Appendix J7

Comparison of Millimole Regression with Weight Regression Regarding Prediction of Toxicity (LD_{50}) for Low or High Molecular Weight Chemicals

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J.7 The Prediction of Toxicity for High and Low Molecular Weight Substances Using Millimole vs. Weight-Based Regressions

The ICCVAM Acute Toxicity Working Group expressed some concern that the RC rat-only weight regression may less accurately (than the RC rat-only millimole regression) predict the toxicity of low molecular weight substances and high molecular weight substances. Using the RC IC_{50} and LD_{50} values for the 282 RC substances with rat oral LD_{50} data, analyses were performed to

- Determine the difference in the over and under-prediction rates of acute oral toxicity (i.e., LD_{50}) from IC_{50} values for low molecular weight substances (i.e., molecular weight ≤100 g/mole) vs. substances with higher molecular weights
- Determine the difference in the over and under-prediction rates of acute oral toxicity from IC_{50} values for high molecular weight substances (i.e., molecular weight ≥400 g/mole) vs. substances with lower molecular weights
- Compare the RC rat-only millimole regression with the RC rat-only weight regression with respect to the over and under-prediction rates of the toxicity of low and high molecular weight substances

J.7.1 Methods

The data used for to evaluate the over- and under-prediction rates of toxicity of low or high molecular weight chemicals were the RC data rather than the NICEATM/ECVAM validation study data because the RC contains data for many more substances. The RC IC_{50} and LD_{50} values for the 282 RC substances with rat oral LD_{50} data were used since substances with rat data are the focus of the BRD with respect to the prediction of oral LD_{50} (and starting dose for acute oral toxicity testing) from IC_{50} (see **Appendix K-3** for the data used). Over- or under-prediction of toxicity was determined by subtracting the predicted LD_{50} in mg/kg (i.e., the rat oral LD_{50} calculated using the RC IC_{50} in the regression equation) from the observed LD_{50} in mg/kg (i.e., the *in vivo* rat oral LD_{50} from the RC that was used to develop the regression). Negative values indicated that toxicity was underpredicted by the regression (i.e., predicted LD_{50} was greater than observed LD_{50}) and positive values indicated that toxicity was overpredicted by the regression (i.e., predicted LD_{50} was less than observed LD_{50}). This analysis assumed that the regressions either underpredicted or overpredicted the toxicity of all of the substances evaluated. In other words, there was a difference between the LD_{50} predicted by the regression and the *in vivo* LD_{50} used to calculate the regression even if it was a tiny fraction (i.e., no substances fit the regression exactly).

The proportion of low or high molecular weight chemicals that were under- and over-predicted in terms of acute oral toxicity (i.e., predicted LD_{50} values were higher or lower than reported *in vivo* LD_{50} values, respectively) using a millimole regression were calculated. These proportions were compared with those for chemicals that did not have low or high molecular weights. The same calculations were then performed for a weight-based regression. The proportions of under- and over-prediction of the toxicity for the millimole and weight-based regressions were compared to determine whether the weight regression increased the proportion of low molecular weight chemicals for which toxicity was underpredicted or the proportion of high molecular weight chemicals for which toxicity was overpredicted.

The millimole regression used was the RC rat-only millimole regression. The RC rat-only regression in millimole units, was calculated using the IC_{50} and oral LD_{50} data from the 282 RC chemicals with rat oral LD_{50} values and is strikingly similar in slope and intercept to the original RC millimole regression, which was based on 347 chemicals (282 chemicals with rat LD_{50} data and 65 chemicals with mouse LD_{50} data) (see **Table J7-1**). The weight-based regression used was the RC rat-only weight regression calculated using the IC_{50} and oral LD_{50} values from the 282 RC chemicals with rat oral LD_{50} values (see **Table J7-1**).

Table J7-1 IC_{50}-LD_{50} Linear Regressions

Moniker	Data Used	Slope	Intercept	R^2
RC millimole regression	347 RC substances with oral rat and mouse LD_{50} data – millimole units[1]	0.435	0.625	0.452
RC rat-only millimole regression	282 RC substances with rat oral LD_{50} data – millimole units[1]	0.439	0.621	0.452
RC rat-only weight regression	282 RC substances with rat oral LD_{50} data – weight units[2]	0.372	2.024	0.325

Abbreviations: RC=Registry of Cytotoxicity; R^2=coefficient of determination
[1]IC_{50} in mM; LD_{50} in mmol/kg.
[2]IC_{50} in μg/mL; LD_{50} in mg/kg.

J.7.2 Results

Figures J7-1 and **J7-2** show either the low molecular weight or high molecular weight chemicals plotted with either the RC rat-only millimole regression or the RC rat-only weight regression. Since LD_{50} is inversely related to toxicity, low LD_{50} values indicate high toxicity and high LD_{50} values indicate low toxicity. The regression lines show the predicted LD_{50} for each IC_{50}. The regression lines underpredict the toxicity of chemicals that are plotted below the lines (i.e., predicted LD_{50} > *in vivo* LD_{50} and predicted toxicity < *in vivo* toxicity). The regression lines overpredict the toxicity of chemicals that are plotted above the lines (i.e., predicted LD_{50} < *in vivo* LD_{50} and predicted toxicity > *in vivo* toxicity).

Of the 282 RC substances with rat oral LD_{50} values, there were 51 substances with molecular weights ≤100 g/mole and 231 substances with molecular weights >100 g/mole. **Figure J7-1** shows the 51 low molecular weight chemicals (i.e., with molecular weight ≤100 g/mole) graphed with both the RC rat-only millimole regression (**Figure J7-1a**) and the RC rat-only weight regression (**Figure J7-1b**). The RC rat-only millimole regression underestimated the toxicity of 20/51 (39%) substances and overestimated the toxicity of 31/51 (61%) substances (see **Table J7-2**). The RC rat-only weight regression underestimated the toxicity of 24/51 (47%) substances and overestimated the toxicity of 27/51 (53%) substances. Fisher's exact test indicated that there was no difference between the millimole and weight regressions for the under and over-prediction rates of toxicity for the 51 low molecular weight substances (two-tailed p=0.549) (see **Table J7-3**).

Figure J7-1 Rat-only Regressions Graphed with 51 Chemicals with Molecular Weight ≤100 g/mole

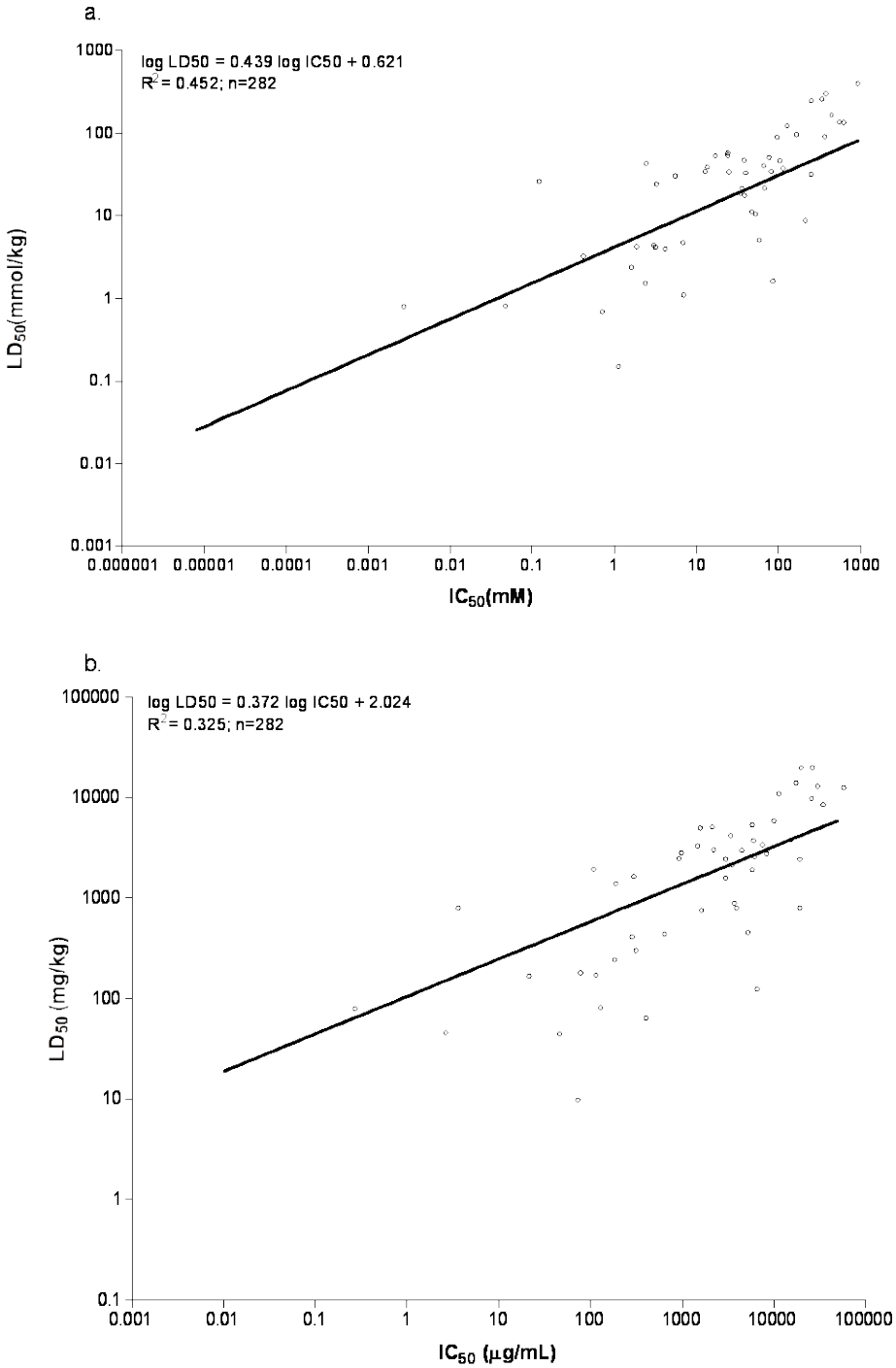

Figure J7-1a shows the RC rat-only millimole regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 20/51 (39%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 31/51 (61%) chemicals. **Figure J7-1b** shows the RC rat-only weight regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 24/51 (47%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 27/51 (53%) chemicals.

Table J7-2 Over- and Under Prediction of Toxicity for Low and High Molecular Weight Chemicals Using RC Rat-only Weight and Millimole Regressions

Regression	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted
	51 Chemicals with Molecular Weight ≤100 g/mole		231 Chemicals with Molecular Weight >100 g/mole	
RC Rat-only Weight	24/51 (47%)	27/51 (53%)	101/231 (44%)	130/231 (57%)
RC Rat-only Millimole	20/51 (39%)	31/51 (61%)	108/231 (47%)	123/231 (53%)
	20 Chemicals with Molecular Weight ≥400 g/mole		262 Chemicals with Molecular Weight <400 g/mole	
RC Rat-only Weight	4/20 (20%)	16/20 (80%)	121/262 (46%)	141/262 (54%)
RC Rat-only Millimole	7/20 (35%)	13/20 (65%)	121/262 (46%)	141/262 (54%)

Table J7-3 Over- and Under Prediction of Toxicity for Low and High Molecular Weight Substances Using RC Rat-Only Weight and Millimole Regressions

Comparison	For	Fisher's Exact Test[1]
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 51 substances with molecular weight ≤100 g/mole	0.549
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 231 substances with molecular weight >100 g/mole	0.575
51 Low molecular weight (≤100 g/mole) substances vs. 231 other substances (>100 g/mole)	RC rat-only millimole regression	0.355
51 Low molecular weight (≤100 g/mole) substances vs. 231 other substances (>100 g/mole)	RC rat-only weight regression	0.756
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 20 substances with molecular weight ≥400 g/mole	0.480
RC rat-only millimole vs. RC rat-only weight regression	Under- and over-prediction of toxicity for 262 substances with molecular weight <400 g/mole	NT
20 High molecular weight substances (≥400 g/mole) vs. 262 other substances (<400 g/mole)	RC rat-only millimole regression	0.362
20 High molecular weight substances (≥400 g/mole) vs. 262 other substances (<400 g/mole)	RC rat-only weight regression	0.033

Abbreviations: RC=Registry of Cytotoxicity; NT=Not tested since the proportions were the same.
Toxicity was underpredicted for 121/262 (46%) substances and overpredicted for 141/262 (54%) substances.
[1]P-values.

For the 231 substances with molecular weights >100 g/mole, the RC rat-only millimole regression underestimated the toxicity of 108/231 (47%) substances and overestimated the toxicity of 123/231 (53%) substances (see **Table J7-2**). The RC rat-only weight regression underestimated the toxicity of 101/231 (44%) substances and overestimated the toxicity of 130/231 (57%) substances. Fisher's exact test indicated that there was no difference between the millimole and weight regressions for the under- and over-prediction rates for the 231 substances with molecular weight >100 g/mole (two-tailed p=0.575; see **Table J7-3**). Additionally, Fisher's exact test also showed that there was no difference in the under- and over-prediction rates for the 51 substances with molecular weight ≤100 g/mole compared to the under- and over-prediction of the toxicity of the 231 substances with molecular weight >100 g/mole (two-tailed p=0.756 for the RC rat-only weight regression and two-tailed p=0.355 for the RC rat-only millimole regression).

Of the 282 RC substances with rat oral LD_{50} values, there were 20 substances with molecular weights ≥400 g/mole and 262 substances with molecular weights <400 g/mole (see **Table J7-2**). **Figure J7-2** shows the 20 chemicals with molecular weights ≥400 g/mole plotted with the RC rat-only milllimole regression (**Figure J7-2a**) and the RC rat-only weight regression (**Figure J7-2b**). The RC rat-only millimole regression underestimated the toxicity of 7/20 (35%) substances and overestimated the toxicity of 13/20 (65%) substances (see **Table J7-2**). The RC rat-only weight regression underestimated the toxicity of 4/20 (20%) substances and overestimated the toxicity of 16/20 (80%) substances. Fisher's exact test indicated that there was no difference between the millimole and weight regressions for the under- and over-prediction of toxicity for the 20 high molecular weight substances (two-tailed p=0.480; see **Table J7-3**).

For the remaining 262 substances with molecular weights <400 g/mole, the RC rat-only millimole and the RC rat-only weight regressions both underestimated the toxicity of 121/262 (46%) substances and overestimated toxicity of 141/262 (54%) substances (see **Table J7-2**). Thus, there was no difference in the two regressions in the rates of under- and over-estimation of toxicity for the 262 substances with molecular weights <400 g/mole. Fisher's exact test also showed that there was no difference in the rates for under- and over-prediction of the toxicity of substances with high molecular weight (≥400 g/mole) compared with the under- and over-prediction of the toxicity of substances with lower molecular weight for the RC rat-only millimole regression (two-tailed p=0.362; see **Table J7-3**). For the RC rat-only weight regression, however, there was a significant difference in the under- and over-prediction rates for substances with high molecular weight (>400 g/mole) compared with the under- and over-prediction rates for substances with lower molecular weight (two-tailed p =0.033). Thus, the weight-based regression overestimated the toxicity of the high molecular weight substances (compared with substances with lower molecular weight) while the millimole regression did not.

Figure J7-2 Rat-only Regressions Graphed with 20 Chemicals with Molecular Weight ≥400 g/mole

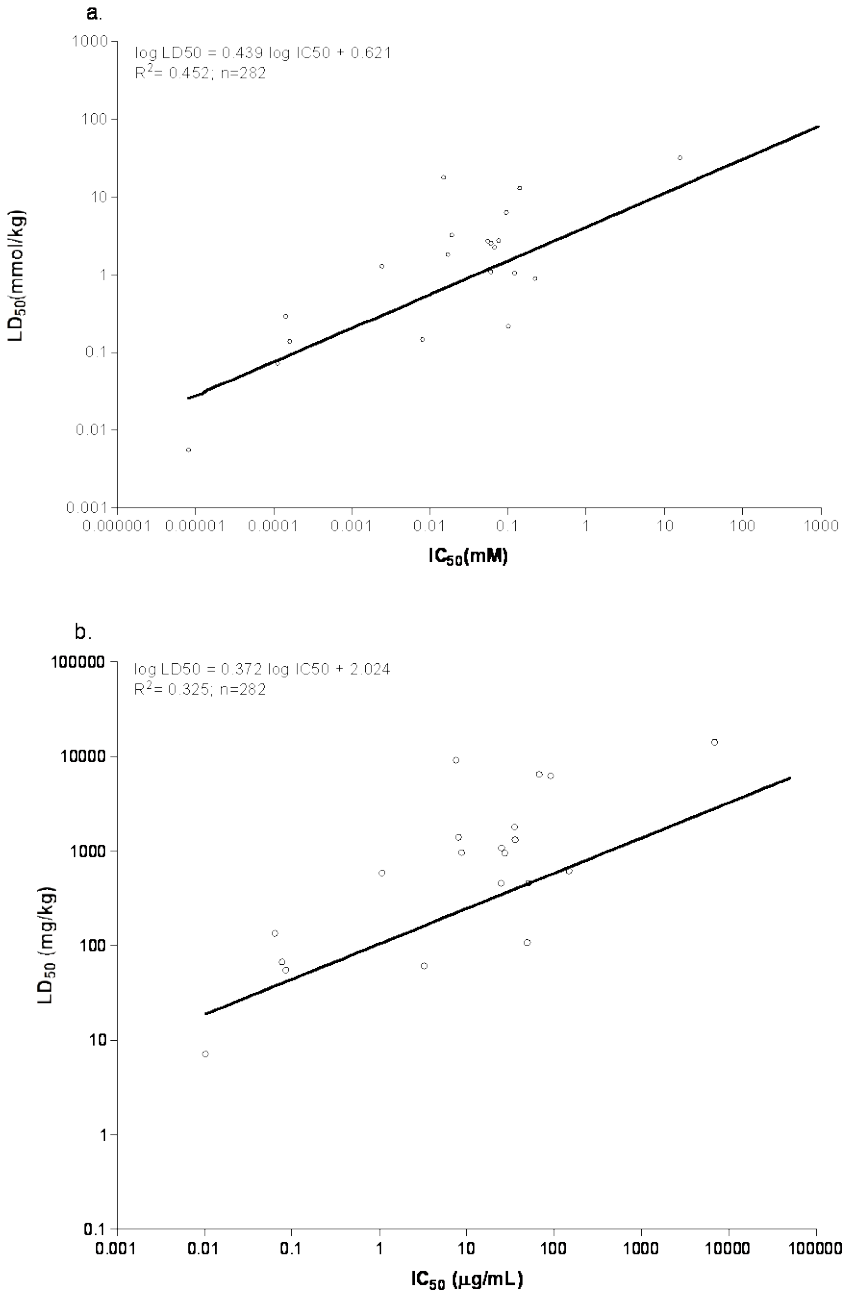

Figure J7-2a shows the RC rat-only millimole regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 7/20 (35%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 13/20 (65%) chemicals. **Figure J7-2b** shows the RC rat-only weight regression. Toxicity is underpredicted (i.e., predicted LD_{50} > *in vivo* LD_{50}) for 4/20 (20%) chemicals. Toxicity is overpredicted (i.e., predicted LD_{50} < *in vivo* LD_{50}) for 16/20 (80%) chemicals.