Prediction of Human Lethal Doses and Concentrations of MEIC Chemicals from Rodent LD_{50} Values: An Attempt to Make Some Reparation

John C. Dearden and Mark Hewitt

Abstract
The prediction of human toxicities from animal toxicity tests is often poor, and is now discouraged and in some cases banned, especially those involving the LD_{50} test. However, there is a vast number of historical LD_{50} data in both public and in-house repositories that are being put to little use. This study examined the correlations between human lethality (doses and concentrations) of 36 MEIC chemicals and the median values of a large number of mouse and rat LD_{50} values obtained for four different routes of administration. The best correlations were found with mouse and rat intraperitoneal LD_{50} values (r^2 = 0.838 and 0.810 for human lethal dose, and r^2 = 0.753 and 0.785 for human lethal concentration). The results show that excellent prediction of human lethal dose and concentration can be made, for this series of chemicals at least, by using uncurated rodent LD_{50} values, thus offering some reparation for the millions of rodent lives sacrificed in LD_{50} testing.

Keywords
correlation, human lethal concentration, human lethal dose, MEIC chemicals, mouse and rat LD_{50} values, QAAR

Introduction
Rodent acute toxicity is a standard requirement in the assessment of the potential acute toxicity of chemicals to humans. However, the correlation of rodent toxicity with human lethal dose (HLD) is often low. Many factors can affect rodent toxicity values, such as species, genetic variability, age, sex, weight, health, diet, administration route, time of assessment after administration, ambient temperature, housing conditions (e.g. isolated or aggregated), time of day/night and time of year. Many of these factors also apply to assessments of HLD values, and thus contribute to uncertainty concerning their accuracy.

Furthermore, concern has been expressed for many years about the use of animals in toxicity testing as a proxy for toxicity in humans, nowhere more so than in regard to lethality tests such as the LD_{50} test, which determines the dose of a chemical that will kill 50\% of test animals in a given time. Because of this concern, many efforts have been made to find acceptable substitutes for the rodent LD_{50} test, such as in vitro cytotoxicity assays.

Bernson et al. proposed a ‘Multicentre Evaluation Study of In vitro Cytotoxicity’ (MEIC), based on a set of 50 very diverse organic and inorganic chemicals. The MEIC chemicals, as they came to be known, have been widely studied. Indeed, it is fair to say that the 50 chemicals can be regarded as a gold standard data set. Ekwall et al. examined the human and rodent acute oral toxicities of the first 10 of these chemicals, and found excellent correlations. They did not give correlation statistics, but from their data, the coefficients of determination r^2 were calculated by the present authors to be 0.917 for human versus mouse oral toxicities and 0.836 for human versus rat oral toxicities. A similar study was carried out on the same 10 chemicals; this study did not give HLD or rodent LD_{50} data, but reported that the human versus mouse oral correlation had a lower prediction error (0.68 log units) than had the human versus rat oral correlation (1.04 log units). For 39 MEIC chemicals, Calleja et al. reported prediction errors of...
0.57 log units for the human versus mouse oral LD50 correlation and 0.61 log units for the human versus rat oral LD50 correlation. Similar prediction errors for a slightly smaller set of MEIC chemicals were reported by Calleja et al.\textsuperscript{16}

Weiss and Sawyer\textsuperscript{17} examined human–rodent acute toxicity correlations for the 27 chemicals for which they could find human lethal dosages (HLDs) from the 50 MEIC chemicals. They used mouse (M) and rat (R), oral (or) and intraperitoneal (ip), LD50 values, and gave the Spearman’s Rank coefficients as: Mor = 0.737, Mip = 0.597, Ror = 0.676 and Rip = 0.439, again indicating that the mouse was a better model for the prediction of HLD.

Ekwall et al.\textsuperscript{18} published rodent and human toxicity data for the 50 MEIC chemicals, and Ekwall et al.\textsuperscript{2} used those rodent LD50 values and results from 61 \textit{in vitro} methods to predict HLD values of the 50 MEIC chemicals. They found that mouse oral LD50 values correlated reasonably well with HLD (coefficient of determination $r^2 = 0.65$), while rat oral LD50 values gave a somewhat poorer correlation with HLD ($r^2 = 0.61$), again indicating that the mouse is a better model than the rat for HLD prediction. Lessigierska et al.\textsuperscript{19} also found that mouse oral LD50 values correlated better ($r^2 = 0.683$) than did rat oral LD50 values ($r^2 = 0.627$) with HLD for 25 MEIC chemicals.

Although the primary aim of the MEIC studies was to examine whether cytotoxicity data could replace rodent LD50 values, recent work\textsuperscript{11,20} indicates that the apparent inherent lack of reliability in some \textit{in vitro} data is an additional reason that the use of cytotoxicity alone may not be enough to model acute toxicity. There is also, of course, much variability in rodent LD50 values. Karmaus et al.\textsuperscript{21} have recently pointed out that \textit{“in vivo} acute systemic toxicity studies can produce variable results, even when conducted according to accepted test guidelines.” They examined over 21,000 rat oral LD50 values, and found that some chemicals had reported LD50 values ranging over at least three orders of magnitude.

It is clear that mouse and rat oral LD50 values correlate reasonably well with HLD values for the MEIC chemicals. However, with the exception of the work of Weiss and Sawyer,\textsuperscript{17} there does not appear to have been any investigation of whether rodent LD50 values obtained using other routes of administration correlate better with HLD values of the MEIC chemicals.

Using average rodent LD50 values, calculated from a very large number of published values, covering mouse and rat oral, intraperitoneal and intravenous routes of administration, Dearden\textsuperscript{22} found that the best correlations of HLD values for up to 18 psychoactive drugs were with mouse subcutaneous ($n = 10$, $r^2 = 0.842$), rat intravenous ($n = 14$, $r^2 = 0.823$) and mouse intravenous ($n = 18$, $r^2 = 0.756$) LD50 values. All other HLD–rodent LD50 correlations had $r^2 < 0.6$.

It should be mentioned that Nendza et al.\textsuperscript{23} stated that while \textit{“in silico} predictions must include an assessment of the error range of experimental (individual) data quality,” they also commented that “less reliable data can still be adequate for risk assessment in combination with other evidence. The pooling of several studies, one or more of which may be inadequate by itself, may collectively satisfy the overall requirement for valid data.” That is probably why the average rodent LD50 values used by Dearden\textsuperscript{22} gave such good HLD predictions.

The HLD–rodent LD50 correlations for the MEIC chemicals discussed above are reasonable but not good, with the exception of those obtained from Ekwall et al.\textsuperscript{13} which could be high because they were based on only 10 chemicals. Encouraged by the results obtained by Dearden\textsuperscript{22} using averaged rodent LD50 values to predict the HLD values of psychoactive drugs, an attempt is made in the present study to use median LD50 values for the MEIC chemicals to try to improve HLD predictions. Median rather than average LD50 values are used,\textsuperscript{2} as they place less weight on the contributions of extreme values.\textsuperscript{24} Although the MEIC data set is relatively small, it is very diverse, representing many closely-studied chemical classes having a wide range of physicochemical and other properties. It is therefore appropriate to use this data set to test the hypothesis that median rodent LD50 values can be used to predict HLD values well. Future work could expand upon the MEIC data set, in order to validate the results of the present study.

It is stressed that this work is an attempt to make some reparation for the millions of rodents sacrificed over many decades, often for little or no purpose. It exemplifies how \textit{existing} data can be used to derive new insights, and in no way does it imply that more rodent LD50 tests should be performed. Madden et al.\textsuperscript{25} recently reviewed the use of \textit{in silico} approaches, such as quantitative structure–activity relationships (QSARs), as alternatives to animal testing, wherein predictions are based on models incorporating molecular structural information. The present study demonstrates the application of an analogous concept — namely, quantitative activity–activity relationships (QAARs). In this approach, new knowledge is derived from existing data from one species to make predictions for another. Maximising the use of data available from in-house or public repositories conforms to the Three Rs principles.\textsuperscript{26}

It is noted that Bailey and Balls\textsuperscript{27} recently stated that: “We reiterate that we welcome any objective efforts to shed light on the value — or lack of value — of animal tests for drugs intended for human use.” The present work accords with that statement.

**Methods**

**Data collection**

Fifty MEIC chemicals were used in the historical study of Ekwall et al.\textsuperscript{18} However, since the present study used...
Rodent LD50 values calculated by software (as outlined below), as well as measured values, inorganic chemicals, xylene (because there are three isomeric xylenes) and paraquat (as the molecule carries a positive charge) were excluded. This left 36 MEIC chemicals to be studied. Rodent LD50 values were collected from a number of sources (see online Supplementary Information 1). HLD values were taken from Ekwall et al.\textsuperscript{18} with the exception of atropine\textsuperscript{28} and digoxin.\textsuperscript{29}

In addition to providing HLD values for the MEIC chemicals, Ekwall et al.\textsuperscript{18} also reported the human lethal blood concentrations (HLCs) of the chemicals in three formats: the clinical, the forensic and the peak values. The present study sought to examine how well median rodent LD50 values modelled HLC values for the 36-chemical data set. As has been reported elsewhere,\textsuperscript{21,30,31} a wide variation in reported rodent LD50 values is often evident. For example, the mouse oral LD50 values for atropine ranged from 32.1 to 1040 mg/kg, the rat oral values for 2,4-dichlorophenoxyacetic acid ranged from 300.6 to 4700 mg/kg, and for lindane from 57 to 6000 mg/kg. All LD50 values collected, and their sources, are provided in the Supplementary Information 1, and the calculation of

### Table 1. Human lethal doses, human forensic lethal concentrations and rodent median LD50 values, for 36 MEIC chemicals.

| Chemical                          | HLD\textsuperscript{a} (mg/kg) | HFLC (mg/l) | Rodent median LD50 values (mg/kg) |
|-----------------------------------|---------------------------------|-------------|----------------------------------|
| Acetylsalicylic acid              | 385.2                           | 801.1       | Mor 231.6 Mip 662.2 Ror 495.0    |
| Amtripryline                      | 37.4                            | 4.2         | Mor 196.1 Mip 70.9 Ror 23.2      |
| Amphetamine                      | 5.0                             | 6.3         | Msc 29.0 Miv 16.3 Ror 43.0       |
| Atropine                          | 1.47                            | 0.20        | Mor 187.0 Mip 60.2 Ror 33.7      |
| Caffeine                          | 140.7                           | 119.7       | Mor 231.6 Mip 163.4 Ror 92.7     |
| Chloramphenicol                   | 2878.0                          | 99.9        | Mor 2355.0 Mip 679.5 Ror 343.2   |
| Chloroform                        | 993.0                           | 97.0        | Mor 246.4 Mip 698.9 Ror 704.0    |
| Chloroquine                       | 219.0                           | 14.0        | Mor 310.5 Mip 54.2 Ror 136.3     |
| Dextropropoxyphene                | 9.1                             | 8.0         | Mor 205.4 Mip 105.4 Ror 708.9    |
| Diazepam                          | 71.5                            | 31.2        | Mor 486.3 Mip 111.5 Ror 489.8    |
| Dichloromethane                   | 1777.4                          | 362.3       | Mor 1316.7 Mip 1172.9 Ror 6460.0 |
| 2,4-Dichlorophenoxyacetic acid    | 384.1                           | 568.2       | Mor 440.5 Mip 182.0 Ror 708.9    |
| Digoxin                           | 0.368                           | 0.016       | Mor 17.9 Mip 3.6 Ror 10.2        |
| Ethanol                           | 4714.3                          | 4824.1      | Mor 6960.4 Mip 4405.4 Ror 10042  |
| Ethylene glycol                   | 1559.1                          | 2587.5      | Mor 8892.0 Mip 3769.9 Ror 7146.0 |
| Hexachlorophene                   | 213.6                           | 34.6        | Mor 115.9 Mip 32.3 Ror 46.0      |
| Isoniazid                         | 172.7                           | 131.0       | Mor 194.7 Mip 120.9 Ror 156.7    |
| Isopropanol                       | 2563.8                          | 1590.6      | Mor 4013.3 Mip 4200.8 Ror 6000.0 |
| Lindane                           | 241.9                           | 1.0         | Mor 95.0 Mip 80.0 Ror 185.4      |
| Methanol                          | 690.2                           | 281.2       | Mor 1109.4 Mip 637.3 Ror 221.0   |
| Nicotine                          | 1569.3                          | 1886.7      | Mor 9686.0 Mip 6370.0 Ror 9800.0 |
| Orphenadrine                      | 41.7                            | 12.0        | Mor 116.1 Mip 58.7 Ror 107.8     |
| Paracetamol                       | 268.8                           | 228.8       | Mor 706.1 Mip 507.1 Ror 697.7    |
| Pentachlorophenol                 | 28.5                            | 99.0        | Mor 106.2 Mip 45.4 Ror 46.0      |
| Phenobarbital                     | 111.2                           | 121.9       | Mor 223.2 Mip 189.9 Ror 193.6    |
| Phenol                            | 156.2                           | 76.5        | Mor 327.3 Mip 180.0 Ror 347.5    |
| Phenylhydrazine                   | 303.3                           | 79.8        | Mor 202.2 Mip 141.8 Ror 95.2     |
| Propranolol                       | 62.2                            | 11.1        | Mor 374.6 Mip 80.0 Ror 165.4     |
| Quinidine                         | 61.8                            | 43.8        | Mor 415.0 Mip 129.9 Ror 400.0    |
| Tetrachloromethane                | 1309.0                          | 227.5       | Mor 10651.0 Mip 1941.0 Ror 30690.0 |
| Theophylline                      | 156.9                           | 149.9       | Mor 315.1 Mip 155.1 Ror 165.1    |
| Thiouracil                        | 54.8                            | 6.4         | Mor 445.3 Mip 104.1 Ror 313.7    |
| 1,1,1-Trichloroethane             | 7165.0                          | 171.9       | Mor 2521.8 Mip 2227.9 Ror 1600.0 |
| Verapamil                         | 114.2                           | 7.9         | Mor 147.3 Mip 60.3 Ror 50.9      |
| Warfarin                          | 106.9                           | 99.8        | Mor 220.6 Mip 361.4 Ror 3576.8   |

\textsuperscript{a}Converted from data in Ekwall et al.\textsuperscript{18}

HLD = human lethal dose; HFLC = human forensic lethal concentration; Mor = mouse, oral administration; Mip = mouse, intraperitoneal administration; Msc = mouse, subcutaneous administration; Miv = mouse, intravenous administration; Ror = rat, oral administration; Rip = rat, intraperitoneal administration; Rsc = rat, subcutaneous administration; Riv = rat, intravenous administration.
median values is presented in the online Supplementary Information 2. The HLD, HLC and median LD50 values are given in Table 1. Rat and mouse LD50 values can also be calculated by a few software programs. Gonella Diaza et al.32 found the best to be the ACD/Labs ToxSuite33 (now called I-Lab 2) and the US Environmental Protection Agency’s Toxicity Estimation Software Tool (TEST) program.34 The former calculates mouse oral (or), intraperitoneal (ip), subcutaneous (sc) and intravenous (iv) LD50 values, as well as rat oral and intraperitoneal LD50 values. The latter calculates rat oral LD50 values only. The ACD/Labs software is a commercial program, but at present it is available free of charge to academics at British universities via the Royal Society of Chemistry website. The present study used these two software programs, and the rodent LD50 values predicted by them are given in Table 2.

All toxicity values were converted to mmol/kg and negative log-transformed for purposes of correlation. Values given for salts were corrected to the corresponding free base or acid. Carai et al.35 have commented that this may not give the correct value for the free acid or base; for example, for a sodium salt, the sodium ion could have affected mortality by changing the electrolytic homeostasis. However, such salt effects could not be corrected in literature data.

### Table 2. ACD I-Lab 2-predicted and TEST-predicted rodent LD50 values for 36 MEIC chemicals.

| Chemical                  | Predicted LD50 values (mg/kg) | ACD Mor | ACD Mip | ACD Msc | ACD Miv | ACD Ror | ACD Rip | TEST Ror |
|---------------------------|-------------------------------|---------|---------|---------|---------|---------|---------|----------|
| Acetylsalicylic acid      | 1100                          | 460     | 760     | 500     | 1400    | 710     | 757     |
| Amitriptyline             | 180                           | 67      | 120     | 26      | 330     | 120     | 309     |
| Amphetamine               | 130                           | 130     | 13      | 28      | 120     | 81      | 770     |
| Atropine                  | 440                           | 110     | 430     | 39      | 760     | 160     | 464     |
| Caffeine                  | 370                           | 260     | 370     | 100     | 150     | 100     | 278     |
| Chloramphenicol           | 2900                          | 710     | 1000    | 260     | 1700    | 1700    | 2059    |
| Chloroform                | 1300                          | 760     | 470     | 110     | 1600    | 950     | 1794    |
| Chloroquine               | 310                           | 140     | 250     | 19      | 550     | 95      | 866     |
| Dextropropoxyphene        | 200                           | 110     | 110     | 40      | 330     | 110     | 346     |
| Diizepam                  | 610                           | 230     | 790     | 51      | 870     | 260     | 1772    |
| Dichloromethane           | 1200                          | 230     | 1300    | 83      | 1200    | 570     | 400     |
| 2,4-Dichlorophenoxyacetic acid | 340          | 510     | 830     | 180     | 480     | 740     | 586     |
| Digoxin                   | 8.6                           | 4.8     | 7.6     | 27      | 24      | 12.7    |
| Ethanol                   | 4400                          | 1700    | 5500    | 1300    | 3500    | 25      | 672     |
| Ethylene glycol           | 12000                         | 4900    | 2700    | 2200    | 10000   | 4600    | 754     |
| Hexachlorophene           | 130                           | 41      | 44      | 37      | 170     | 40      | 308     |
| Isoniazid                 | 240                           | 200     | 310     | 290     | 1200    | 180     | 528     |
| Isopropanol               | 4100                          | 2000    | 4900    | 890     | 3800    | 2000    | 2217    |
| Lindane                   | 220                           | 220     | 12      | 43      | 710     | 130     | 513     |
| Malathion                 | 1100                          | 920     | 120     | 210     | 1600    | 100     | 459     |
| Methanol                  | 4300                          | 2500    | 5900    | 1500    | 3400    | 2600    | 171     |
| Nicotin                   | 68                            | 57      | 45      | 7.9     | 220     | 59      | 1019    |
| Orphenadrine              | 290                           | 94      | 350     | 38      | 530     | 100     | 720     |
| Paracetamol               | 830                           | 520     | 410     | 220     | 2300    | 950     | 1807    |
| Pentachlorophenol         | 140                           | 57      | 100     | 56      | 110     | 100     | 227     |
| Phenobarbital             | 450                           | 240     | 290     | 390     | 180     | 200     | 539     |
| Phenol                    | 450                           | 100     | 370     | 87      | 260     | 160     | 416     |
| Phenytoin                 | 570                           | 280     | 310     | 87      | 1600    | 1100    | 1228    |
| Propranolol               | 280                           | 100     | 240     | 34      | 750     | 84      | 1793    |
| Quinidine                 | 730                           | 390     | 170     | 75      | 380     | 110     | 553     |
| Tetrachloromethane        | 2800                          | 460     | 1900    | 99      | 3500    | 280     | 1803    |
| Theophylline              | 160                           | 96      | 270     | 130     | 61      | 120     | 171     |
| Thoridazine               | 460                           | 140     | 320     | 54      | 790     | 110     | 1360    |
| 1,1,1-Trichloroethane     | 2100                          | 840     | 2000    | 77      | 3500    | 280     | 3469    |
| Verapamil                 | 220                           | 69      | 64      | 2.9     | 380     | 57      | 365     |
| Warfarin                  | 1000                          | 390     | 200     | 85      | 170     | 480     | 47      |

ACD = ACD/Labs; TEST = US Environmental Protection Agency’s Toxicity Estimation Software Tool; Mor = mouse, oral administration; Mip = mouse, intraperitoneal administration; Msc = mouse, subcutaneous administration; Miv = mouse, intravenous administration; Ror = rat, oral administration; Rip = rat, intraperitoneal administration.
Table 3. Correlations of the HLD values of the MEIC chemicals with experimental and calculated rodent LD50 values.

| Eqn | Correlation | n  | r^2 | q^2 | s    | F   |
|-----|-------------|----|-----|-----|------|-----|
| 1   | \(\log 1/\text{HLD} = 0.516 (0.101) + 1.119 (0.104) \log 1/\text{Mor}\) | 36 | 0.773 | 0.743 | 0.572 | 116.1 |
| 2   | \(\log 1/\text{HLD} = 0.189 (0.081) + 1.084 (0.082) \log 1/\text{Mip}\) | 36 | 0.838 | 0.815 | 0.484 | 175.8 |
| 3   | \(\log 1/\text{HLD} = 0.490 (0.113) + 0.940 (0.099) \log 1/\text{Msc}\) | 34 | 0.737 | 0.702 | 0.634 | 89.8 |
| 4   | \(\log 1/\text{HLD} = -0.003 (0.126) + 1.001 (0.110) \log 1/\text{Miv}\) | 26 | 0.776 | 0.729 | 0.601 | 83.1 |
| 5   | \(\log 1/\text{HLD} = 0.695 (0.115) + 1.122 (0.114) \log 1/\text{Ror}\) | 36 | 0.739 | 0.701 | 0.614 | 96.4 |
| 6   | \(\log 1/\text{HLD} = 0.252 (0.088) + 1.046 (0.087) \log 1/\text{Rip}\) | 36 | 0.810 | 0.781 | 0.524 | 145.0 |
| 7   | \(\log 1/\text{HLD} = 0.542 (0.151) + 0.790 (0.193) \log 1/\text{Rsc}\) | 26 | 0.411 | 0.237 | 0.765 | 16.7 |
| 8   | \(\log 1/\text{HLD} = -0.040 (0.143) + 0.961 (0.126) \log 1/\text{Riv}\) | 27 | 0.699 | 0.650 | 0.683 | 58.1 |
| 9   | \(\log 1/\text{HLD} = 0.702 (0.111) + 1.244 (0.120) \log 1/\text{ACDMor}\) | 36 | 0.759 | 0.733 | 0.590 | 107.3 |
| 10  | \(\log 1/\text{HLD} = 0.282 (0.103) + 1.254 (0.128) \log 1/\text{ACDMip}\) | 36 | 0.739 | 0.708 | 0.614 | 96.3 |
| 11  | \(\log 1/\text{HLD} = 0.392 (0.122) + 1.050 (0.133) \log 1/\text{ACDMsc}\) | 36 | 0.648 | 0.594 | 0.714 | 62.5 |
| 12  | \(\log 1/\text{HLD} = -0.196 (0.130) + 1.088 (0.141) \log 1/\text{ACDMiv}\) | 36 | 0.635 | 0.577 | 0.726 | 59.2 |
| 13  | \(\log 1/\text{HLD} = 0.808 (0.138) + 1.240 (0.149) \log 1/\text{ACDRor}\) | 36 | 0.671 | 0.633 | 0.690 | 69.4 |
| 14  | \(\log 1/\text{HLD} = 0.221 (0.141) + 1.110 (0.189) \log 1/\text{ACDRip}\) | 36 | 0.504 | 0.448 | 0.847 | 34.5 |
| 15  | \(\log 1/\text{HLD} = 0.756 (0.188) + 1.255 (0.243) \log 1/\text{TESTRor}\) | 36 | 0.439 | 0.378 | 0.901 | 26.6 |

n = number of chemicals in the model; r^2 = coefficient of determination (a measure of goodness of fit); q^2 = cross-validated coefficient of determination (a measure of robustness of the correlation); s = standard error of the prediction; F = Fisher statistic, or variance ratio (a measure of predictivity). The numbers in brackets are the standard errors on each coefficient.

HLD = human lethal dose; ACD = ACD/Labs; TEST = US Environmental Protection Agency’s Toxicity Estimation Software Tool; Mor = mouse, oral administration; Mip = mouse, intraperitoneal administration; Msc = mouse, subcutaneous administration; Miv = mouse, intravenous administration; Ror = rat, oral administration; Rip = rat, intraperitoneal administration; Rsc = rat, subcutaneous administration; Riv = rat, intravenous administration.

**Statistical analysis**

Statistical analysis was carried out by using regression analysis in Minitab 19 software. It is now accepted practice to carry out an external validation of a predictive correlation, which is achieved by removing, say, 20% of the chemicals from the training data set, re-developing the correlation with the remaining 80% of chemicals, and using that correlation to predict the endpoint values of the removed chemicals (i.e. the test set). Selection of the test set chemicals was done by the well-used method of removal of every fifth chemical of the alphabetical list, namely: caffeine, diazepam, ethylene glycol, malathion, pentachlorophenol, quinidine and verapamil.

**Results and Discussion**

**Correlation of the HLD and rodent LD50 values**

Correlations of the HLD values with the median measured rodent LD50 values, as well as the calculated rodent LD50 values for the whole data set, are given in Table 3 (see Figure 1 for the best correlation between the values). Table 4 shows the best training set QAARs for HLD values correlated with both measured and calculated rodent LD50 values. The best training set QAARs were used to predict the HLD values of the seven test set chemicals, and these values are given in Table 5.

Equations 16–19 gave predicted HLD values within 1 standard deviation (sd), with the exception of verapamil, for which three predictions were between 1 and 2 sd (see Table 5). This is still acceptable, bearing in mind the wide range of some rodent LD50 values, and Lipnick’s comment that only predicted values with errors over 3 sd should be regarded as unacceptable. Hence, equations 16–19 have been successfully externally validated.

HLD predictions from equations 20–22, based on ACD/Labs-predicted rodent LD50 values, were also mostly within 1 sd, with the exception of two values with an error greater than 1 sd and two values with an error greater than...
Therefore, on the whole, the ACD/Labs I-Lab 2 software yielded good HLD predictions, with 20 out of 21 predictions being acceptable.

It can be seen from Table 3 that mouse intraperitoneal (Mip) and rat intraperitoneal (Rip) LD50 values correlate excellently with HLD values; it is generally acknowledged that $r^2$ values of about 0.8 are as good as can be expected from *in vivo* data. Mouse oral (Mor) and rat oral (Ror) LD50 values also correlate well with HLD values. These correlations are much better than that reported for HLD–Ror LD50 correlation ($r^2 = 0.571$) for a 30-chemical data set. Figure 1 illustrates the HLD–Mip LD50 correlation obtained in the present work. It is interesting to note that Fry et al. reported that rodent intraperitoneal LD50 values were more widely available than were those for other routes of administration. However, in the *Supplementary Information* 2 of the present work, it can be seen that more LD50 values were found for oral administration than for intraperitoneal administration.

The training set QAARs in Table 4 are very similar to those developed for the full data set, given in Table 3. The predicted HLD values in Table 5 are almost all within 1 sd of the observed HLD values, and only one equation (from the ACDMor prediction) has an error of more than 2 sd. Thus, any of the equations 16–22, and particularly equations 16–19 based on median measured rodent LD50 values, can be used to predict human lethal doses of MEIC chemicals.

HLD values also correlated well with ACD/Labs-predicted Mor and Mip LD50 values, but less well with ACD/Labs-predicted LD50 values via other routes of administration. As can be seen from Table 6, the ACD/Labs-predicted LD50 values all correlated very well ($r^2 > 0.88$) with the respective median measured LD50 values, with the exception of the Rip LD50 values ($r^2 = 0.70$). One reason for these good correlations may be that, with one exception, all the MEIC chemicals are in the ACD/Labs I-Lab 2 database. However, it should be noted that all but one of the ACD/Labs I-Lab 2-predicted LD50 values were different from the measured values in their database.

The TEST-predicted Ror LD50 values correlated very poorly with HLD values ($r^2 = 0.439$), which is disappointing. The TEST software-predicted Ror LD50 values also correlated only poorly ($r^2 = 0.611$) with the median measured Ror LD50 values. Thus, it can be concluded that the

### Table 4. Best correlations of the HLD values of the MEIC training set chemicals with experimental and ACD-calculated rodent LD50 values.

| Eqn | Correlation | n | $r^2$ | $q^2$ | s | F |
|-----|-------------|---|-------|-------|---|---|
| 16  | $\log 1/\text{HLD} = 0.525 (0.120) + 1.167 (0.122) \log 1/\text{Mor}$ | 29 | 0.773 | 0.734 | 0.616 | 91.9 |
| 17  | $\log 1/\text{HLD} = 0.208 (0.098) + 1.120 (0.096) \log 1/\text{Mip}$ | 29 | 0.835 | 0.806 | 0.525 | 136.9 |
| 18  | $\log 1/\text{HLD} = 0.757 (0.139) + 1.177 (0.133) \log 1/\text{Ror}$ | 29 | 0.742 | 0.693 | 0.657 | 77.7 |
| 19  | $\log 1/\text{HLD} = 0.283 (0.106) + 1.085 (0.102) \log 1/\text{Rip}$ | 29 | 0.807 | 0.770 | 0.568 | 112.9 |
| 20  | $\log 1/\text{HLD} = 0.721 (0.124) + 1.329 (0.136) \log 1/\text{ACDMor}$ | 29 | 0.773 | 0.747 | 0.609 | 95.2 |
| 21  | $\log 1/\text{HLD} = 0.264 (0.116) + 1.353 (0.146) \log 1/\text{ACDMip}$ | 29 | 0.753 | 0.720 | 0.635 | 85.4 |
| 22  | $\log 1/\text{HLD} = 0.868 (0.159) + 1.326 (0.173) \log 1/\text{ACDRor}$ | 29 | 0.677 | 0.635 | 0.726 | 58.8 |

$n =$ number of chemicals in the model; $r^2 =$ coefficient of determination (a measure of goodness of fit); $q^2 =$ cross-validated coefficient of determination (a measure of robustness of the correlation); $s =$ standard error of the prediction; $F =$ Fisher statistic, or variance ratio (a measure of predictivity). The numbers in brackets are standard errors on each coefficient.

HLD = human lethal dose; ACD = ACD/Labs; Mor = mouse, oral administration; Mip = mouse, intraperitoneal administration; Ror = rat, oral administration; Rip = rat, intraperitoneal administration.

### Table 5. Observed HLD values of the MEIC test set chemicals, and those predicted from equations 16–22.

| Chemical         | Observed HLD (mg/kg) | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|------------------|----------------------|----|----|----|----|----|----|----|
| Caffeine         | 140.7                | 71.4 | 81.0 | 64.4 | 80.5 | 87.0 | 156.9 | 18.7* |
| Diazepam         | 71.5                 | 159.0 | 61.5 | 71.9 | 57.1 | 149.0 | 116.1 | 169.7 |
| Ethylene glycol  | 1559.1               | 6066 | 3829 | 2897 | 3793 | 12894** | 2473 | 7104 |
| Malathion        | 690.2                | 405.9 | 426.4 | 229.3 | 356.3 | 310.6 | 719.1 | 362.6 |
| Pentachlorophenol| 28.5                 | 27.3 | 22.7 | 13.9 | 26.5 | 21.5 | 18.0 | 13.6 |
| Quinidine        | 61.8                 | 129.4 | 71.9 | 84.7 | 50.2 | 181.1 | 226.6 | 54.2 |
| Verapamil        | 114.2                | 36.6 | 29.2* | 16.3* | 26.8* | 32.9 | 19.3* | 48.6 |

HLD = human lethal dose.

*Prediction error > 1 standard deviation; **Prediction error > 2 standard deviations.
ACD/Labs-predicted mouse and rat oral LD$_{50}$ values are the better choice of the two to predict the HLD values of the MEIC chemicals.

Ekwall et al.\cite{Ekwall2000} found that rat and mouse oral LD$_{50}$ values correlated well with each other ($r^2 = 0.852$) for the 50 MEIC chemicals, a finding also reported by Hoffmann et al.\cite{Hoffmann2000} in their study based on 40 MEIC chemicals ($r^2 = 0.800$). Table 7 shows rat–mouse LD$_{50}$ correlations for the log-transformed values of the data given in Table 1, with a better correlation value ($r^2 = 0.883$) than those calculated by Ekwall et al.\cite{Ekwall2000} and by Hoffmann et al.\cite{Hoffmann2000}. It is not surprising that rat–mouse LD$_{50}$ values correlate better than do HLD–rodent values, since the two rodent species share more similar physiology. Indeed, some researchers have used rat and mouse LD$_{50}$ values interchangeably in studies.\cite{Hoffmann2000}

Table 7 shows that the correlation between log 1/Rsc and log 1/Msc is very poor. Table 3 shows that the correlation between log 1/HLD and log 1/Rsc ($r^2 = 0.411$) is much poorer than the correlation between log 1/HLD and log 1/Msc ($r^2 = 0.737$), suggesting that it is the Rsc data that are largely at fault. This could be because only relatively few Rsc LD$_{50}$ values were found (see Supplementary Information 2), with only a single Rsc LD$_{50}$ value having been found for many of the studied MEIC chemicals.

The use of rodent LD$_{50}$ values to model human lethal blood concentrations

Of the three HLC formats (i.e. forensic, clinical and peak), the forensic values correlated best with rodent LD$_{50}$ values. For example, $r^2$ values for the correlation of forensic, clinical and peak HLC values with Mip values were 0.753, 0.683 and 0.651, respectively. Therefore, only human forensic lethal concentration (HFLC) values were subsequently used, and their correlations with median measured and calculated rodent LD$_{50}$ values for the whole data set are given in Table 8 (see Figure 2 for the best correlation between the values). Table 9 gives the best training set QAARs for HFLC values correlated with both measured and calculated rodent LD$_{50}$ values. The best training set QAARs were used to predict the HFLC values of the seven test set chemicals, and these values are given in Table 10.

Table 7. QAARs of rat–mouse LD$_{50}$ values.

| Eqn | Correlation                                                                 | n  | $r^2$ | $q^2$ | s       | F       |
|-----|-----------------------------------------------------------------------------|----|-------|-------|---------|---------|
| 45  | $\log 1/Mor = -0.184 (0.056) + 0.917 (0.057) \log 1/Mor$                   | 36 | 0.883 | 0.870 | 0.316   | 255.3   |
| 46  | $\log 1/Rip = -0.061 (0.035) + 0.997 (0.036) \log 1/Mip$                   | 36 | 0.958 | 0.954 | 0.212   | 772.3   |
| 47  | $\log 1/Rsc = -0.017 (0.112) + 0.819 (0.151) \log 1/Msc$                   | 25 | 0.562 | 0.488 | 0.542   | 29.5    |
| 48  | $\log 1/Riv = 0.046 (0.061) + 0.919 (0.052) \log 1/Miv$                    | 25 | 0.932 | 0.919 | 0.282   | 315.1   |
| 49  | $\log 1/ACDMor = -0.141 (0.057) + 0.874 (0.061) \log 1/ACDMor$             | 36 | 0.857 | 0.847 | 0.300   | 204.2   |
| 50  | $\log 1/ACDRip = 0.024 (0.068) + 0.792 (0.085) \log 1/ACDMip$              | 36 | 0.721 | 0.666 | 0.406   | 87.8    |
Table 8. Correlations of the HFLC values of the MEIC chemicals with experimental and calculated rodent LD_{50} values.

| Eqn | Correlation | n  | r^2   | q^2   | s     | F  |
|-----|-------------|----|-------|-------|-------|----|
| 23  | log I/HFLC = 3.930 (0.138) + 1.188 (0.142) log I/Mor | 36 | 0.672 | 0.619 | 0.784 | 69.7 |
| 24  | log I/HFLC = 3.583 (0.113) + 1.170 (0.115) log I/Mip | 36 | 0.753 | 0.716 | 0.680 | 103.7 |
| 25  | log I/HFLC = 3.846 (0.148) + 0.953 (0.130) log I/Msc | 34 | 0.628 | 0.563 | 0.829 | 54.1 |
| 26  | log I/HFLC = 3.259 (0.175) + 1.112 (0.152) log I/Miv | 26 | 0.689 | 0.610 | 0.834 | 53.2 |
| 27  | log I/HFLC = 4.126 (0.151) + 1.204 (0.149) log I/Ror | 36 | 0.657 | 0.605 | 0.802 | 65.1 |
| 28  | log I/HFLC = 3.655 (0.106) + 1.172 (0.105) log I/Rip | 36 | 0.785 | 0.749 | 0.635 | 123.8 |
| 29  | log I/HFLC = 3.939 (0.205) + 0.850 (0.261) log I/Rsc | 26 | 0.307 | 0.111 | 1.033 | 10.6 |
| 30  | log I/HFLC = 3.188 (0.179) + 1.123 (0.158) log I/Riv | 27 | 0.670 | 0.615 | 0.854 | 50.8 |
| 31  | log I/HFLC = 4.158 (0.133) + 1.393 (0.144) log I/ACDMor | 36 | 0.734 | 0.704 | 0.706 | 94.0 |
| 32  | log I/HFLC = 3.693 (0.110) + 1.457 (0.136) log I/ACDMip | 36 | 0.771 | 0.742 | 0.655 | 114.4 |
| 33  | log I/HFLC = 3.823 (0.131) + 1.232 (0.142) log I/ACDMsc | 36 | 0.689 | 0.648 | 0.763 | 75.5 |
| 34  | log I/HFLC = 3.108 (0.120) + 1.353 (0.131) log I/ACDMiv | 36 | 0.759 | 0.714 | 0.672 | 106.9 |
| 35  | log I/HFLC = 4.231 (0.180) + 1.298 (0.194) log I/ACDRor | 36 | 0.568 | 0.507 | 0.899 | 44.8 |
| 36  | log I/HFLC = 3.624 (0.153) + 1.324 (0.204) log I/ACDRip | 36 | 0.554 | 0.498 | 0.914 | 42.2 |
| 37  | log I/HFLC = 4.222 (0.216) + 1.411 (0.279) log I/TESTRor | 36 | 0.429 | 0.341 | 1.035 | 25.5 |

n = number of chemicals in the model; r^2 = coefficient of determination (a measure of goodness of fit); q^2 = cross-validated coefficient of determination (a measure of robustness of the correlation); s = standard error of the prediction; F = Fisher statistic, or variance ratio (a measure of predictivity). The numbers in brackets are standard errors on each coefficient.

HFLC = human forensic lethal concentration; ACD = ACD/Labs; TEST = US Environmental Protection Agency’s Toxicity Estimation Software Tool; Mor = mouse, oral administration; Mip = mouse, intraperitoneal administration; Msc = mouse, subcutaneous administration; Miv = mouse, intravenous administration; Ror = rat, oral administration; Rip = rat, intraperitoneal administration; Rsc = rat, subcutaneous administration; Riv = rat, intravenous administration.

with only two correlations having r^2 values of < 0.65. Two correlations based on ACD/Labs-predicted values also had r^2 values of < 0.65. Table 9 shows the best of those correlations developed from the training set, and Table 10 gives the predicted HFLC values derived from equations 38–43 for the test set chemicals. With the exception of those for pentachlorophenol, the predicted HFLC values were all within 1 s.d. of the observed values, and even the pentachlorophenol values were less than 3 s.d. in error, which is acceptable under Lipnick’s guidance.28 Hence, any of the equations 38–43 can be used to predict human forensic lethal concentrations of MEIC chemicals with acceptable accuracy.

Ekwall et al.2 also examined correlations between the HLC values of the MEIC chemicals and the IC_{50} values from 61 in vitro cytotoxicity tests, and found that on the whole they were poor, with only 5 out of 150 correlations having r^2 values of ≥ 0.7. It should be noted that in these five instances, IC_{50} values from fewer than the full set of 50 MEIC chemicals were used in the correlations, implying perhaps that some data were omitted to improve the extent of correlation.

**The use of ‘lowest’ rodent LD_{50} values to model human acute toxicity values**

Several studies8,17,41 have used the lowest available rodent LD_{50} values, in order to obtain worst case estimates of toxicity. From the very large number of rodent LD_{50} values collected for the present study (see Supplementary Information 2), lowest values were used to determine whether better predictions of HLD could be obtained from these data than from the use of median LD_{50} values. The QAARs developed from these data are given in Table 11.

A comparison of the QAARs given in Table 11 with equations 1–8 in Table 3 shows that almost all those in Table 3 have better statistics than do those in Table 11, indicating that the use of median rodent LD_{50} values is preferable to the use of the worst case (i.e. ‘lowest’) rodent LD_{50} values for the prediction of HLD and probably other toxicity endpoints. The main exceptions involve intravenous LD_{50} values, as equations 4 and 54 are almost identical. It can be seen from Supplementary Information 2 that...
not only are there generally fewer intravenous LD50 values available, but their range is usually much narrower than those obtained from other routes of administration. It would thus appear that the intravenous route gives more accurate LD50 values than does administration by other routes.

Weiss and Sawyer\textsuperscript{17} used the 50 MEIC chemicals in their study of the correlation of HLD values with worst case Mor, Mip, Ror and Rip LD50 values, so their results can be compared directly with those found in the present study. The r^2 values from their data were found to be: 0.636 (Mor); 0.784 (Mip); 0.578 (Ror); and 0.769 (Rip). In each case, these r^2 values are appreciably lower than those of the corresponding equations 1, 2, 5 and 6 in Table 3, again indicating that the use of median rodent LD50 values leads

| Table 9. Best correlations of the HFLC values of the MEIC training set chemicals with experimental and ACD-calculated rodent LD50 values. |
|---|---|---|---|---|---|
| Eqn | Correlation | n | r^2 | q^2 | s | F |
| 38 | log 1/HFLC = 3.979 (0.165) + 1.210 (0.167) log 1/Mor | 29 | 0.661 | 0.591 | 0.845 | 52.6 |
| 39 | log 1/HFLC = 3.651 (0.135) + 1.191 (0.132) log 1/Mip | 29 | 0.751 | 0.705 | 0.724 | 81.3 |
| 40 | log 1/HFLC = 4.231 (0.179) + 1.244 (0.172) log 1/Ror | 29 | 0.660 | 0.647 | 0.846 | 52.3 |
| 41 | log 1/HFLC = 3.736 (0.124) + 1.203 (0.120) log 1/Rip | 29 | 0.789 | 0.746 | 0.667 | 100.8 |
| 42 | log 1/HFLC = 4.224 (0.154) + 1.466 (0.167) log 1/ACDMor | 29 | 0.740 | 0.707 | 0.740 | 76.7 |
| 43 | log 1/HFLC = 3.727 (0.127) + 1.558 (0.159) log 1/ACDMip | 29 | 0.781 | 0.749 | 0.679 | 96.2 |

n = number of chemicals in the model; r^2 = coefficient of determination (a measure of goodness of fit); q^2 = cross-validated coefficient of determination (a measure of robustness of the correlation); s = standard error of the prediction; F = Fisher statistic, or variance ratio (a measure of predictivity). The numbers in brackets are standard errors on each coefficient.

HFLC = human forensic lethal concentration; ACD = ACD/Labs; Mor = mouse, oral administration; Mip = mouse, intraperitoneal administration; Ror = rat, oral administration; Rip = rat, intraperitoneal administration.

| Table 10. Observed HFLC values of the MEIC test set chemicals, and those predicted from equations 38–43. |
|---|---|---|---|---|---|---|---|---|
| Chemical | Observed HFLC (mg/kg) | HFLC predicted from equation number: | 38 | 39 | 40 | 41 | 42 | 43 |
| Caffeine | 119.7 | 25.2 | 35.3 | 22.4 | 27.7 | 29.8 | 57.3 |
| Diazepam | 31.2 | 57.1 | 20.8 | 45.7 | 18.1 | 51.9 | 38.3 |
| Ethylene glycol | 2587 | 2648 | 1845 | 1336 | 2243 | 8335 | 10517 |
| Malathion | 281.2 | 150.3 | 161.4 | 83.2 | 135.8 | 115.1 | 305.5 |
| Pentachlorophenol | 99.0 | 9.2** | 7.2** | 4.4** | 7.8** | 6.2** | 4.5** |
| Quinidine | 43.8 | 45.8 | 24.4 | 29.1 | 15.5 | 63.5 | 81.1 |
| Verapamil | 7.9 | 12.2 | 9.2 | 5.0 | 7.5 | 9.4 | 4.5 |

HFLC = human forensic lethal concentration.
*Prediction error > 1 standard deviation; **Prediction error > 2 standard deviations.

| Table 11. Correlations of the HLD values of the MEIC chemicals with minimum (worst case) experimental rodent LD50 values. |
|---|---|---|---|---|---|---|
| Eqn | Correlation | n | r^2 | q^2 | s | F |
| 51 | log 1/HLD = 0.113 (0.120) + 0.914 (0.117) log 1/Mor | 36 | 0.642 | 0.591 | 0.719 | 61.0 |
| 52 | log 1/HLD = -0.150 (0.101) + 1.093 (0.103) log 1/Mip | 36 | 0.769 | 0.732 | 0.578 | 113.0 |
| 53 | log 1/HLD = -0.370 (0.106) + 0.927 (0.092) log 1/Msc | 34 | 0.760 | 0.725 | 0.607 | 101.1 |
| 54 | log 1/HLD = -0.158 (0.133) + 0.945 (0.104) log 1/Mip | 26 | 0.775 | 0.719 | 0.601 | 82.8 |
| 55 | log 1/HLD = 0.257 (0.138) + 0.876 (0.141) log 1/Ror | 36 | 0.534 | 0.440 | 0.821 | 38.9 |
| 56 | log 1/HLD = 0.063 (0.091) + 1.040 (0.091) log 1/Rip | 36 | 0.794 | 0.760 | 0.546 | 131.1 |
| 57 | log 1/HLD = 0.457 (0.147) + 0.814 (0.177) log 1/Rsc | 26 | 0.468 | 0.310 | 0.727 | 21.1 |
| 58 | log 1/HLD = -0.107 (0.153) + 0.895 (0.124) log 1/Riv | 27 | 0.674 | 0.623 | 0.711 | 51.8 |

n = number of chemicals in the model; r^2 = coefficient of determination (a measure of goodness of fit); q^2 = cross-validated coefficient of determination (a measure of robustness of the correlation); s = standard error of the prediction; F = Fisher statistic, or variance ratio (a measure of predictivity). The numbers in brackets are standard errors on each coefficient.

HLD = human lethal dose; Mor = mouse, oral administration; Mip = mouse, intraperitoneal administration; Msc = mouse, subcutaneous administration; Miv = mouse, intravenous administration; Ror = rat, oral administration; Rip = rat, intraperitoneal administration; Rsc = rat, subcutaneous administration; Riv = rat, intravenous administration.
to better correlations with HLD values than does the use of ‘lowest’ rodent LD50 values. It should be noted that both approaches require extensive literature searches.

**Do more rodent LD50 values yield more accurate median LD50 values?**

It might be expected that the more rodent LD50 values that can be found for a given endpoint, the more accurate should be their median value. Equations 1–8 (Table 3) were developed using median rodent LD50 values (given in Supplementary Information 2), while equations 51–58 (Table 11) were developed using single worst-case LD50 values (given in Supplementary Information 2). The difference in the statistics between corresponding equations (e.g. 1 and 51) in Tables 3 and 11 should be a function of the number of rodent LD50 values employed to calculate the median LD50 values used in equations 1–8. The following correlations were obtained:

\[
\text{Diff. in } r^2 = -0.0766(0.0194) + 0.0323(0.0043) \text{ (mean no. of LD50 values)}
\]

\[
\begin{align*}
\text{n} &= 8 \\
\text{r}^2 &= 0.903 \\
\text{q}^2 &= 0.820 \\
\text{s} &= 0.0289 \\
\text{F} &= 55.6
\end{align*}
\]

Equations 59 and 60 show clearly that the more rodent LD50 values are available for calculation of median values, the greater the improvement in prediction of HLD values. It follows that studies carried out using only a single set of LD50 values, rather than using average or median values, may be less accurate, although this would be dependent on the quality of the individual data set used. The advantage of the approach demonstrated here is that use of median values improves predictivity without the need for extensive data quality assessment.

**Conclusions**

Human lethal dosage (HLD) and human forensic lethal concentration (HFLEC) values for 36 organic MEIC chemicals have been shown to correlate very well with mouse and rat median intraperitoneal LD50 values, and slightly less well with mouse and rat median oral LD50 values. The correlations of HLD values with subcutaneous and intravenous LD50 values were not as good. The use of median LD50 values gave better results than the use of lowest available LD50 values, with the advantage that no curation of data is required. Therefore, it appears feasible that publicly available median rodent LD50 values could be effectively used to obtain very good predictions of human toxicity, and thus make some reparation for the millions of rodent lives lost in LD50 testing. To this end, it would be admirable if other researchers could also undertake such investigations embracing a wider range of endpoints, in order to maximise — and indeed extend — the utility of the existing data.

**Acknowledgements**

The authors thank Dr Grace Patlewicz and Dr David Ebbrell for help and advice given.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical approval**

Informed consent was not required for this research paper.

**Informed consent**

Informed consent was not required for this research paper.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

**Supplemental material**

Supplemental material for this article is available online.

**References**

1. Gadaleta D, Vuković K, Toma C, et al. SAR and QSAR modeling of a large collection of LD50 rat acute oral toxicity data. *J Cheminform* 2019; 11: 58.
2. Ekwall B, Barile FA, Castano A, et al. MEIC evaluation of acute systemic toxicity. Part VI. The prediction of human toxicity by rodent LD50 values and results from *in vitro* methods. *Altern Lab Anim* 1998; 26 (Suppl 2): 617–658.
3. Clark M and Steger-Hartmann T. A big data approach to the concordance of the toxicity of pharmaceuticals in animals and humans. *Regul Toxicol Pharmacol* 2018; 96: 94–105.
4. Zbinden G and Flury-Roversi M. Significance of the LD50 test for toxicological evaluation of chemical substances. *Arch Toxicol* 1981; 47: 77–99.
5. Balls M and Horner SA. The FRAME interlaboratory programme on *in vitro* cytotoxicity. *Food Chem Toxicol* 1985; 23: 209–213.
6. Clothier RH, Hulme LM, Smith M, et al. Comparison of the *in vitro* cytotoxicities and acute *in vivo* toxicities of 59 chemicals. *Mol Toxicol* 1987; 1: 571–577.
1. Karmaus A, Fitzpatrick J, Allen D, et al. Variability of LD50 values from rat oral acute toxicity studies: implications for alternative model development. In: NICEATM Abstract, Society of Toxicology 2018 Annual Meeting, San Antonio, TX. 11–15 March 2018.

2. Dearden JC. Prediction of human lethality of psychoactive drugs from rodent LD50 values. Int J Quant Struct-Prop Relat 2019; 4(2): 1–27.

3. Nedza M, Aldenberg T, Benfenati E, et al. Data quality assessment for in silico methods: survey of approaches and needs. In: Cronin MTD and Madden JC (eds) In silico toxicology: principles and applications. Cambridge: RSC Publishing, 2010, pp. 59–117.

4. Rowe PH. Essential statistics for the pharmaceutical sciences, 2nd edn. Chichester: John Wiley, 2016, 409 pp.

5. Madden JC, Enoch SJ, Paini A, et al. A review of in silico tools as alternatives to animal testing: principles, resources and applications. Altern Lab Anim 2020; 48: 146–172.

6. Russell WMS and Burch RL. The principles of humane experimental technique. London: Methuen, 1959, 238 pp.

7. Bailey J and Balls M. Recent efforts to elucidate the scientific validity of animal-based drug tests by the pharmaceutical industry, pro-testing lobby groups, and animal welfare organisations. BMC Med Ethics 2019; 20: 16.

8. Arena JM. Atropine poisoning: a report of two cases from Jimson Weed. Clin Pediatr 1963; 2: 182–184.

9. National Institutes of Health. Toxnet, https://www.nlm.nih.gov/toxnet/index.html (2021, accessed 22 January 2021).

10. Hoffman S, Kinsner-Ovaskainen A, Prieto P, et al. Acute oral toxicity: variability, reliability, relevance and interspecies comparison of rodent LD50 data from literature surveyed for the ACuteTox project. Regul Toxicol Pharmacol 2010; 58: 395–407.

11. Zhu H, Martin TM, Ye L, et al. Quantitative structure–activity relationship (QSAR/QSPR). SAR QSAR Environ Res 2015; 26: 1–27.

12. Anon. ACD/Labs, https://www.acdlabs.com (undated, accessed 22 January 2021).

13. United States Environmental Protection Agency. Toxicity estimation software tool (TEST), https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test (undated, accessed 22 January 2021).

14. Caral MAM, Colombo G and Gessa GL. Protection by the GABAB receptor antagonist SCH50911, of γ-hydroxybutyric acid-induced mortality in mice. Eur J Pharmacol 2004; 503: 77–80.

15. Anon. Minitab, https://www.minitab.com/en-us/ (undated, accessed 22 January 2021).

16. Dearden JC, Cronin MTD and Kaiser KLE. How not to develop a quantitative structure–activity or structure–property relationship (QSAR/QSPR). SAR QSAR Environ Res 2009; 20: 241–266.
38. Raevsky OA, Grigor’ev VYu, Weber EE, et al. Classification and quantification of the toxicity of chemicals to Guppy, Fathead Minnow and Rainbow Trout. Part 1. Nonpolar narcosis mode of action. *QSAR Comb Sci* 2008; 27: 1274–1281.

39. Lipnick RL. Outliers: their origin and use in the classification of molecular mechanisms of toxicity. *Sci Total Environ* 1991; 109–110: 131–153.

40. Fry JR, Garle MJ and Hammond AH. Choice of acute toxicity measures for comparison of in vivo/in vitro toxicity. *Altern Lab Anim* 1988; 18: 175–179.

41. Freidig AP, Dekkers S, Verwei M, et al. Development of a QSAR for worst case estimates of acute toxicity of chemically reactive compounds. *Toxicol Lett* 2007; 170: 214–222.