
- Tukey's ANOVA indicated a similar sensitivity (~80%) for the assays.
- The toxicity of many chemicals increased with exposure time, making it necessary to perform a test at several exposure times to fully characterize the cytotoxicity.
- In general, human cytotoxicity was predicted well by animal cytotoxicity.
- Prediction of human cytotoxicity by ecotoxicological tests was only fairly good.
- One organotypic endpoint (muscle cell contractility) gave different results to those obtained with viability/growth assays.
- Sixteen comparisons of similar test systems involving different cell types and exposure times revealed similar toxicities, regardless of cell type.
- Nine of ten comparisons of test systems with identical cell types and exposure times revealed similar toxicities, regardless of the viability or growth endpoint measurement used.
- Nine comparisons of similar test systems employing different primary cultures and cell lines indicated that they shared similar toxicities.
- A high correlation between an intracellular protein denaturation test and average human cell line toxicity test suggested that denaturation may be a frequently occurring mechanism in basal cytotoxicity.

The following results were based on comparisons between *in vitro* data and *in vivo* data:

- Simple human cell tests were shown to be relevant for human acute lethal action for as many as 43 of the 50 MEIC reference chemicals (86%). The exceptions were atropine, digoxin, malathion, nicotine, cyanide, paracetamol, and paraquat -- all specific receptor-mediated toxicants.
- A battery of three of these human cell line tests (nos. 1, 9, 5/16) was found to be highly predictive (R^2 = 0.77) of the peak human lethal blood concentrations (LC50) of chemicals. The prediction increased markedly (R^2 = 0.83) when a simple

algorithm based on the knowledge of passage across the blood-brain barrier was used to adapt *in vitro* to *in vivo* concentrations (**Appendix 7**). The battery involved four endpoints and two exposure times (protein content/24 hours; ATP content/24 hours; inhibition of elongation of cells/24 hours; pH change/7 days). Prediction was better than the prediction of human lethal doses by rat and mouse LD50-values (R^2 = 0.65). The correlation between calculated oral LD50 doses in rats and mice and acute lethal dose in humans is presented graphically in **Appendix 10**, while the correlation between IC50 values and peak lethal blood concentrations in humans is presented graphically in **Appendix 11**.

- In the *in vitro -- in vivo* MEIC evaluation of chemicals that do easily not cross the blood-brain barrier, the 24 hour cytotoxic concentrations for rapidly acting chemicals correlated well with the human lethal peak blood concentrations, while the corresponding cytotoxicity for the slow-acting chemicals did not correlate as well with the peak concentrations. The prediction of human toxicity by the tests of slow-acting chemicals was much improved when 48-hour cytotoxic concentrations were compared with 48-hour human lethal blood concentrations. Thus, an *in vitro* test providing a discrimination between a rapid and a slow cytotoxic action would increase the predictive power of a cell test battery on acute toxicity.
- The findings from both the *in vitro-in vitro* comparisons and the *in vitro-in vivo* comparisons strongly supported the basal cytotoxicity concept.

8.0 MEIC Conclusions and Recommendations

Based on the analyses conducted, the MEIC management team made the following conclusions:

- The MEIC 1, 9, 5/16 test battery can be used directly as a surrogate for a LD50 test. However, since the battery predicts lethal blood concentrations, not lethal dosages, it is not a direct counterpart of the animal LD50 test. Thus, the 1, 9, 5/16 battery must be supplemented with data on gut absorption as well as the distribution volumes (Vd) of chemicals. Vd essentially depends on whether chemicals penetrate cells or not, and the degree of accumulation in the cell for chemicals that enter cells. Binding to proteins, lipids, bone and intracellular matrix will also influence Vd. Probably, a simple test of accumulation in cells over time would provide adequate Vd data. There is sufficient *knowledge of kinetics and Vd to enable an evaluation of results from such an assay for most of the 50 MEIC chemicals.
- An ongoing evaluation is being conducted to address the issue of predicting human oral lethal doses rather than human lethal blood concentrations. One MEIC manuscript in preparation will focus on the importance of the kinetic determinants of target organs for basal cytotoxicity. A second MEIC manuscript will describe how human lethal doses may be predicted by cellular tests on basal cytotoxicity (the 1, 9, 5/16 battery) and kinetic data.
- If human lethal doses are shown to be well predicted by the 1, 9, 5/16 battery, when combined with absorption and distribution data, a new but simple *in vitro* test to predict distribution volumes must be developed. An effective *in vitro* test on absorption is stated to already exist. Development of new *in vitro* methods is not addressed by MEIC, which only evaluated existing methods.
- In MEIC, only two of the 50 reference chemicals (ethylene glycol and methanol) were biotransformed to more toxic metabolites, contributing to the acute lethal action. The occurrence of toxic metabolites for the two chemicals did not affect the prediction of human lethal peak concentrations by human cell line inhibitory concentrations, but seemed to interfere with the correlation between *in vitro* delayed effects and the prediction of

later lethal effects of the chemicals. These results confirm the proposed usefulness of an *in vitro* test that could measure the formation and release of a toxic metabolite by metabolically competent cells within the time frame of acute toxicity. One design of such a test would be to use human hepatocytes in co-cultures with a target cell line. Since so few metabolically active chemicals were tested in MEIC, future studies will need to include additional metabolically activated chemicals.

9.0 Evaluation-Guided Development of *In Vitro* Tests (EDIT)

In recognition that additional *in vitro* tests were needed to enhance the accuracy of the proposed *in vitro* battery for predicting human acute toxicity, a second voluntary multicenter program was initiated by the CTLU. The CTLU has designed a blueprint for an extended battery and has invited all interested laboratories to develop the "missing" tests of this battery within the framework of the EDIT program (**Appendix 12** and **13**). The EDIT research program is published on the Internet (www.ctlu.se). The aim of EDIT is to provide a full replacement of the animal acute toxicity tests. The most urgently needed developments are assays on the accumulation of chemicals in cells (test of Vd), passage across the intestinal and blood-brain barriers, and biotransformation to more toxic metabolites. CTLU will provide interested laboratories with human reference data and will evaluate results as single components of complex models. The Internet version of the general EDIT research program contains additional, regularly updated information on the project. Purported advantages of the project are as follows. First, the evaluation-guided test development in EDIT is rational since tests are designed according to obvious needs and as elementary tests of single events integrated into whole models, which is the potential strength of the *in vitro* toxicity testing strategy. Second, the direct testing of MEIC chemicals in newly developed *in vitro* assays will lead to a rapid evaluation of the potential value of each assay.

10.0 Recommended Integration of MEIC/EDIT into the EPA High Production Volume (HPV) Program

Dr. Ekwall, the principle scientist for the MEIC program, has provided several suggestions for using MEIC results and the forthcoming EDIT results to reduce animal testing in the HPV program. These suggestions include the following:

1. Formal validation by ECVAM/ICCVAM of the existing 3 test MEIC battery. If considered validated, use of the battery to test every chemical in the HPV program would provide inexpensive and useful supplementary data.
2. Evaluate some of the HPV chemicals in a battery of *in vitro* toxicity and toxicokinetic tests on acute toxicity (EDIT and similar models) as follows:
    - Engage poison information experts to select a set of HPV chemicals with sound human acute toxicity data, including time-related lethal blood concentrations.
    - Give priority to standard testing of the same chemicals in the HPV program.
    - Testing of the same chemicals in the newly developed *in vitro* systems (EDIT, etc.), including modeling of acute toxicity by the new assays.
    - Comparison of HPV standard animal data and the *in vitro* data with the human data for the selected set of chemicals.

    If the new *in vitro* models can be shown to predict human acute toxicity better than the HPV animal tests, *in vitro* batteries may totally replace the animal acute toxicity tests in further HPV testing.

11.0 MEIC Evaluation Guidelines Checklist

A complete and formal assessment of the validation status of MEIC in regard to the ICCVAM evaluation guidelines would require the following to be reviewed and evaluated:

ICCVAM Evaluation Guidelines

1.0 Introduction and Rationale of each Test Method
1.1 Scientific basis for each test method
1.1.1 Purpose of each proposed method, including the mechanistic basis
1.1.2 Similarities and differences of modes and mechanisms of action in each test system as compared to the species of interest (e.g., humans for human health-related toxicity testing).
1.2. Intended uses of each proposed test method.
1.2.1 Intended regulatory use(s) and rationale.
1.2.2 Substitute, replace, or complement existing test methods.
1.2.3 Fits into the overall strategy of hazard or safety assessment. If a component of a tiered assessment process, indicate the weight that will be applied relative to other measures.
1.2.4 Intended range of materials amenable to test and/or limits according to chemical class or physico-chemical factors.
2.0 Proposed Each Test Method Protocol(s)
2.1 Detailed protocol for each test method, duration of exposure, know limits of use, and nature of the response assessed, including:
2.1.1 Materials, equipment, and supplies needed
2.1.2 Suggested positive or negative controls.
2.1.3 Detailed procedures for conducting the test
2.1.4 Dose-selection procedures, including the need for any dose range-finding studies or acute toxicity data prior to conducting the test, if applicable;
2.1.5 Endpoint(s) measured
2.1.6 Duration of exposure
2.1.7 Known limits of use
2.1.8 Nature of the response assessed
2.1.9 Appropriate vehicle, positive and negative controls and the basis for their selection
2.1.10 Acceptable range of vehicle, positive and negative control responses
2.1.11 Nature of the data to be collected and the methods used for data collection
2.1.12 Type of media in which data are stored
2.1.13 Measures of variability
2.1.14 Statistical or non-statistical method(s) used to analyze the resulting data (including methods to analyze for a dose response relationship). The method(s) employed should

be justified and described
2.1.15 Decision criteria or the prediction model used to classify a test chemical (e.g., positive, negative, or equivocal), as appropriate
2.1.16 Information that will be included in the test report
2.2 Basis for each test system
2.3 Confidential information
2.4 Basis for the decision criteria established for each test
2.5 Basis for the number of replicate and repeat experiments; provide the rationale if studies are not replicated or repeated
2.6 Basis for any modifications to each proposed protocol that were made based on results from validation studies
3.0 Characterization of Materials Tested
3.1 Rationale for the chemicals/products selected for evaluation. Include information on suitability of chemicals selected for testing, indicating any chemicals that were found to be unsuitable
3.2 Rationale for the number of chemicals that were tested
3.3 The chemicals/products evaluated, including:
3.3.1. Chemical or product name; if a mixture, describe all components.
3.3.2 CAS number(s)
3.3.3 Chemical or product class
3.3.4 Physical/chemical characteristics
3.3.5 Stability of the test material in the test medium
3.3.6 Concentration tested.
3.3.7 Purity; presence and identity of contaminants.
3.3.8 Supplier/source of compound.
3.4 If mixtures were tested, constituents and relative concentrations should be provided whenever possible
3.5 Describe coding used (if any) during validation studies.
4.0 Reference Data Used for Performance Assessment
4.1 Clear description of the protocol for the reference test method. If a specific guideline has been followed, it should also be provided. Any deviation should be indicated, including the rationale for the deviation.
4.2. Provide reference data used to assess the performance of the proposed test method.
4.3 Availability of original datasheets for the reference data
4.4 Quality of the reference test data, including the extent of GLP compliance and any use of coded chemicals.
4.5 Availability and use of relevant toxicity information from the species of interest.
5.0 Test Method Data and Results
5.1 Complete, detailed protocol used to generate each set of data for each proposed test method.

Any deviations should be indicated, including the rationale for the deviation. Any protocol modifications made during the development process and their impact should be clearly stated for each data set.
5.2 Provide all data obtained using each proposed test method. This should include copies of original data from individual animals and/or individual samples, as well as derived data. The laboratory's summary judgement as to the outcome of each test should be indicated. The submission should also include data (and explanations) from unsuccessful, as well as successful, experiments.
5.3 Statistical approach used to evaluate the data from each proposed test method
5.4 Provide a summary, in graphic or tabular form, of the results.
5.5 For each set of data, indicate whether coded chemicals were tested, experiments were conducted blind, and the extent to which experiments followed GLP procedures.
5.6 Indicate the lot-to-lot consistency of the test materials, the time frame of the various studies, and the laboratory in which the study or studies were done. A coded designation for each laboratory is acceptable.
5.7 Any data not submitted should be available for external audit, if requested
6.0 Test Method Performance Assessment
6.1 Describe performance characteristics (e.g., accuracy, sensitivity, specificity, positive and negative predictivity, and false positive and negative rates) of each proposed test method separately and in combination compared with the reference test method currently accepted by regulatory agencies for the endpoint of interest. Explain how discordant results from each proposed test were considered when calculating performance values.
6.2 Results that are discordant with results from the reference method.
6.3 Performance characteristics of each proposed test method compared to data or recognized toxicity from the species of interest (e.g., humans for human health-related toxicity testing), where such data or toxicity classification is available. In instances where the proposed test method was discordant from the reference test method, describe the frequency of correct predictions of each test method compared to recognized toxicity information from the species of interest.
6.4 Strengths and limitations of the method, including those applicable to specific chemical classes or physical/chemical properties
6.5 Salient issues of data interpretation, including why specific parameters were selected for inclusion
7.0 Test Method Reliability (Repeatability/Reproducibility)
7.1 Rationale for the chemicals selected to evaluate intra- and inter-laboratory reproducibility for each test method, and the extent to which they represent the range of possible test outcomes.
7.2 Analyses and conclusions reached regarding inter- and intra-laboratory repeatability and reproducibility for each test method
7.3 Summarize historical positive and negative control data for each test method, including number of trials, measures of central tendency and variability.
8.0 Test Method Data Quality
8.1 Extent of adherence to GLPs

8.2. Results of any data quality audits
8.3 Impact of deviations from GLPs or any non-compliance detected in data quality audits
9.0 Other Scientific Reports and Reviews
9.1 All data from other published or unpublished studies conducted using the proposed test method should be included.
9.2 Comment on and compare the conclusions published in independent peer-reviewed reports or other independent scientific reviews of the test method. The conclusions of such scientific reports and/or reviews should be compared to the conclusions reached in this submission. Any other ongoing evaluations of the method should be mentioned.
10.0 Animal Welfare Considerations (Refinement, Reduction, and Replacement)
10.1 Describe how the proposed test methods will refine (reduce pain or distress), reduce, and/or replace animal use compared to the current methods used.
11.0 Other Considerations
11.1 Aspects of test method transferability. Include an explanation of how this compares to the transferability of the reference test method.
11.1.1 Facilities and major fixed equipment needed to conduct the test.
11.1.2 Required level of training and expertise needed for personnel to conduct the test.
11.1.3 General availability of other necessary equipment and supplies.
11.2 Cost involved in conducting each test. Discuss how this compares to the cost of the reference test method.
11.3 Indicate the amount of time needed to conduct each test and discuss how this compares with the reference test method.
12.0 Supporting Materials
12.1 Provide copies of all relevant publications, including those containing data from the proposed test method or the reference test method.
12.2 Include all available non-transformed original data for both each proposed test method and the reference test method.
12.3 Summarize and provide the results of any peer reviews conducted to date, and summarize any other ongoing or planned reviews.
12.4 Availability of laboratory notebooks or other records for an independent audit. Unpublished data should be supported by laboratory notebooks.

12.0 MEIC Related Publications (in chronological order)

Bernson, V., Bondesson, I., Ekwall, B., Stenberg, K., and Walum, E. (1987) A multicentre evaluation study of in vitro cytotoxicity. ATLA, 14, 144-145.

Bondesson, I., Ekwall, B., Stenberg, K., Romert, L. and Walum, E. (1988) Instruction for participants in the multicentre evaluation study of in vitro cytotoxicity (MEIC). ATLA, 15, 191-193.

Bondesson, I., Ekwall, B., Hellberg, S., Romert, L., Stenberg, K., and Walum, E. (1989) MEIC - A new international multicenter project to evaluate the relevance to human toxicity of in vitro cytotoxicity tests. Cell Biol. Toxicol., 5, 331-347.

Ekwall, B. (1989) Expected effects of the MEIC-study. In Report from The MEIC In Vitro Toxicology Meeting, Stockholm 9/3 1989, (Eds. T. Jansson and L.Romert), pp 6-8, Swedish National Board for Technical Development.

Ekwall, B., Gómez-Lechón, M.J., Hellberg, S., Bondsson, I., Castell, J.V., Jover, R., Högberg, J., Ponsoda, X., Stenberg, K., and Walum, E. (1990) Preliminary results from the Scandinavian multicentre evaluation of in vitro cytotoxicity (MEIC). Toxicol. In Vitro, 4, 688-691.

Hellberg, S., Bondesson, I., Ekwall, B., Gómez-Lechón, M.J., Jover, R., Högberg, J., Ponsoda; X., Romert, L., Stenberg, K., and Walum, E. (1990) Multivariate validation of cell toxicity data: The first ten MEIC chemicals. ATLA, 17, 237-238.

Hellberg, S., Eriksson, L., Jonsson, J., Lindgren, F., Sjöström, M., Wold, S., Ekwall, B., Gómez-Lechón, J.M., Clothier, R., Accomando, N.J., Gimes, G., Barile, F.A., Nordin, M., Tyson, C.A., Dierickx, P., Shrivastava, R.S., Tingsleff-Skaanild, M., Garza-Ocanas, L., and Fiskesjö, G. (1990) Analogy models for prediction of human toxicity. ATLA, 18, 103-116.

Shrivastava, R., Delomenie, C., Chevalier, A., John, G., Ekwall, B., Walum, E., and Massingham, R. (1992) Comparison of in vivo acute lethal potency and in vitro cytotoxicity of 48 chemicals. Cell Biol. Toxicol., 8(2), 157-170.

Ekwall, B., Abdulla, E., Barile, F., Bondesson, I., Clemedson, C., Clothier, R.,Curren, R., Dierickx, P., Fiskesjö, G., Garza-Ocanas, L., Gómez-Lechón, M.J., Gülden, M., Imai, K., Janus, J., Kristen, U., Kunimoto,, M., Kärenlampi, S., Lavrijsen, K., Lewan, L.,Malmsten, A., Miura, T., Nakamura, M., Ohno, T., Ono, H., Persoone, G., Rouget, R., Romert, L., Sandberg, M., Sawyer, T., Seibert, H., Shrivastava, R., Stammati, A., Tanaka, N., Walum, E., Wang, X & Zucco, F. (1992) Acute lethal toxicity in man predicted by cytotoxicity in 55 cellular assays and by oral LD50 tests in rodents for the first 30 MEIC chemicals, In Proc. of JSAAE (Japanese Society for Alternatives to Animal Experiments) 6th annual meeting in Tokyo, Dec 17-18, 1992, (Ed. S. Sato), pp 114-115, Tokyo.

Ekwall, B., Abdulla, E., Barile, F.,Chesne, C., Clothier, Cottin, M., Curren, R., Daniel- Szolgay, E., Dierickx, P., Ferro, M., Fiskesjö, G., Garza-Ocanas, L., Gómez-Lechón, M.J., Gülden, M. Isomaa, B., Kahru, A., Kemp, R.B., Kerszman, G., Kristen, U., Kunimoto,, M., Kärenlampi, S., Lavrijsen, K., Lewan, L., Ohno, T., Persoone, G., Pettersson, R., Rouget, R., Romert, L., Sawyer, T., Seibert, H., Shrivastava, R., Sjöström, M., Tanaka, N., Zucco, F., Walum, E., & Clemedson, C. (1994) A comparative cytotoxicity analysis of the results from tests of the first 30 MEIC reference chemicals in 68 different in vitro toxicity systems, pp 117-118 in Alternatives Research - Proceedings of the 8th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Nov. 28-29, 1994, Tokyo.

Ekwall, B. (1995) The basal cytotoxicity concept, pp 721-725. In Proceedings of the World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing. Alternative Methods in Toxicology and the Life Sciences, Vol 11. Mary Ann Liebert, New York, 1995.

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Appendix I
First Fifty Reference Chemicals

Acetaminophen
Aspirin
Ferrous sulfate
Diazepam
Amitriptyline
Digoxin
Ethylene glycol
Methyl alcohol
Ethyl alcohol
Isopropyl alcohol
1,1,1-Trichloroethane
Phenol
Sodium chloride
Sodium fluoride
Malathion
2,4-Dichlorophenoxyacetic acid
Xylene
Nicotine
Potassium cyanide
Lithium sulfate
Theophylline
Dextropropoxyphene HCl
Propranolol HCl
Phenobarbital
Paraquat
Arsenic trioxide
Cupric sulfate
Mercuric chloride
Thioridazine HCl
Thallium sulfate
Warfarin
Lindane
Chloroform
Carbon tetrachloride
Isoniazid
Dichloromethane
Barium nitrate
Hexachlorophene
Pentachlorophenol
Varapamil HCl
Chloroquine phosphate
Orphenadrine HCl
Quinidine sulfate
Diphenylhydantoin
Chloramphenicol
Sodium oxalate
Amphetamine sulfate
Caffeine
Atropine sulfate
Potassium chloride

Appendix II: Descriptions of the Essential Traits of 67 *in vitro* Methods

Table I: Descriptions of the essential traits of 67 *in vitro* methods

Method No.	Old No.[a]	Cell type/ test system	Tissue of origin	Species	Endpoint	Incubation time	Testing laboratory[b]	Reference
Human cell lines								
1.	II:1	Hep G2	Hepatoma	Human	Protein content/Lowry	24 hours	Dierickx	3
2.	III:2	Hep G2	Hepatoma	Human	Protein content/ Sulphorhodamine B	24 hours	Hall, Cambridge & James	5
3.	II:2	Hep G2	Hepatoma	Human	MTT	24 hours	Gómez-Lechón, Jover, Ponsoda & Castell[c]	3, 12
4.	II:4	WI-1003/Hep G2[d]	Lung/Hepatoma	Human	Morphology	24 hours	Garza-Ocañas & Torres-Alanis	3
5.	II:3	Chang liver cells	Liver	Human	Morphology	24 hours	Garza-Ocañas & Torres-Alanis	3
6.	II:5	HeLa	Cervical carcinoma	Human	Morphology	24 hours	Ekwall & Malmsten	3
7.	II:6	Hep 2	Epithelial carcinoma of larynx	Human	Protein content/ Coomassie blue staining	24 hours	Stammati, Zucco, Zanetti & De Angelis	3
8.	II:7	Hep 2	Epithelial carcinoma of larynx	Human	LDH release	24 hours	Stammati, Zucco, Zanetti & De Angelis	3
9.	II:8	HL-60	Promyelocytic leukaemia	Human	ATP content	24 hours	Tanaka, Wakuri, Izumi, Sasaki & Ono	3
10.	III:10	HFL1	Fetal lung cells	Human	MTT	24 hours	Barile & Sookhoo[e]	5, 13
11.	III:11A	SQ-5	Lung squamous carcinoma	Human	LDH content[f]	48 hours	Ohno, Wang, Sasaki & Hirano	3, 14
12.	III:12	SQ-5	Lung squamous carcinoma	Human	Killing index[g]	48 hours	Ohno, Wang, Sasaki & Hirano	3, 14
13.	II:10	NB-1	Neuroblastoma	Human	Protein content/ Crystal violet staining	48 hours	Kunimoto, Miura, Aoki & Kunimoto	3
14.	II:11	McCoy	Epithelial cells from synovial fluid	Human	Morphology/Trypan blue exclusion[h]	72 hours	Shrivastava & Chevalier	3
15.	II:13	WI-1003/Hep G2[d]	Lung/Hepatoma	Human	Morphology/pH changes	168 hours	Garza-Ocañas & Torres-Alanis	3

Source: Clemedson et al. 1998. MEIC Evaluation of Acute Systemic Toxicity. Part IV. ATLA 26:131-183. (reprinted with permission from the editor)

16.	II:12	Chang liver	Liver	Human	Morphology/pH changes	168 hours	Garza-Ocañas & Torres-Alanis	3
17.	II:14	HeLa	Cervical carcinoma	Human	pH changes (phenol red)	168 hours	Ekwall & Malmsten	3
18.	II:15	MRC-5 (finite cell line)	Epithelial cells from embryonic lung	Human	Protein content/Lowry	6 weeks	Dierickx	3, 15
Human primary cultures								
19.	III:21	Polymorphonuclear leukocytes[i]	Blood	Human	Viable cell count fluorescein diacetate/ Ethidium bromide	3 hours	Valentino, Monaco, Pieragostini, Amati & Governa	5
20.	III:22	Polymorphonuclear leukocytes[i]	Blood	Human	Locomotion stimulated by chemotactic peptide	3 hours	Valentino, Monaco, Pieragostini, Amati & Governa	5
Animal cell lines								
21.	II:19	ELD	Subline of Ehrlich ascites tumour cells	Mouse	ATP leakage	10 minutes	Lewan & Andersson	3
22.[j]	II:20	ELD	Subline of Ehrlich ascites tumour cells	Mouse	ATP leakage	10 minutes	Lewan & Andersson	3
23.	II:23	HTC	Hepatoma	Rat	Macromolecular content	24 hours	Ferro, Bassi & Canepa[k]	3
24.	II:25	L2	Lung epithelial cells	Rat	[^{3}H]-proline uptake	24 hours	Barile, Borges, Arjun & Hopkinson[l]	3, 16
25.	II:30	3T3	Fibroblasts	Mouse	MTT	24 hours	Gómez-Lechón, Jover, Ponsoda & Castell[c]	3, 42
26.	III:40	LLC-PK1	Kidney	Pig	Protein content/ Sulphorhodamine B	24 hours	Hall, Cambridge & James	5
27.	II:31	BP8	Ascites sarcoma	Mouse	Cell number/ Coulter counter	48 hours	Romert, Jansson & Jenssen	3
28.	II:32	PC12h	Pheochromocytoma	Rat	Protein content	48 hours	Kunimoto, Miura, Aoki & Kunimoto	3
29.	II:33	MDBK	Kidney	Bovine[m]	Morphology/Trypan blue exclusion[h]	72 hours	Shrivastava & Chevalier	3
30.	II:34	Hepa-1c1c7 (Sub-clone of Hepa-1)	Hepatoma	Mouse	Protein content/ Coomassie blue staining	72 hours	Kärenlampi & Malmivuori	3 *INVITTOX* protocol 112[m]

Table I: continued

No.	Old No.[a]	Cell type/ test system	Tissue of origin	Species	Endpoint	Incub-ation time	Testing laboratory[b]	Refer-ence
Method								
31.	II:35	3T3-L1 (Sub-clone of 3T3)	Embryonal fibroblasts	Swiss mouse	Protein content/Kenacid blue staining	72 hours	Clothier	3
32.	II:36	Balb 3T3 A31-1-1	Whole embryo	Balb/c mouse	Colony formation	168 hours	Tanaka, Wakuri, Izumi, Sasaki & Ono	3
Animal primary cultures								
33.		Muscle cells	Skeletal muscle	Rat	Spontaneous contractility	1 hour	Gülden, Seibert & Voss	3, *INVITTOX* protocol 93[m]
34.	II:45A	Neurons	Embryonal forebrain	Chicken	Neutral red uptake	20 hours	Sawyer & Weiss	3
35.	II:46A	Neurons	Embryonal forebrain	Chicken	MTT	21 hours	Sawyer & Weiss	3
36.	II:50	Hepatocytes[a]	Liver	Male rat	MTT	24 hours	Gómez-Lechón, Jover, Ponsoda & Castell[c]	3, 12
37.	II:51	Hepatocytes[a]	Liver	Male rat	Morphology/Trypan blue exclusion/LDH release[h]	24 hours	Shrivastava & Chevalier	3
38.	II:52	Erythrocytes	Peripheral blood of 9-week males	Balb/c mouse	ATP content	24 hours	Tanaka, Wakuri, Izumi, Sasaki & Ono	3
39.		Muscle cells	Skeletal muscle	Rat	Intracellular creatine kinase activity	24 hours	Gülden, Seibert & Voss	3, *INVITTOX* protocol 93[m]
40.		Muscle cells	Skeletal muscle	Rat	Glucose consumption	24 hours	Gülden, Seibert & Voss	3, *INVITTOX* protocol 93[m]
41.		Muscle cells	Skeletal muscle	Rat	Spontaneous contractility	24 hours	Gülden, Seibert & Voss	3, *INVITTOX* protocol 93[m]

Appendix III: Oral LD50 Doses for Rat and Mouse and Mean Oral Lethal Doses for Humans and Toxicity Categories

Oral LD50 Doses for Rat and Mouse and Mean Oral Lethal Doses for Humans

Chemical Number	Chemical	Rat LD50		Mouse LD50		Ave. Human Dose	
		mg/kg	umol/kg	mg/kg	umol/kg	mg/kg	umol/kg
28	Mercuric chloride	**1**	**4**	6	22	25.7	94.7
31	Warfarin	**2**	**5**	3	10	107.1	347.4
18	Potassium cyanide	**5**	**77**	9	131	2.9	43.9
26	Arsenic trioxide	**15**	**74**	31	159	4.1	20.9
30	Thallium sulfate	**16**	**32**	24	47	14.0	27.7
39	Pentachlorophenol	**27**	**101**	28	105	28.6	107.3
6	Digoxin	**28**	**36**	18	23	0.1	0.17
17	Nicotine	**50**	**308**	3	21	0.7	4.4
13	Sodium fluoride	**52**	**1238**	57	1357	92.8	2210.9
47	Amphetamine sulfate	**55**	**149**	24	65	20.0	54.3
38	Hexachlorophene	**56**	**138**	67	165	214.3	526.6
32	Lindane	**76**	**261**	44	151	242.9	835.1
21	Propoxyphene HCL	**84**	**223**	255	678	24.6	65.4
25	Paraquat	**100**	**537**	120	644	40.0	214.7
40	Varapamil HCL	**108**	**220**	163	331	122.3	249.1
23	Penobarbital	**162**	**697**	137	590	111.4	479.7
48	Caffeine	**192**	**989**	127	654	135.7	698.8
2	Acetylsalicylic acid	**200**	**1110**	232	1287	385.7	2140.5
20	Theophylline	**244**	**1354**	235	1304	157.1	872.1
42	Orphenadrine HCL	**255**	**834**	100	327	50.0	163.4
43	Quinidine sulfate	**258**	**610**	286	676	79.2	187.4
14	Malathion	**290**	**878**	190	575	742.8	2248.4
11	Phenol	**317**	**3369**	270	2869	157.2	1670.0
3	Ferrous sulfate	**319**	**2100**	680	4477	392.1	2581.0
5	Amitriptyline	**320**	**1154**	140	505	37.1	133.8
4	Diazepam	**352**	**1236**	45	159	71.4	250.8
37	Barium nitrate	**355**	**1358**	266	1016	37.1	142.1
15	2,4-Dichlorophenoxy-acetic acid	**375**	**1697**	347	1570	385.8	1745.3
22	Propamolol HCL	**466**	**1575**	320	1082	71.5	241.7
27	Cupric sulfate	**469**	**1880**	502	2012	290.6	1163.6
19	Lithium sulfate	**492**	**4478**	1190	10,828	1065.5	9691.8
49	Altropine sulfate	**585**	**864**	456	674	1.7	2.5
41	Chloroquine phosphate	**623**	**1208**	500	969	84.3	163.4
33	Chloroform	**908**	**7605**	36	302	999.8	8375.2
29	Thioridazine HCL	**995**	**2445**	385	946	68.6	1684
35	Isoniazid	**1250**	**9117**	133	970	171.5	1250.4
36	Dichloromethane	**1601**	**18,846**	873	10,280	1386.2	16,321.7
44	Diphenylhydantoin	**1635**	**6480**	150	595	300.0	1189.1
34	Carbon tetrachloride	**2350**	**15,280**	8264	53,726	1314.4	8545.4
1	Paracetamol	**2404**	**15,899**	338	2235	271.4	1795.2
45	Chloramphenicol	**2500**	**7735**	1500	4641	285.7	884.0
50	Potassium chloride	**2598**	**34,853**	1499	20,107	285.5	3830.0
12	Sodium chloride	**3002**	**51,370**	4003	68,493	2287.3	39,138.9

Oral LD50 Doses for Rat and Mouse and Mean Oral Lethal Doses for Humans

16	Xylene	**4299**	**40,490**	2119	19,953	899.8	8474.6
7	Ethylene glycol	**4698**	**75,684**	5498	88,567	1570.9	25,304.8
8	Methanol	**5619**	**175,327**	7289	227,414	1569.0	48,954.2
9	Ethanol	**7057**	**153,145**	3448	74,837	4712.2	102,262.2
46	Sodium oxalate	**11160**	**83,284**	5095	38,019	357.1	2665.3
10	1,1,1-Trichloroethane	**11196**	**83,927**	7989	59,884	5707.6	42,785.8
Source: E. Walum. 1998. Acute oral toxicity. EHP 106:497-503. (reprinted with permission from the editor)							

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Chemical Number	Chemical	Rat LD50		Mouse LD50		Ave. Human Dose	
		mg/kg	umol/kg	mg/kg	umol/kg	mg/kg	umol/kg
31	Warfarin	2	5	**3**	**10**	107.1	347.4
17	Nicotine	50	308	**3**	**21**	0.7	4.4
28	Mercuric chloride	1	4	**6**	**22**	25.7	94.7
18	Potassium cyanide	5	77	**9**	**131**	2.9	43.9
6	Digoxin	28	36	**18**	**23**	0.1	0.2
30	Thallium sulfate	16	32	**24**	**47**	14.0	27.7
47	Amphetamine sulfate	55	149	**24**	**65**	20.0	54.3
39	Pentachlorophenol	27	101	**28**	**105**	28.6	107.3
26	Arsenic trioxide	15	74	**31**	**159**	4.1	20.9
33	Chloroform	908	7605	**36**	**302**	999.8	8375.2
32	Lindane	76	261	**44**	**151**	242.9	835.1
4	Diazepam	352	1236	**45**	**159**	71.4	250.8
13	Sodium fluoride	52	1238	**57**	**1357**	92.8	2210.9
38	Hexachlorophene	56	138	**67**	**165**	214.3	526.6
42	Orphenadrine HCL	255	834	**100**	**327**	50.00	163.4
25	Paraquat	100	537	**120**	**644**	40.00	214.7
48	Caffeine	192	989	**127**	**654**	135.7	698.8
35	Isoniazid	1250	9117	**133**	**970**	171.5	1250.4
23	Penobarbital	162	697	**137**	**590**	111.4	479.7
5	Amitriptyline	320	1154	**140**	**505**	37.1	133.8
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8	Methanol	5619	175,327	**7289**	**227,414**	1569.0	48,954.2
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17	Nicotine	50	308	3	21	**0.7**	**4.4**
49	Altropine sulfate	585	864	456	674	**1.7**	**2.5**
18	Potassium cyanide	5	77	9	131	**2.9**	**43.9**
26	Arsenic trioxide	15	74	31	159	**4.1**	**20.9**
30	Thallium sulfate	16	32	24	47	**14.0**	**27.7**
47	Amphetamine sulfate	55	149	24	65	**20.0**	**54.3**
21	Propoxyphene HCL	84	223	255	678	**24.6**	**65.4**
28	Mercuric chloride	1	4	6	22	**25.7**	**94.7**
39	Pentachlorophenol	27	101	28	105	**28.6**	**107.3**
5	Amitriptyline	320	1154	140	505	**37.1**	**133.8**
37	Barium nitrate	355	1358	266	1016	**37.1**	**142.1**
25	Paraquat	100	537	120	644	**40.0**	**214.7**
42	Orphenadrine HCL	255	834	100	327	**50.0**	**163.4**
29	Thioridazine HCL	995	2445	385	946	**68.6**	**168.5**
4	Diazepam	352	1236	45	159	**71.4**	**250.8**
22	Propamolol HCL	466	1575	320	1082	**71.5**	**241.7**
43	Quinidine sulfate	258	610	286	676	**79.2**	**187.4**
41	Chloroquine phosphate	623	1208	500	969	**84.3**	**163.4**
13	Sodium fluoride	52	1238	57	1357	**92.8**	**2210.9**
31	Warfarin	2	5	3	10	**107.1**	**347.4**
23	Penobarbital	162	697	137	590	**111.4**	**479.7**
40	Varapamil HCL	108	220	163	331	**122.3**	**249.1**
48	Caffeine	192	989	127	654	**135.7**	**698.8**
20	Theophylline	244	1354	235	1304	**157.1**	**872.1**
11	Phenol	317	3369	270	2869	**157.2**	**1670.0**
35	Isoniazid	1250	9117	133	970	**171.5**	**1250.4**
38	Hexachlorophene	56	138	67	165	**214.3**	**526.6**
32	Lindane	76	261	44	151	**242.9**	**835.1**
1	Paracetamol	2404	15,899	338	2235	**271.4**	**1795.2**
50	Potassium chloride	2598	34,853	1499	20,107	**285.5**	**3830.0**
45	Chloramphenicol	2500	7735	1500	4641	**285.7**	**884.0**
27	Cupric sulfate	469	1880	502	2012	**290.6**	**1163.6**
44	Diphenylhydantoin	1635	6480	150	595	**300.0**	**1189.1**
46	Sodium oxalate	11160	83,284	5095	38,019	**357.1**	**2665.3**
2	Acetylsalicylic acid	200	1110	232	1287	**385.7**	**2140.5**
15	2,4-Dichlorophenoxy-acetic	375	1697	347	1570	**385.8**	**1745.3**
3	Ferrous sulfate	319	2100	680	4477	**392.1**	**2581.0**
14	Malathion	290	878	190	575	**742.8**	**2248.4**
16	Xylene	4299	40,490	2119	19,953	**899.8**	**8474.6**
33	Chloroform	908	7605	36	302	**999.8**	**8375.2**
19	Lithium sulfate	492	4478	1190	10,828	**1065.5**	**9691.8**
34	Carbon tetrachloride	2350	15,280	8264	53,726	**1314.4**	**8545.4**
36	Dichloromethane	1601	18,846	873	10,280	**1386.2**	**16,321.7**
8	Methanol	5619	175,327	7289	227,414	**1569.0**	**48,954.2**
7	Ethylene glycol	4698	75,684	5498	88,567	**1570.9**	**25,304.8**
12	Sodium chloride	3002	51,370	4003	68,493	**2287.3**	**39,138.9**
9	Ethanol	7057	153,145	3448	74,837	**4712.2**	**102,262.2**
10	1,1,1-Trichloroethane	11196	83,927	7989	59,884	**5707.6**	**42,785.8**

Source: E. Walum. 1998. Acute oral toxicity. EHP 106:497-503. (reprinted with permission from the editor)

Toxicity Categories

Category	Signal Word	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)	Inhalation LD_{50} (mg/L)[2]	Oral Lethal Dose	Eye Irritation	Skin Irritation
I - Highly Toxic	DANGER, POISON (skull & crossbones), WARNING	0 to 50	0 to 200	0 to 0.05	A few drops to a teaspoonful	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Corrosive (tissue destruction into the dermis and/or scarring)
II - Moderately Toxic	CAUTION	>50 to 500	>200 to 2,000	> 0.05 to 0.5	Over a teaspoonful to one ounce	Corneal involvement or irritation clearing in 8-21 days	Severe irritation at 72 hours (severe erythema or edema)
III - Slightly Toxic	CAUTION	>500 to 5,000	>2,000 to 20,000	>0.5 to 2	Over one ounce to one pint	Corneal involvement or irritation clearing in 7 days or less	Moderate irritation at 72 hours (moderate erythema)
IV - Relatively Non-toxic	none	>5,000	>20,000	> 2	Over one pint to one pound	Moderate irritation at 72 hours (moderate erythema)	Mild or slight irritation at 72 hours (no irritation or slight erythema)

1 EPA/OPP does not currently use the inhalation toxicity values in 40 CFR 150.10(h). Instead, OPP uses values that are from a 2/1/94 Health Effects Division paper entitiled "Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies".

[2] Four hour exposure.

Sources:
(1) U.S. EPA, Office of Pesticide Programs. Label Review Manual. Chapter 8: Precautionary Labeling. http://www.epa.gov/oppfead1/labeling/lrm/chap-0.8.htm.
(2) National Ag Safety Database. Toxicity of Pesticides. http://www.cdc.gov/niosh/nasd/docs2/as18700.html.
(3) 40 CFR 156.10(h) – Labeling Requirements for Pesticides and Devices. Warnings and precautionary statements.

Appendix IV: Oral Acute Single Lethal Doses in Humans

Table II: Oral acute single lethal doses in humans

No.	Chemical	LD/ MLD	Dose values (g) Reference numbers 10	11	12	13	14	15	16	17	18	19	Other references	Mean doses
1.	Paracetamol	LD	19											19
		MLD	10	10	>10	17.5	22.5	>10	17.5			10		15
2.	Acetylsalicylic acid	LD	33.6	17.5	30			25						27
		MLD	35	17.5			20							22
3A.	Fe^{2+} in iron (II) sulphate	LD	16.8	17.5			6	15.7	11.5	7.7	23.2			14
		MLD			2.1	1.5	1.5				4.28			2.3
3B.	Iron (II) sulphate	LD												38
4.	Diazepam	LD												E5*
		MLD						>2				2		2
5.	Amitriptyline hydrochloride	LD	5					1	1.75					2.6
		MLD			>2.1	2	2							2
6.	Digoxin	LD				0.005		0.015	0.0075					0.0092
		MLD			0.001									0.0011
7.	Ethylene glycol	LD		111	100			111		111	111			110
		MLD	111						111			111		110
8.	Methanol	LD		70	123		67	150	123	119				110
		MLD	23.8	23.8							17.5	59		31
9.	Ethanol	LD	455	280	276			455	455					330
		MLD											98 (9, 59)	98
10.	Isopropanol	LD	132	188	196		196	188	157	188				180
		MLD										196		196
11.	1,1,1-Trichloroethane	LD											193 (60), 802 (61)	500
		MLD		>42	>6.7									24
12.	Phenol	LD	20	4.5							8.8			11
		MLD	4.8		2	11.5						8		6.6
13.	Sodium chloride	LD	140	140									210[b]	160
		MLD												nr

Source: Ekwall et al. 1998. MEIC Evaluation of Acute Systemic Toxicity. Part V. ATLA 26:571-616. (reprinted with permission from the editor)

14.	Sodium fluoride	LD	7.5			5	5	7.5	7.5		4.5			6.2
		MLD		4.6	1.2		2	1		7.5		1		2.9
15.	Malathion	LD	60					17.5		60			70 (62)	52
		MLD											25 (9, 59)[c]	25
16.	2,4-Dichloro-	LD	28[d]					24.1		28				27[e]
	phenoxyacetic acid	MLD	5.6		6.5								5.6 (9)[c]	5.9
17.	Xylene	LD			245			19.4	53					63[e]
		MLD	120							12.9		21.5		51
18.	Nicotine	LD	0.060			0.040		0.060	0.045	0.05	0.05	0.045		0.05
		MLD	0.050	0.045		0.045		0.005						0.036
19.	Potassium cyanide	LD	0.25	0.20	0.14			0.20			0.25			0.21
		MLD								0.20		0.2		0.20
20A.	Lithium	LD						9.4[f]						9.4
		MLD												nr
20B.	Lithium sulphate	LD								42[g]				58
21.	Theophylline	LD								11[g]			11 (63)	11
		MLD							4			7	5.2 (9, 59)[c]	5.4
22.	Dextropropoxyphene	LD							0.78[h]		0.64			0.71
	hydrochloride	MLD	1.1		0.5	0.75		1.28		0.65				0.86
23.	Propranolol	LD				E9.6		E5.1	4		1.2			5
	hydrochloride	MLD			>1									1
24.	Phenobarbital	LD	8	8			8	7.5	7.5					7.8
		MLD			1.5					6	5	5		4.8
25.	Paraquat	LD	4.5	2.1					3.1	1.5		1.5		2.5
		MLD			0.28							0.075		0.18
26.	Arsenic trioxide	LD						0.25	0.33				0.3[h]	0.29
		MLD	0.21	0.23	0.12				0.2	0.2		0.1		0.18
27.	Copper (II) sulphate	LD						15	15	15	10			14
		MLD										15	3.5 (59)[c]	9.3
28.	Mercury (II) chloride	LD		2.1	1			2.5		1	1			1.5
		MLD		0.5				0.5				0.5		0.5
29.	Thioridazine	LD	4.8						3.5					4.2
	hydrochloride	MLD							>3					3
30.	Thallium sulphate	LD	1	0.85	1			1	1	1	1			0.98
		MLD		0.56								0.8		0.68

Table II: continued

No.	Chemical	LD/ MLD	Dose values (g): Reference numbers 10	11	12	13	14	15	16	17	18	19	Other references	Mean doses
31.	Warfarin	LD							7.5[i]	7.5[i]				7.5
		MLD												nr
32.	Lindane	LD	15					8.75		28				17
		MLD			3.5									3.5
33.	Chloroform	LD						44			96			70
		MLD	44	14.8	14.8					14.8		22		22
34.	Carbon tetrachloride	LD	151					32.8[e,f]						92
		MLD	12		6.4			3.2	6.4			6.4		6.9
35.	Isoniazid	LD	12.5					14	8	12.5[f]	14			12
		MLD	8	8		8		8				10		8.4
36.	Dichloromethane	LD			33.2				146[e]			110[e]		97
		MLD									24			24
37.	Barium nitrate	LD		2	2					3.9[e]				2.6
		MLD										1		1
38.	Hexachlorophene	LD			5					17.5	21			15
		MLD										2.25		2.3
39.	Pentachlorophenol	LD								2			2 (62)	2
		MLD	2		1									1.5
40.	Verapamil hydrochloride	LD							8.6[f]					8.6
		MLD		3					>3	3.8[g]				3.4
41.	Chloroquine phosphate	LD	2.5			7.2	8		5.6		6.4			5.9
		MLD											2.2 (9)[c]	2.2
42.	Orphenadrine hydrochloride	LD	2.8	2.8	5.5[d]	2.8			2.8					3.3
		MLD								1				1
43.	Quinidine sulphate	LD				11.5			8		11.5			10
		MLD	4						>6	8[g]	2			5

44.	Diphenylhydantoin	LD			E21									21
		MLD	E7.5	9.1					8.5[j]			2		6.8
45.	Chloramphenicol	LD	20							20				20
		MLD											10 (62), 28 (9)[e]	19
46.	Sodium oxalate	LD		30	15					23				23
		MLD									15	5	5 (64)	8.3
47.	Amphetamine sulphate	LD							0.5		1.4[d]			0.95
		MLD		0.1	0.1			0.15	0.12			0.25		0.14
48.	Caffeine	LD	7.5		15	7.5	7.5	10	12					9.9
		MLD	E6.5	10							10	10		9.1
49.	Atropine sulphate	LD	E0.10[k]		E0.1[k]	E0.2[k]					0.1[h]	0.075		0.12[k]
		MLD						0.10	0.2	0.050				0.12
50.	Potassium chloride	LD							E45		16.2		24 (65)	28
		MLD								18[h]				18

[a]*high variability as well as tolerance makes it difficult to establish human LD.*
[b]*POISINDEX™, Information Systems (ed. B.H. Rumack & D.G. Spoerke), Micromedex (Denver, CO, USA).*
[c]*Low LD.*
[d]*Extrapolated from animal dosage.*
[e]*Geometric mean value, when the quotient between original values (range) is larger than ten.*
[f]*Two lethal poisonings.*
[g]*One survivor and one dead.*
[h]*One death.*
[i]*12.5mg/kg lethal in 14 days (16),1g lethal in 13 days (17).*
[j]*Several survivors.*
[k]*Very variable.*
LD = mean lethal dose; MLD = minimal lethal dose; E = extrapolated; nr = not reported.

Appendix V: Clinically Measured Acute Lethal Serum Concentrations in Humans

Table III: Clinically measured acute lethal serum concentrations in humans

No.	Chemical	LC/MLC	Concentrations (mg/l) References numbers 10	11	12	13	14	15	16	17	18	19	Other references	Mean concentration (mg/ml)
1.	Paracetamol	LC	300[a]	300[a]		300[a]						400		330
		MLC		160[a]	300[a]		300[a]							250[a]
2.	Salicylic acid	LC	1300[b]									600		950
		MLC		1000	900[b]	800[b]	1000							930
3.	Iron	LC										8		8
		MLC	10[c]	10[c]	5			5	8.1[c]					7.6
4.	Diazepam	LC										20		20
		MLC	15	20						20				18
5.	Amitriptyline	LC		5								10		7.5
		MLC								2.5[d]				2.5
6.	Digoxin	LC								0.027		0.01		0.018
		MLC	0.015						0.003		0.003			0.007
7.	Ethylene glycol	LC	4370							4370		2000		3600
		MLC												nr
8.	Methanol	LC	1750	1000						1600	1800	800		1400
		MLC						500		400				450
9.	Ethanol	LC		5000			4000	5000				4500		4600
		MLC	5000		5000				5000		3700			4600
10.	Isopropanol	LC	3400	2000								3000		2800
		MLC						1500	2000					1800
11.	1,1,1-Trichloro-ethane	LC											180 (26)[e]	E180
		MLC												nr
12.	Phenol	LC												nr
		MLC	42						50					46[f]

Source: Ekwall et al. 1998. MEIC Evaluation of Acute Systemic Toxicity. Part V. ATLA 26:571-616. (reprinted with permission from the editor)

13.	Sodium in	LC												nr
	sodium chloride	MLC	10800	10800			10800							11000[f]
14.	Fluoride	LC			3				14.2	14[d,g]		3		8.6
		MLC												nr
15.	Malathion	LC											4.4 (26)[e]	E4.4
		MLC								0.35[d,g]				E0.35
16.	2,4-Dichlorophenoxy	LC	416							600[d]				510
	acetic acid	MLC												nr
17.	Xylene	LC							E50				43 (66)	47
		MLC											11[h,i]	11
18.	Nicotine	LC										5		5
		MLC		10				10						10[f]
19.	Cyanide	LC											5	5
		MLC	2.5					3		3.1[d]				2.9
20.	Lithium	LC						69[j]		77[e]				72[k]
		MLC		24		24								24[k]
21.	Theophylline	LC		183[l]					135	130[m]	150			150
		MLC	100[n]				100		64			50		79
22.	Dextropropoxy-	LC	6									10		8
	phene	MLC			2				2	1.8[d]				1.9
23.	Propranolol	LC	16	3.3[h]					3.3[h]	3[e]				6.4
		MLC		4.7			4[d]							3.9
24.	Phenobarbital	LC	115							117	110	200		136
		MLC		80					120					100
25.	Paraquat	LC	2											2[f]
		MLC		0.2						0.2	0.1			0.17
26.	Arsenic	LC			3.9[d]						1.5	2[e]		2.5
		MLC												nr
27.	Copper	LC									4.5	6		5.5
		MLC												nr

Table III: continued

No.	Chemical	LC/ MLC	Concentrations (mg/l): References numbers 10	11	12	13	14	15	16	17	18	19	Other references	Mean concentration (mg/ml)
28.	Mercury	LC								0.65[e]		2	14.3 (67)[d,g]	2.6[h]
		MLC	0.22		>0.1									0.22
29.	Thioridazine	LC								7.1[e]		20		14
		MLC												nr
30.	Thallium	LC								1.5[d,h]				1.5[h]
		MLC	0.3											0.3
31.	Warfarin	LC											110 (26)[d,g]	E110[e]
		MLC									107[d]			107
32.	Lindane	LC								0.92[e]				E0.92
		MLC							0.5					0.5
33.	Chloroform	LC										400		400
		MLC								165[a]		200[a]		180
34.	Carbon tetrachloride	LC								20[d,g]		1		4.5[h]
		MLC								1.8[d,g]				1.8
35.	Isoniazid	LC								77[e]				E77
		MLC		10										10
36.	Dichloromethane	LC										300[f]		300
		MLC												nr
37.	Barium	LC											97 (26)[d]	E97
		MLC								5.6[d]				5.6
38.	Hexachlorophene	LC	35.6[e]							52[e]				44
		MLC												nr
39.	Pentachlorophenol	LC								74[e]				74
		MLC	40											40

40.	Verapamil	LC	3[d,g]		*			4.1	4[d,g]				3.7
		MLC											nr
41.	Chloroquine	LC	10[d]			8		9	22[d]		4		11
		MLC											nr
42.	Orphenadrine	LC		6					3.6[e]				4.8
		MLC	6										6[f]
43.	Quinidine	LC		16.8[d]					14.6[d]		40		24
		MLC	14		10		9						11
44.	Diphenylhydantoin	LC	95						98		80		91
		MLC	60	50									55
45.	Chloramphenicol	LC							190[n]				E190
		MLC				75[t]		68[t]	68[t]				70
46.	Oxalate	LC							20[n]		20	20 (26)[e]	20
		MLC											nr
47.	Amphetamine	LC								4	2		3
		MLC											nr
48.	Caffeine	LC	150							160[d,g]	150		150
		MLC				150			135[d]				140
49.	Atropine	LC							0.13[d,g]				E0.13[*]
		MLC											nr
50.	Potassium	LC	397						364[d]	352			370
		MLC	293						313				300

[a]After 4 hours. [b]After 6 hours. [c]After 3 hours. [d]As judged from high survived concentrations. [e]SD analysis. [f]This value will substitute for the presented LC value in calculations based on LC values. [g]Based on one case only. [h]Geometrical mean value from a range of values with a quotient larger than ten. [i]TOMES® Information Services (ed. B.H. Rumack & D.G. Spoerke), Micromedex (Denver, CO, USA). [j]Also 69mg/l as judged from high survived concentrations in reference 16. [k]May include acute chronic dosage. [l]Peak concentration. [m]S/D: 90/170 = 130 mg/l (17). [n]Acute dosage. [o]In blood. [p]Represents acute on chronic dosage: no reports on single-dose lethal poisonings. [q]Plane 4 anaesthesia. [r]Value probably originating from forensic medicine data. [s]Reported value of 90mg/l, which seems too high. [t]Grey baby syndrome.

E = estimated/extrapolated; LC = mean lethal serum concentration; MLC = minimal lethal serum concentration; S/D = high survived and lethal concentrations from case reports, with a resulting mean value; nr = not reported.

Appendix VI: Post-Mortem Acute Lethal Concentrations in Humans

Table IV: Post-mortem acute lethal concentrations in humans

No.	Chemical	LC/ MLC	Concentrations (mg/l) Reference numbers 17	20	21	22	23	24	25	Other references	Mean concentration (mg/ml)
1.	Paracetamol	LC	248		250	280[a]			160		230
		MLC		160	160		150	250			180
2.	Salicylic acid	LC	661	500	500	732			700		620
		MLC					450	450			450
3.	Iron	LC	9.0[b]	35							22
		MLC									nr
4.	Diazepam	LC	18							10 (68)	14
		MLC		20	5	50				50 (69)	31
5.	Amitriptyline	LC	3.7	6.32[a]	3.3[c]	5.58[a]			2		4.2
		MLC			0.55[d]		1.5	1.75			1.3
6.	Digoxin	LC	0.025	0.015	0.0103[c]				0.015		0.016
		MLC			0.0015[d]		0.005	0.005			0.0038
7.	Ethylene glycol	LC	2400	3000	2400						2600
		MLC			300						300
8.	Methanol	LC	1900		1900						1900
		MLC		890	200				900		660
9.	Ethanol	LC	5500		4000[c]				5000		4800
		MLC		3500	2250[d]	4000					3300
10.	Isopropanol	LC	1500								1500
		MLC			1000						1000
11.	1,1,1-Trichloroethane	LC	126		80[c]				316[e]		170
		MLC			15[d]						15
12.	Phenol	LC	49	90					90		76
		MLC									nr
13.	Sodium in sodium chloride	LC								13000 (26)[e]	13000
		MLC									nr

Source: Ekwall et al. 1998. MEIC Evaluation of Acute Systemic Toxicity. Part V. ATLA 26:571-616. (reprinted with permission from the editor)

14.	Fluoride	LC	15	2	3				2		5.5
		MLC									nr
15.	Malathion	LC	281								280
		MLC									nr
16.	2,4-Dichlorophenoxy-acetic acid	LC	464			669					570
		MLC									nr
17.	Xylene	LC	43	10.9	13.4[a,b]				10.9		20
		MLC									nr
18.	Nicotine	LC	29	16[a]	25	17.7[a]					22
		MLC			5			13.6			9.3
19.	Cynnide	LC	24.7	3.7		7.6[a]			3.7		9.9[f]
		MLC			5						5[f]
20.	Lithium sulphate	LC	31.9[e]	34					35		34[g]
		MLC			13.9			14			14[g]
21.	Theophylline	LC	150	150					150		150
		MLC			50		50	50			50
22.	Dextropropoxyphene	LC	4.7	4.1[a]	15	7.7[a]					7.9
		MLC			2			1.5			1.8
23.	Propranolol	LC	14	10	9	16			7		11
		MLC			4		7	7			6
24.	Phenobarbital	LC	97		45[f]	210			125		120
		MLC		80	4.3[d]		80	55			35[e]
25.	Paraquat	LC	1.2[a,h]	35	1.2				2		3.2[e]
		MLC									nr
26.	Arsenic	LC	3.3	12	15	2.36[a]					8.2
		MLC									nr
27.	Copper	LC	36	12.5[a]							24
		MLC									nr
28.	Mercury	LC	4.2[i]			0.58					2.4
		MLC							0.6		0.6
29.	Thioridazine	LC	5.1	4.24[a]	5	7				3.3 (27)[c]	4.9
		MLC			2		10	11.5		2.4 (27)[d]	6.5
30.	Thallium	LC	4.0[j]	0.5							2.3
		MLC									nr

Table IV: continued

No.	Chemical	LC/MLC	Concentrations (mg/l): Reference numbers 17	20	21	22	23	24	25	Other references	Mean concentration (mg/ml)
31.	Warfarin	LC								100 (28)	100
		MLC			>10		> 10	> 10	> 11		> 10
32.	Lindane	LC									nr
		MLC	0.02[e,k]								0.02
33.	Chloroform	LC	64	390	30[c]	29			390		97[e]
		MLC			7[d]						7
34.	Carbon tetrachloride	LC	274[b]		260				150		230
		MLC									nr
35.	Isoniazid	LC	117[b]		150[e]						130
		MLC					100	100			100
36.	Dichloromethane	LC	364	280	395[b]	496			280		360
		MLC									nr
37.	Barium	LC									nr
		MLC	1.9[e,j]							< 20[e,l,m]	1.9
38.	Hexachlorophene	LC	35[e]	35					35		35
		MLC									nr
39.	Pentachlorophenol	LC	107		99						100
		MLC		46	46				45		46
40.	Verapamil	LC	11	6.4					6		7.8
		MLC						2.5			2.5
41.	Chloroquine	LC	30.5	17.2[a]	10	11.2[a]			3		14
		MLC			3		4.5	3			3.5
42.	Orphenadrine	LC	20.6	6	9	16.7			6		12
		MLC			4		7	3.6			4.9
43.	Quinidine	LC	45[e]	40	55	40			40		44
		MLC			30		15				23
44.	Diphenylhydantoin	LC	54[e,n]		94				100		83
		MLC		100	70		50	50			68

Appendix VII: Human Kinetic Data

Table V: Human kinetic data[a]

No.	Chemical	Absorption in the gut[b]	Time to peak (ingestion)	Kinetics	T½[c]	Vd l/kg	Passage of blood-brain barrier	Accumulation in vital organs	Blood protein binding	References[d]
1.	Paracetamol	Good	0.5–> 4 hours[e]	First-order[f]	> 12 hours[e]	0.9	Free?	Liver,[f] kidney[f]	20–50%[e]	[g]
2A.	Acetylsalicylic acid	Good		Zero-order	0.27 hours	0.2	Restricted	None	50–90%	
2B.	Salicylic acid	[h]	12–24 hours[e]	Zero-order	27 hours[e]	0.17	Restricted	None	< 80%	30
3.	Iron (II) sulphate	Good[e,i]	2–4 hours[e]	Biphasic	nr	nr[j]	Restricted	Blood, liver	100%	16, 30
4.	Diazepam	Complete	1–3 hours	Biphasic	96 hours[e]	1.1	Free	CNS, liver,[f] kidney[k]	99%	
5.	Amitriptyline hydrochloride	Good[l]	20 hours[e]	Biphasic	8 and 27 hours[e]	15	Free	Liver,[f] kidney, lung, heart, CNS[k]	95%	
6.	Digoxin	Moderate	2–5 hours[e]	Biphasic	48 hours[e]	6	Restricted	Heart, kidney, liver[f,m]	29%	
7.	Ethylene glycol	Complete	1–4 hours	First-order?	8.4 hours[e]	0.65	Free	Liver,[f] kidney[f]	None	
8.	Methanol	Good	0.5–1.5 hours	Zero-order	27 hours[e]	0.65	Free	Kidney, liver[f]	None	[g]
9.	Ethanol	Good	0.5–> 3 hours[e]	Zero-order	4 hours[e]	0.6	Free	None	None	
10.	Isopropanol	Complete	1 hour	First-order	5.4 hours[e]	0.6	Free	None	nr	31
11.	1,1,1-Trichloroethane	Complete	1 hour?	Triphasic	0.7, 6 and 53 hours	> 1[e]	Free	CNS[k]	30–70%	32, 33 [g]
12.	Phenol	Complete	E0.5 hours[e]	Biphasic?	2.8 hours	nr	Free	CNS	30–70%?	34, 35
13.	Sodium chloride	Complete	5 hours[e]	Zero-order	nr	0.64	Restricted	None	None	
14.	Sodium fluoride	Complete	> 1 hours[e]	Biphasic	5.5 hours	0.6	Restricted	None (bone only)	None	36
15.	Malathion	Good	1–5 hours[e]	Multiphasic	nr[n]	nr[n]	Free	Kidney, liver,[f] CNS[m]	nr[n]	37–39
16.	2,4-Dichlorophenoxy-acetic acid	Complete	7–24 hours[e]	First-order	58 hours[e,p]	0.2[e]	Restricted	Liver, kidney	High	
17.	Xylene	Good	1.5 hours	Biphasic	1 and 25 hours	nr[j]	Free	Lipid-rich organs[k]	High	15
18.	Nicotine	Complete[l]	> 0.5 hours?	Biphasic	10 minutes and 2.2 hours	2	Free	CNS, liver,[f] kidney[f,k]	High	
19.	Potassium cyanide	Complete	< 1 hour[e]	Biphasic	1 and 6–66 hours[e]	1	Free	Erythrocytes[k]	5%	15
20.	Lithium sulphate	Complete	nr	Biphasic	3–12 and 8–65 hours[e]	0.9	Restricted	Liver, kidney[k]	None	16

Source: Ekwall et al. 1998. MEIC Evaluation of Acute Systemic Toxicity. Part V. ATLA 26:571-616. (reprinted with permission from the editor)

21.	Theophylline	Complete	2-8 hours*	Biphasic*	17 minutes and 6 hours*	0.5	Free	None	56%	63
22.	Dextropropoxyphene hydrochloride	Complete[j]	1-2 hours	Biphasic*	5 and 15 hours*	16	Free	CNS, liver,[f] lung, kidney[h]	78%	*
23.	Propranolol hydrochloride	Complete[j]	1-2 hours	Biphasic?	3.9 and 16 hours*?	4.3	Free	CNS, liver,[f] kidney[h]	80-95%	26
24.	Phenobarbital	Complete	nr	First-order	100 hours*	0.6	Free	Liver[f]	50%	16, 30
25.	Paraquat	Moderate*	< 4 hours*	Biphasic	5 and 84 hours*	1.4*	Free?	Lung, liver, kidney	nr	40
26.	Arsenic trioxide	Good	1 hour	Biphasic*[j]	1-2 and 30 hours*	0.2?	Restricted	Liver, kidney, heart, GIT[m]	nr	
27.	Copper (II) sulphate	Poor	nr	Biphasic	2-3 hours and 26 days*	2	Restricted	Blood, liver[f]	95%	16, 41
28.	Mercury (II) chloride	Moderate[j]	nr	Biphasic	2 and 24-50 days*	> 1	Restricted	Blood, kidney, liver, heart	nr*	67*
29.	Thioridazine hydrochloride	Good[j]	2-4 hours	Multiphasic	26 hours	18	Free	CNS, lung, liver,[f] kidney[h]	96-99%	16, 30, 42
30.	Thallium sulphate	Good	2-4 hours	Biphasic*	48 and 96 hours?*	4.6	Restricted	Kidney, heart, liver, CNS[m]	None	16, 30, 43
31.	Warfarin	Good	3-9 hours	First-order	22-96 hours*	0.11*	Restricted	none	99%	15, 16, 44
32.	Lindane	Good[k]	6 hours	Biphasic*	21 hours and 10 days?*	nr[j]	Free	CNS, liver, kidney, (fat)	nr	16, 26
33.	Chloroform	Complete	1 hour	First-order?	1.5 hours	2.6	Free	CNS, liver, kidney, (fat)[h]	nr	16
34.	Carbon tetrachloride	Good	nr	Biphasic*	11 and 43 hours*	nr[j]	Free	Liver,[f] kidney,[f] (fat)	nr	16, 45
35.	Isoniazid	Complete	1.5-3 hours*	First-order	2.4 and 5 hours*[j]	0.6	Free	Liver,[f] kidney, lung, skin	10%	16
36.	Dichloromethane	Complete	2 hours	First-order?	40 minutes	0.6?	Free	None	nr	16
37.	Barium nitrate	Good	> 2 hours*	Triphasic Biphasic?*	3.6, 34 and 1033 days**	nr	Restricted	Muscle, lung, (bone)	54%	15, 26
38.	Hexachlorophene	Good	3-6 hours?	First-order?	24 hours*	nr	Restricted	Liver,[f] kidney	92%*	46, 47
39.	Pentachlorophenol	Good	4 hours	First-order	13 hours to 16 days	0.35*	Restricted	Liver,[f] kidney	> 96%	16, 48
40.	Verapamil hydrochloride	Good[j]	2 hours	Biphasic	23 minutes and 5 hours	5	Restricted?	Liver[f, h]	90%	

Table V: continued

No.	Chemical	Absorption in the gut[b]	Time to peak (ingestion)	Kinetics	T½[c]	Vd l/kg	Passage of blood-brain barrier	Accumulation in vital organs	Blood protein binding	References[d]
41.	Chloroquine phosphate	Good	1-3 hours*	Triphasic	2, 7 and 45 days**	94	Free	Heart, liver, kidney, lung, erythrocytes[k]	55-61%	16, 49
42.	Orphenadrine hydrochloride	Good[l]	3 hours	First-order Biphasic?*	15 hours 6 and 15 hours*	6	Free	CNS, liver, lung[k]	20-95%	16, 50, 51
43.	Quinidine sulphate	Good[l]	> 2 hours*	First-order?	> 7.8 hours*	2.7*	Restricted	Liver,[f] kidney, heart[k]	60-90%	15, 16
44.	Diphenyl-hydantoin	Poor/good	30-120 hours*	Zero-order and first-order*	24-230 hours**	0.6*	Free	Liver,[f] kidney, CNS	60%*	52
45.	Chloramphenicol	Good	2-3 hours	First-order	2.5 hours	1.2	Free	Liver,[f] kidney	55%	
46.	Sodium oxalate	Poor	6 hours?	First-order?	4 hours?*	E0.4*	Restricted	Kidney, liver	nr	26, 64
47.	Amphetamine sulphate	Complete	1-4 hours*	First-order?	7-34 hours[p]	3-6.1	Free	Liver, kidney	16%	15, 16
48.	Caffeine	Complete	1 hour	First-order?	9-16 hours*	0.6	Free	None (liver 2x)	35-60%	
49.	Atropine sulphate	Good	> 2 hours*	First-order?	3.5 hours	3	Free	Kidney, liver	50%	53, 54
50.	Potassium chloride	Complete	0.5 hours	Multiphasic	nr	nr	Free?	None	None	65[s]

[a]Data for the overdose situation are indicated by an asterisk. [b]Absorption: complete = 100% and rapid, good = 80%, moderate = 20-80%, and poor = 0-20%. [c]One value indicates T½ of the elimination phase. Successive values represent separate phases (alpha, beta, etc.). [d]Other than references 10, 11, 13, 14 and 17. [e]Non-linear in overdose? [f]Also a biotransforming organ. [g]POISINDEX®, Information Systems (ed. B.H. Rumack & D.G. Spoerke), Micromedex (Denver, CO, USA). [h]Absorbed as acetylsalicylic acid. [i]Due to corrosivity. [j]Probably large, i.e. around 5l/kg; [k]Early accumulation. [l]Documented first therapeutic doses, i.e. bioavailability is decreased by rapid binding in the liver of a large fraction of the absorbed dose (25-85%). For most such chemicals, passage of the intestinal mucosa is probably complete. However, the term "good" is often used in this table, based on literature reports on the total absorption (the sum of intestinal passage and first pass reduction of bioavailability). [m]Slow accumulation. [n]Alpha phase: 2.9 hours. [o]Probably large Vd and protein binding. [p]pH-dependent. [q]Dependent on formulation. [r]Biphasic up to 160 hours. [s]TOMES®, Information Systems (ed. B.H. Rumack & D.G. Spoerke), Micromedex (Denver, CO, USA). [t]Varies between rapid and slow acetylators. [u]Alpha-phase: 3 hours in overdose. [v]Dose-dependent.*

nr = non reported; CNS = central nervous system (brain); GIT = gastrointestinal tract (gut); T½ = plasma half-life; Vd = distribution volume.

Appendix VIII: Peaks from Approximate 50% Lethal Concentration (LC50) Curves

Table VI: Peaks from approximate 50% lethal concentration (LC50) curves[a]

No. Chemical	Time to peak (hours)	Peak conc. mg/l	Type of curve	Case reports: Sub-lethal	Case reports: Lethal (clinical)	Case reports: Lethal (post-mortem)	Case reports: Total
1. Paracetamol	4	358	LC50	81	62	0	143
2. Salicylic acid	20	1070	LC50	31	46	1	78
3. Iron	4	43.5	LC50	15	12	0	27
4. Diazepam	2	19.9	LC100	4	0	0	4
5. Amitriptyline	6	1.69	LC50	8	6	10	24
6. Digoxin	3	0.071	LC50	15	9	1	25
7. Ethylene glycol	2.5	1550	LC50	28	12	9	49
8. Methanol	2	3790	LC50	76	37	7	120
9. Ethanol	1	8440	LC50	20	1	143	164
10. Isopropanol	1	4960	LC50	13	2	2	17
11. 1,1,1-Trichloro-ethane	1	231	LC50	3	0	2	5
12. Phenol	0.5	80	LC50	3	0	4	7
13. Sodium in sodium chloride	5	11700	LC50	3	9	1	13
14. Fluoride	3	19.4	LC0	3	3	7	13
15. Malathion	5	1.88	LC0	2	1	11	14
16. 2,4-Dichloro-phenoxyacetic acid	14	1125	LC50	7	1	4	12
17. Xylene	1	110	LC0	3	0	1	4
18. Nicotine	0.5	13.5	LC0	1	1	3	5
19. Cyanide	0.5	16.4	LC50	12	9	10	31
20. Lithium	3	97.2	LC100	4[b]	0	0	4[b]
21. Theophylline	12	180	LC50	57	18	1	76
22. Dextropropoxy-phene	2	8	LC0	2	1	6	9
23. Propranolol	4	3.11	LC50	6	2	1	9
24. Phenobarbital	15	230	LC50	20	1	0	21
25. Paraquat	2.5	12.6	LC50	23	66	16	105
26. Arsenic	4	1.65	LC50	10	8	3	21
27. Copper	11	15.9	LC50	10	5	1	16
28. Mercury	12	40.1	LC50	12	2	4	18
29. Thioridazine	4	4.08	LC50	1	1	4	6
30. Thallium	24	7.35	LC50	25	5	2	32

[a]*From reference 26.*
[b]*Documented single-dose cases (not overdose on previous medication).*

Source: Ekwall et al. 1998. MEIC Evaluation of Acute Systemic Toxicity. Part V. ATLA 26:571-616. (reprinted with permission from the editor)

Table VI: continued

No. Chemical	Time to peak (hours)	Peak conc. mg/l	Type of curve	Case reports Sub-lethal	Lethal (clinical)	Lethal (post-mortem)	Total
31. Warfarin	6	200	LC0	3	0	0	3
32. Lindane	6	1.3	LC0	5	2	1	8
33. Chloroform	2	490	LC50	2	0	5	7
34. Carbon tetrachloride	6	5.8	LC50	5	1	1	7
35. Isoniazid	3	167	LC50	24	3	4	31
36. Dichloromethane	3	344	LC0	0	0	9	9
37. Barium	2	305	LC100	9	0	0	9
38. Hexachlorophene	5	116	LC50	2	1	1	4
39. Pentachlorophenol	10	79.1	LC50	1	0	3	4
40. Verapamil	2	13.2	LC50	10	9	4	23
41. Chloroquine	2	9.41	LC50	4	1	9	14
42. Orphenadrine	2	11.3	LC50	6	1	8	15
43. Quinidine	6	26	LC50	4	2	0	6
44. Diphenylhydantoin	34	202	LC50	13	1	0	14
45. Chloramphenicol	6	180	LC0	5	4	0	9
46. Oxalate	6	110	LC0	1	1	0	2
47. Amphetamine	2	15.5	LC50	1	1	5	7
48. Caffeine	3	179	LC50	6	0	4	10
49. Atropine	3	4.05	LC100	2	0	0	2
50. Potassium	1	375	LC0	4	3	1	8

[b]*Documented single-dose cases (not overdose on previous medication).*

a few organs are routinely screened for chemicals, such as blood, heart, liver, kidney, brain and lung. Thus, the information on body distribution is often limited to these organs.

The qualitative human toxicity data

The human toxicity data presented in Table IX are the result of a study of references 10–17, in a few instances supplemented by data from other sources. In the same way as the kinetic data in Table V, the toxicity data represent the sum of the information from all the handbooks consulted. The classification of lethal symptoms into main causes and other causes of death, as well as the classification of lethal action into known, unknown and hypothetical mechanisms, represent judgements by the handbook authors. However, the lists of lethal symptoms in various handbooks have been extensively edited to provide uniform terminology. The handbook authors have used a plethora of terms for essentially the same type of event. To mention only one example, circulatory failure in Table IX stands for vascular collapse, vasomotor collapse, shock, circulatory shock, hypovolaemic shock, hypotensive shock, and so on.

Potentially the most controversial data in Table IX are those that are based on mecha-

Appendix IX: Human Acute, Single-Dose Toxicity Data

Table IX: Human acute, single-dose toxicity data

No.	Chemical	Lethal symptoms[a]	Mean time to death	Danger over	Target organs	Toxic metabolites[b]	Lethal mechanisms	References[c]
1.	Paracetamol	Hypoglycemic coma Liver failure M Kidney failure	3–5 days	nr	Liver P Kidney P (CNS)	More toxic intracellular metabolites	Known: Covalent NAPQI binding and lipid peroxidation	
2.	Acetylsalicylic acid	Metabolic acidosis M Cerebral bleedings Pulmonary oedema Cardiovascular failure	48 hours	nr	Kidney P Liver P CNS P Lung P GIT P	Salicylic acid is the reactive metabolite of the parent compound	Known: General cell poison. Uncoupling of oxidative phosphorylation, inhibition of Kreb's cycle dehydrogenases	
3.	Iron (II) sulphate	Haematemesis GIT perforation Pulmonary oedema CNS excitation/depression Circulatory failure Liver and kidney failure	6 or 48 hours	72 hours	GIT P Liver P Kidney CNS CVS Lung P	tp	Known: General cell poison. Inhibition of oxidative phosporylation and ATP; lipid peroxidation	
4.	Diazepam	CNS depression M	2 hours	3 hours	CNS	(Nordiazepam)	Unknown	
5.	Amitriptyline hydrochloride	CNS excitation/ depression Heart arrythmias/arrest M	< 12 hours	6 days	CNS Heart	(Nortriptyline)	Hypothetical: Blocks noradrenaline, 5-HT and dopamine presynaptic uptake; prevents reuptake of heart noradrenaline	
6.	Digoxin	Heart arrythmias/ arrest M Hyperkalaemia	7 hours	20 hours	Heart GIT CNS	(Metabolites)	Known: Impairing ion transport and increasing sarcoplasmic Ca by binding to Na/K ATPase, increasing automaticity of cells	

Source: Ekwall et al. 1998. MEIC Evaluation of Acute Systemic Toxicity. Part V. ATLA 26:571-616. (reprinted with permission from the editor)

7.	Ethylene glycol	1–12 hours: CNS excitation/depression M 12–24 hours: heart failure 24–72 hours: kidney failure	17 hours	72 hours	CNS Heart P Kidney P	Glyoxalate Glycolate Oxalate	Hypothetical: Metabolites inhibit mitochondria, leading to metabolic acidosis. Oxalate decreases S-Ca	
8.	Methanol	CNS depression M Metabolic acidosis Cardiovascular failure	32 hours[d] 173 hours[f]	nr	CNS P[e] Pancreas P Liver P Kidney P Heart P	Formaldehyde Formic acid	Hypothetical: Accumulation of formic acid leads to metabolic acidosis. Lactate inhibits mitochondrial respiration	
9.	Ethanol	CNS depression M Cardiovascular failure	6 hours[d]	12 hours	CNS CVS	(Acetaldehyde)	Hypothetical: Interference with cell membrane fluidity, pertubing proteins, such as ion channels. Depression of postsynaptic potentials in CNS	
10.	Isopropanol	CNS depression M Cardiovascular failure Pneumonia	3 hours	48 hours	CNS CVS Lung P	tp	Unknown	60[g]
11.	1,1,1-Tri-chloroethane	CNS depression M Heart arrythmias Cardiovascular failure Pneumonia	3 hours	4 hours	CNS P CVS Lung P	tp	Unknown	
12.	Phenol	CNS excitation/depression M Heart arrest/pulmonary oedema Liver and kidney failure	1 hour	24 hours	CNS Heart Liver Kidney GIT P	tp	Known: General protoplasmic poison that denaturates proteins	18, 34
13.	Sodium chloride	CNS excitation/depression M Cerebral bleedings Cardiovascular failure Pulmonary oedema Vasculitis	20 hours	25 hours	CNS P Lungs Kidney VS P	tp	Known: Acute dehydration of brain cells caused by osmotic shift of water to the outside of the blood-brain barrier	

Table IX: continued

No.	Chemical	Lethal symptoms[a]	Mean time to death	Danger over	Target organs	Toxic metab-olites[b]	Lethal mechanisms	Refer-ences[c]
14.	Sodium fluoride	Cardiovascular failure CNS excitation/depression	2–4 hours	20 hours	Heart[b] CNS[b] Liver Kidney	tp	Hypothetical: Protoplasmic poison interfering with many enzymes. May lower S-Ca and induce potassium efflux from cells	
15.	Malathion	Early: Cholinergic crisis/ respiratory failure M Later: Heart failure Heart arrythmias/arrest	0.5–6 hours	24 hours	CNS Muscles Heart P	Maloxon	Known: Inhibition of acetylcholine esterase resulting in acetlycholine accumulation in CNS and effector organs	
16.	2,4-Dichloro-phenoxyacetic acid	Hyperthermia/myotonia CNS excitation/depression Metabolic acidosis Heart failure Liver failure	8–96 hours	48 hours	CNS P Liver P Kidney P Heart	tp	Hypothetical: Hypermetabolism due to uncoupling of oxidative phosphorylation. Direct toxin to striated muscle	
17.	Xylene	CNS depression M Heart arrythmias/arrest Heart failure Pulmonary oedema	1–2 hours?	72 hours	CNS P Heart Lung P Liver P	tp	Unknown: Heart failure caused by sensi-tisation of myocardium to endogenous catecholamines?	
18.	Nicotine	CNS excitation/depression M Cardiovascular failure	minutes -1 hour	4 hours	CNS PNS	tp	Known: Cholinergic block causing polarisation of CNS and PNS synapses	
19.	Potassium cyanide	CNS excitation/depression M Metabolic acidosis Circulatory failure	0.5–1 hour	4 hours	CNS P Heart VS	tp	Known: General enzyme inhibition. High affinity for ferric ion. Inhibits cytochrome oxidase and thereby cell respiration	

20.	Lithium sulphate	CNS depression Circulatory failure Kidney failure	1–7 days	7 days	CNS Heart Kidney	tp	Unknown: Partial substitution for normal cations of cells may disturb energy processes?
21.	Theophylline	CNS excitation M Metabolic acidosis Heart arrythmias Electrolyte disturbances GIT bleedings	1–5 days	nr	CNS Heart (GIT)	tp	Unknown: Inhibits prostaglandins and cGMP metabolism. Adenosine receptor antagonist
22.	Dextropropoxyphene hydrochloride	CNS excitation/depression Heart arrythmias/arrest Cardiovascular failure	0.5–2 hours	24 hours	CNS Heart	(Norpropoxyphene)	Hypothetical: Binds to morphine receptors. Stabilises cell membranes. Norpropoxyphene is a primary cardiotoxin
23.	Propranolol hydrochloride	CNS excitation/depression Cardiovascular failure Bronchospasm	0.5–2 hours	4–20 hours	CNS Heart VS	tp?	Unknown: Beta-adrenergic blockade?
24.	Phenobarbital	CNS depression M Circulatory failure	5 hours–7 days	10 days	CNS Heart	tp	Hypothetical: CNS depression through inhibition of GABA synapses? Inhibits hepatic NADH cytochrome oxidoreductase
25.	Paraquat	Early (24 hours): CNS excitation Pulmonary oedema Heart failure Kidney failure M Liver failure Later (48 hours–6 days): Pulmonary fibrosis M	3 hours–4 weeks	nr	Lung P Kidney P Heart P Liver P CNS P	tp	Hypothetical: Multisystem failure due to depletion of superoxide disputase, formation of free-radicals, and lipid peroxidation. Lung fibrosis due to accumulation of paraquat in this oxygen-rich organ

Table IX: continued

No.	Chemical	Lethal symptoms[a]	Mean time to death	Danger over	Target organs	Toxic metabolites[b]	Lethal mechanisms	References[c]
26.	Arsenic trioxide	Gastroenteritis Circulatory failure Heart failure Pulmonary oedema Intravascular haemolysis Kidney failure Liver failure CNS excitation/depression	1 hour–4 days	4 days	Kidney P Heart Liver P VS P CNS P GIT P	tp	Known: Cellular poison. Multisystem failure due to uncoupling of oxidative phosphorylation and inhibition of pyruvate and succinate oxidative pathways	
27.	Copper (II) sulphate	Liver failure Kidney failure Intravascular haemolysis Circulatory failure CNS excitation depression	3 hours–7 days	4 days	Liver P Kidney VS	tp	Hypothetical: Cupric copper is reduced to cuprous form by thiol groups in cell membranes. Superoxide is formed by reoxidation of cuprous copper, which induces lipid peroxidation	18
28.	Mercury (II) chloride	Gastroenteritis Circulatory failure Kidney failure	3 hours–14 days	14 days	Kidney P VS GIT P	tp	Hypothetical: Changes membrane potentials and blocks enzyme reactions in cells by targeting the sulphydryl part of active sites of some enzymes	
29.	Thioridazine hydrochloride	CNS depression Heart arrythmias/arrest M	2–10 hours	nr	CNS Heart	(Mesoridazine?)	Unknown	
30.	Thallium sulphate	Gastroenteritis Cardiovascular failure M Respiratory failure Kidney failure Liver failure CNS excitation depression	24 hours–3 weeks	4–5 weeks	Heart P VS Kidney P Liver P CNS P PNS	tp	Hypothetical: Enzyme inhibition by binding to sulphydryl groups of mitochondrial membranes. Interference with oxidative phosphorylation by inhibition Na/K ATPase	18

31. Warfarin	Bleeding M[i]	36-48 hours	nr	Liver VS	(Metabolites?)	Known: Inhibition of liver synthesis of vitamin K-requiring clotting factors, notably prothrombin. Direct action on capillaries?
32. Lindane	CNS excitation/depression M Pulmonary oedema Metabolic acidosis	1 hour–8 days	8 days	CNS Heart VS P Kidney P Muscle P	tp?	Unknown: CNS depression through inhibition of TBPS binding to the GABA receptor linked chloride channel, leading to blockade of chloride influx into neurons?
33. Chloroform	CNS depression M Heart arrythmias/arrest Liver failure Kidney failure	10 minutes–5 days	5 days	CNS Heart P Liver P Kidney P	More toxic intracellular metabolites?	Hypothetical: Liver and/or kidney injury through covalent binding of toxic metabolites, for example, phosgene, to cell proteins and lipids
34. Carbon tetrachloride	CNS depression[k] Kidney failure[l] Liver failure[l] Heart arrythmias/arrest	24 hours–7 days[l]	7 days	CNS P Heart Kidney P Liver P Pancreas	More toxic intracellular metabolites?	Hypothetical: Covalent binding of toxic intracellular metabolites (see above). Free-radicals inducing lipid peroxidation?
35. Isoniazid	CNS excitation M Metabolic acidosis Circulatory failure CNS depression Liver failure	14 hours–3 days	nr	CNS Liver P	(Intracellular metabolites)	Hypothetical: Interference with metabolism of vitamin B6 reduces GABA and seizure threshold. Conversion of acetylhydrazine (ICM) to alkylating agent
36. Dichloromethane	CNS depression M Heart arrythmias Pulmonary oedema Metabolic acidosis	2 hours	3 hours	CNS Heart	(Carbon monoxide)	Unknown: Carbon monoxide–haemoglobin complex formation?

Table IX: continued

No.	Chemical	Lethal symptoms[a]	Mean time to death	Danger over	Target organs	Toxic metab-olites[b]	Lethal mechanisms	Refer-ences[c]
37.	Barium nitrate	Muscle paralysis/ respiratory failure Heart arrythmias/arrest High blood pressure Convulsions	2–3 hours or 2–3 days	24 hours	Muscle[m] Heart (Kidney)	tp	Hypothetical: Neuromuscular depolarisation. Potassium is forced into cells by an action on Na/K ATPase?	19
38.	Hexachlorophene	Early: Gastroenteritis Hyperthermia Circulatory failure 12–18 hours: CNS excitation/depression 48–60 hours: Heart arrythmias/arrest	4–60 hours	3 days	GIT VS Heart CNS[a]	tp	Hypothetical: Uncoupling of oxidative phosphorylation in cells. Binding to proteins in cytoplasma membrane and cell organelles	47
39.	Pentachloro-phenol	Hyperthermia CNS excitation/depression Circulatory failure Myotonia Metabolic acidosis	4–24 hours	24 hours	Heart P VS CNS Liver P Kidney P	tp	Hypothetical: Uncoupling of oxidative phosphorylation. Protein binding, including selective enzyme inhibition (liver/kidney P450)	
40.	Verapamil hydrochloride	Circulatory failure Heart arrythmias/arrest Metabolic acidosis CNS depression Hypoglycaemia	24 hours	36 hours	VS Heart	(Metabolites)	Known: Inhibition of transmembrane Ca flux in excitatory tissues. Also alpha-adrenergic blocking	
41.	Chloroquine phosphate	Cardiovascular failure Cardiac arrythmias/arrest M CNS excitation/depression Hypokalaemia	1–24 hours	24 hours	Heart VS CNS	tp	Hypothetical: Stabilisation of cell membranes leading to reduction of excitation and conduction in heart. Interference with mitochondria	

42.	Orphenadrine hydrochloride	CNS excitation/depression (max. 2–5 hours) M Heart arrythmias (max. 12–18 hours) Heart failure Liver failure	1–48 hours	24 hours	CNS Heart Liver P	tp?	Unknown
43.	Quinidine sulphate	Early: Heart failure Heart arrythmias/arrest M Later: CNS excitation/depression Kidney failure	6 hours?	nr	Heart VS CNS Kidney	tp?	Unknown: Decreased electrolyte permeability of cell membranes leading to depression of heart excitability, conduction velocity and contractility.
44.	Diphenyl-hydantoin	(Nystagmus/ataxia) CNS excitation/depression M Heart arrythmias/arrest[a]	30 hours–14 days	14 days	CNS (Cerebellum) Heart	tp	Unknown: Binds to specific receptors in neuronal cell membranes. Inhibits voltage-dependent sodium channels
45.	Chloramphenicol	Cardiovascular failure CNS excitation/depression Metabolic acidosis (Liver and kidney failure)	5 hours–2 days	nr	Heart VS CNS Liver Kidney	tp	Hypothetical: Binds to mitochondrial ribosomes and inhibits enzyme synthesis, for example, enzymes necessary for oxidative phosphorylation
46.	Sodium oxalate	Initially (minutes): Gastroenteritis Circulatory failure Later (hours): CNS excitation/depression Heart arrythmias/arrest Later (2 days): Kidney failure	3 hours	nr	GIT CNS[b] Heart[b] Kidney	tp	Hypothetical: Calcium-complexing action, depressing the level of ionized calcium in body fluids. The direct action on GIT, VS and kidney cannot explained that way. Corrosivity is not caused by acidity.
47.	Amphetamine sulphate	(Hyperthermia) Cardiac arrythmias/arrest CNS excitation/depression M Metabolic acidosis	2–4 hours	nr	CNS P[a] Heart P Liver P Kidney	tp	Hypothetical: Release of biogenic amines (dopamine, norepinephrine) from nerve terminal stores. Direct action as false transmitter

Table IX: continued

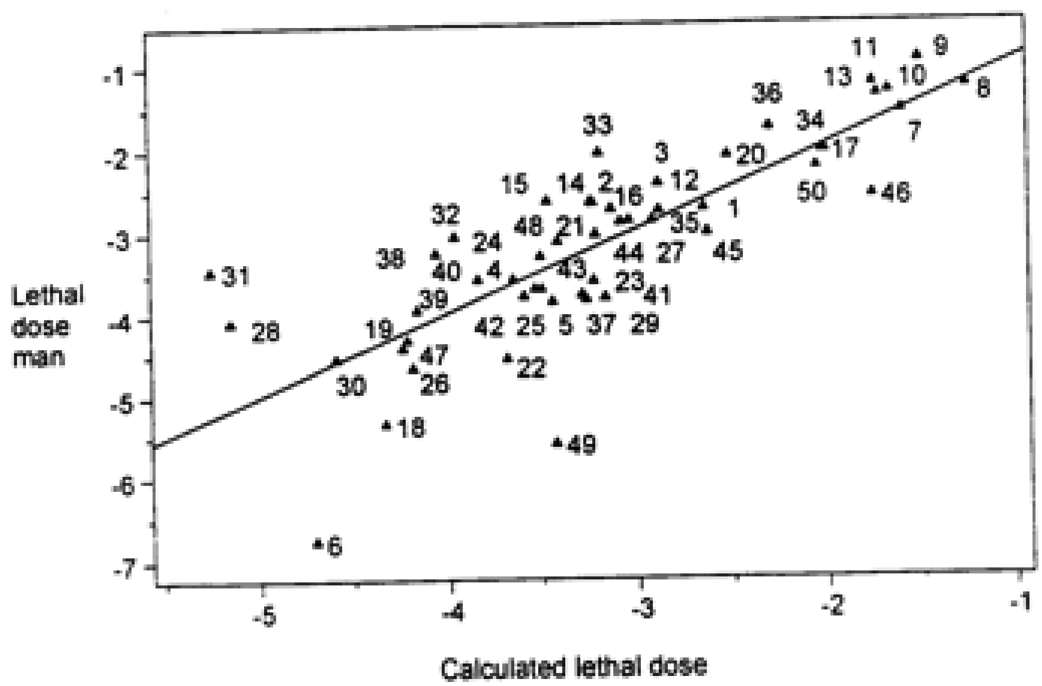
No.	Chemical	Lethal symptoms[a]	Mean time to death	Danger over	Target organs	Toxic metabolites[b]	Lethal mechanisms	References[c]
48.	Caffeine	Initially (3 hours): Heart arrythmias/arrest Pulmonary oedema Later (3 hours–3 days): CNS excitation/depression	3 hours–3 days	nr	Heart CNS	tp	Hypothetical: Inhibition of phosphodiesterase leading to AMP accumulation. Translocation of intracellular calcium? Adenosine receptor antagonism?	
49.	Atropine sulphate	(Psychosis/hyperthermia) CNS excitation/depression Heart arrythmias/arrest M	15 hours	24–48 hours	CNS Heart PNS	tp	Known: Antimuscarinic, anticholinergic action. Competitive antagonism of acetylcholine at cardiac and CNS receptor sites	19
50.	Potassium chloride	CNS excitation/depression Paralysis Heart arrythmias/arrest M	2 hours	nr	Heart CNS (Muscle)	tp	Known: Essential cellular electrolyte maintains normal trans-membrane potential, necessary for heart conduction	

[a]Arranged in order of appearance, when possible. Characteristic but non-lethal symptoms have generally been omitted. CNS excitation stands for seizures, and CNS depression stands for all phases of coma including final respiratory arrest. For chemicals with multisystem failure or a very rapid action, it is difficult to indicate the main cause of death. [b]Metabolites with higher toxicity than the parent compound. Metabolites with the same toxicity as the parent compound are bracketed. TP indicates toxicity from the parent compound, only. [c]Other than references 10–17. [d]Post-mortem cases. [e]Including the eye (blindness). [f]Clinical cases. [g]POISINDEX®, Information Systems (ed. B.H. Rumack & D.G. Spoerke), Micromedex (Denver, CO, USA). [h]Targets of a decreased blood calcium level? [i]TOMES®, Information Systems (ed. B.H. Rumack & D.G. Spoerke), Micromedex (Denver, CO, USA). [j]Cerebral bleeding is most life-threatening. [k]Inhalation. [l]Ingestion. [m]Motor end-plates of muscles. [n]Repeated dermal exposure. [o]Intravenous administration. [p]Vasculitis, haemorrhages.

M = main causes of death; P = histopathological organ lesions; CNS = central nervous system (brain); CVS = cardiovascular system; VS = vascular system (blood vessels/capillaries); GIT = gastrointestinal tract (gut); PNS = peripheral nervous system; tp = toxicity of parent compound only; nr = not reported.

Appendix X: Plot of Acute Lethal Dosage in Humans Against Values Calculated by a PLS Model Based on Rat Oral LD50 and Mouse Oral LD50

Figure 1: Plot of acute lethal dosage in humans against values calculated by a PLS model based on rat oral LD50 and mouse oral LD50.



Source: Ekwall et al. 1999. MEIC Evaluation of Acute Systemic Toxicity. Part VIII.

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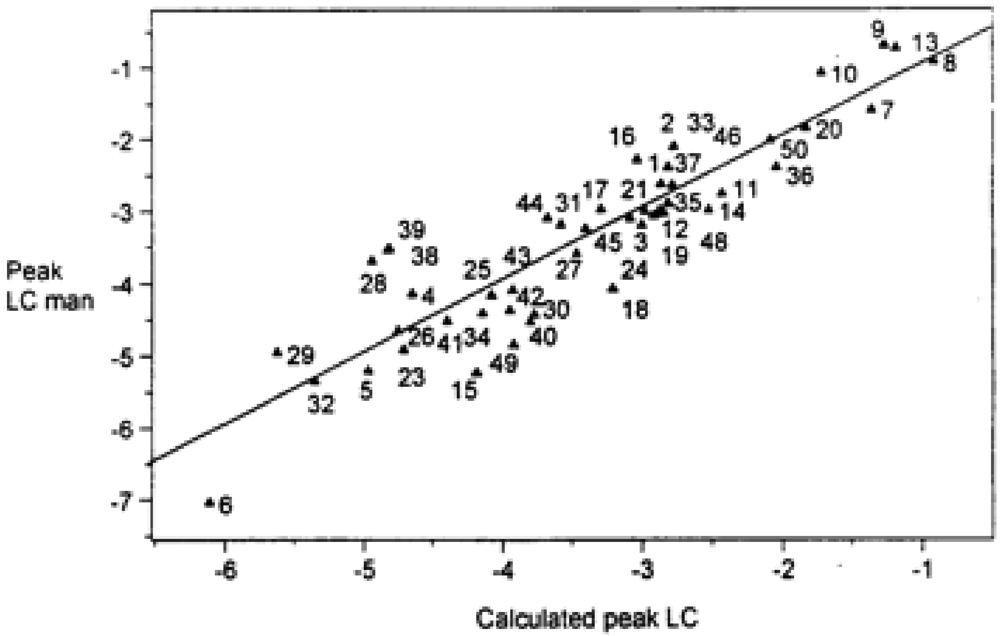
Appendix XI: Plot of Peak Lethal Blood Concentrations in Man Against IC50 Values

Figure 10: Plot of peak lethal blood concentrations in man against IC-50 values calculated by a PLS model based on peak lethal blood concentrations in man, all 50 chemicals, and "blood-brain barrier compensated results" from assays 1, 5, 9 and 16.



Source: Ekwall et al. 1999. MEIC Evaluation of Acute Systemic Toxicity. Part VIII.
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Appendix XII: Priority Areas for Development and Evaluation of New *In Vitro* Tests

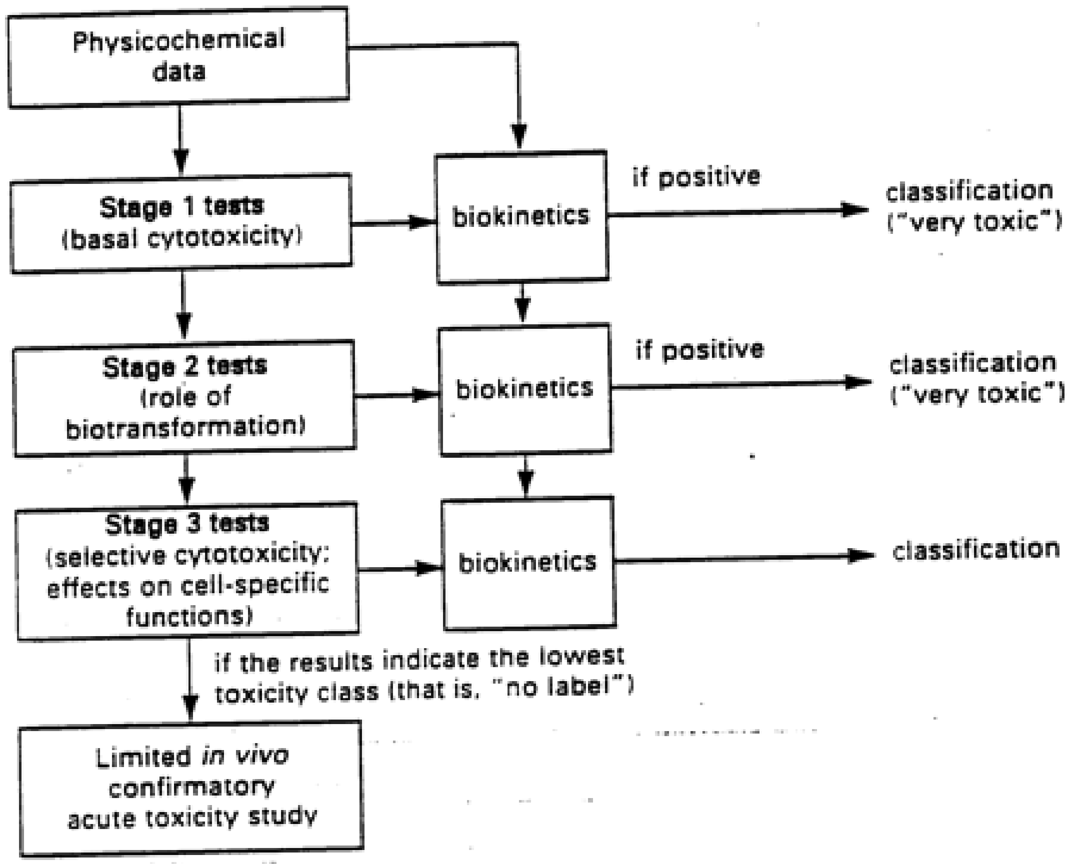
Table I: Priority areas for development and evaluation of new *in vitro* tests on systemic toxicity

No.	Subproject
1.	Repeat dose toxicity
2.	Mechanism studies:
	a) protein denaturation
	b) morphology of injury to cell lines
	c) differential cytotoxicity 30 minutes/24 hours
	d) toxicity to aerobic cells
	e) time-frames for cytotoxic effects
3.	Extracellular receptor toxicity
4.	Excitatory toxicity
5.	Reversibility of cytotoxicity
6.	Passage across blood–brain barrier
7.	Absorption in the gut
8.	Blood protein binding
9.	Distribution volumes (Vd)
10.	More-toxic metabolites

Source: Ekwall et al. 1999. EDIT: A new international multicentre programme to develop and evaluate batteries of *in vitro* tests for acute chronic systemic toxicity. ATLA 27:339-349. (reprinted with permission from the editor)

Appendix XIII: Proposed Testing Scheme for the Classification and Labelling of Chemicals

Figure 1: Proposed testing scheme for the classification and labelling of chemicals according to their potential acute toxicities



Source: Ekwall et al. 1999. EDIT: A new international multicentre programme to develop and evaluate batteries of *in vitro* tests for acute chronic systemic toxicity. ATLA 27:339-349. (reprinted with permission from the editor)