

## **Appendix L-3**

### **Analysis of Outliers by Halle (1998) for the RC Millimole Regression**

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### L.3 Analysis of Outliers for the RC Millimole Regression

The RC millimole regression was constructed from the *in vitro* IC<sub>50X</sub> cytotoxicity data from multiple cell lines and the *in vivo* acute toxicity data from rats and mice (i.e., LD<sub>50</sub> values) for 347 chemicals (Halle 1998). Halle (1998) investigated the 95 (27.4%) chemicals for which the observed log LD<sub>50</sub> values were greater than 0.699 (i.e., 0.5 log) from predicted log LD<sub>50</sub> values. Of the 95 outliers, 46 were positive outliers and 49 were negative outliers. The positive outliers have IC<sub>50X</sub> values that predict a far higher *in vivo* toxicity (i.e., lower LD<sub>50</sub>) than the actual animal experiment. The negative outliers are more important since the IC<sub>50X</sub> values predict lower toxicity (i.e. higher LD<sub>50</sub>) than the observed *in vivo* toxicity. It seems that Halle (1998) was not concerned about the positive outliers since the prediction erred in a health protective direction. Halle (1998) was much more concerned about trying to explain the reasons for the negative outliers since the error was in a nonconservative direction.

Halle (1998) investigated three factors that could have explained the negative outliers.

1. Variation in the oral LD<sub>50</sub> values

Reported oral LD<sub>50</sub> values for a particular chemical might vary by factor 4 to 14 even when experiments were highly standardized. They found LD<sub>50</sub> values from other sources for 23 of the 95 outliers. They found that the variations in the LD<sub>50</sub> values (difference between the RTECS® value and the “new” value found for the 23 chemicals) were larger for the negative outliers than for the positive outliers.

2. Species-specificity of the oral LD<sub>50</sub> values.

Halle (1998) compared an IC<sub>50X</sub>–LD<sub>50</sub> regression using mouse LD<sub>50</sub> values (242 values) with a regression using rat LD<sub>50</sub> values (285 values) and found no significant difference between the two regressions. The RC millimole regression with 347 chemicals has 285 rat values and 65 mouse values and is not statistically different from either the rat or mouse regressions.

3. The cell culture(s) used may have been unsuitable for the detection of cytotoxic potential or it may have been unable to simulate the complex process of toxicity *in vivo*.

Halle (1998) expected, *a priori*, that three classes of compounds, insecticides (**Table L3-1**), neurotoxins (**Table L3-2**), and those requiring metabolic activation for toxicity (**Table L3-3**), would not fit the RC millimole regression (i.e., cytotoxicity data would not predict *in vivo* toxicity). Sixty-two of the 347 chemicals belong to these three classes. Twenty-three (37.1%) of the 62 chemicals were negative outliers. Of the 23, 10 were insecticides, 5 were neurotoxins, and 8 required metabolic activation. No positive outliers were identified in the three classes.

Of the 49 negative outliers, 23 (46.9%) belonged to the three classes of concern. Examination of these classes showed that the RC millimole prediction was accurate (i.e., predicted log LD<sub>50</sub> [mmol/kg] was within 0.699 of observed log LD<sub>50</sub> in [mmol/kg]) for 50% of the insecticides (**Table L3-1**) and chemicals that required metabolic activation (**Table L3-3**). For neurotoxins (**Table L3-2**), the results were even better, since 21 (80.8%) fell within the prediction interval. Halle (1998) felt that the ability to predict the acute LD<sub>50</sub> for 50% of the insecticides and xenobiotics requiring metabolic activation and for 81% of the neurotoxic xenobiotics was sufficiently accurate for practical purposes.

Of the 49 negative outliers in the RC millimole regression, 23 (46.9%) of these belonged to the three classes of concern that may explain the false negative IC<sub>50X</sub> values. Findings were contrary to Halle's assumption that *in vitro* cytotoxicity would not predict *in vivo* toxicity for these types of chemicals. The RC millimole prediction of LD<sub>50</sub> was applicable to 50% of the insecticides and chemicals that required metabolic activation. For neurotoxic chemicals the results were even better, since 21 (80.8%) fell within the prediction interval. Halle felt that the ability to predict the acute LD<sub>50</sub> for 50% of the insecticides and chemicals requiring metabolic activation and for 81% of the neurotoxic chemicals was sufficiently accurate for practical purposes.

In separate analyses, Halle (1998) considered the physicochemical properties of chemicals (i.e., molecular weight and the octanol/water partition coefficient) as independent variables in a multiple regression analysis, but they did not improve the prediction of LD<sub>50</sub> by IC<sub>50</sub>.

### L3-1 The Error of Prediction<sup>a</sup> of 20 of The Most Important Insecticides in the RC Ordered According to Their Chemical Characteristics<sup>b</sup>

Chemical class	RC No	Name	LD <sub>50</sub> Error of Prediction <sup>a</sup>
<b>Chlorinated hydrocarbon</b>			
	26	Kelthane	0.340
	40	Chlordan	-0.046
	43	Aldrin	<b>-1.074<sup>b</sup></b>
	61	DDT	<b>-0.775</b>
	167	DDD	-0.378
	185	Heptachlor	<b>-1.050</b>
	195	DDA	0.133
	197	DDE	0.251
	207	Dieldrin	<b>-1.223</b>
	223	Lindane	<b>-1.043</b>
<b>Organophosphorus compounds</b>			
	49	Parathion	<b>-2.339</b>
	51	Disulfoton	<b>-2.346</b>
	67	Malathion	0.106
	75	Trichlorfon	-0.136
	96	Cygon	<b>-0.848</b>
<b>Carbamate compounds</b>			
	73	Carbaryl	-0.279
	186	Zineb	<b>1.185</b>
<b>Other compounds</b>			
	134	Rotenone	0.583
	173	Pentachlorophenol	<b>-0.720</b>
	235	Paraquat	<b>-1.019</b>

<sup>a</sup> defined as observed log LD<sub>50</sub> (mmol/kg) - predicted log LD<sub>50</sub> (mmol/kg)

<sup>b</sup> modified from Table 10 of Halle (1998)

**bold numbers:** outliers (i.e., observed log LD<sub>50</sub> (mmol/kg) - predicted log LD<sub>50</sub> (mmol/kg) > 0.699)

**Table L3-2 The Error of Prediction<sup>a</sup> of 26 Neurotoxic Xenobiotics in the RC Ordered According to Their *In Vivo* Potency<sup>b</sup>**

Chemical Class	RC No	Name	LD <sub>50</sub> Error of Prediction <sup>a</sup>
<b>Sedative, hypnotic, CNS depressants</b>			
	69	Secobarbital sod.	-0.651
	83	Thiopental	-0.119
	84	Amobarbital	-0.335
	87	Pentobarbital sodium	-0.654
	101	Gluthetimide	-0.270
	118	Phenobarbital	<b>-1.035<sup>b</sup></b>
	247	(+)-Thalidomide	-0.397
	264	Chloral hydrate	-0.349
	317	Barbital sodium	-0.591
<b>Antidepressant</b>			
	38	Imipramine HCl	-0.093
	90	Iproniazid	-0.273
	183	Amitriptyline	0.021
<b>Antipsychotic, anxiolytic</b>			
	27	Chlorpromazine	-0.176
	44	Hydroxyzine HCl	0.248
	63	Diazepam	0.116
	170	Thioridazine HCl	-0.013
<b>Stimulants</b>			
	112	Caffeine	<b>-0.815</b>
	262	Amphetamine sulfate	<b>-1.579</b>
<b>Anticonvulsants</b>			
	82	Diphenylhydantoin	-0.551
<b>Analgetic (general anesthesia)</b>			
	229	Dextropropoxyphene HCl	<b>-1.150</b>
<b>Anticholinergic</b>			
	251	Scopolamine * HBr	-0.123
	296	Homatropine methylbromide	-0.532
<b>Other Neurotoxins (not insecticide)</b>			
	102	Acrylamide	-0.338
	137	Triethyltin chloride	<b>-0.852</b>
	142	Methylmercury chloride	0.105
	316	Toluene	0.571

<sup>a</sup> defined as observed log LD<sub>50</sub> (mmol/kg) - predicted log LD<sub>50</sub> (mmol/kg)

<sup>b</sup> modified from Table 11 of Halle (1998)

**bold numbers:** outliers (i.e., observed log LD<sub>50</sub> (mmol/kg) - predicted log LD<sub>50</sub> (mmol/kg) > 0.699)

**Table L3-3 The Error of Prediction<sup>a</sup> of the 16 Xenobiotics in the RC that Require Metabolic Activation<sup>b</sup>**

RC No	Name	LD <sub>50</sub> error of prediction <sup>a</sup>
13	Cycloheximide	<b>-1.370<sup>b</sup></b>
33	p-Chloromercuribenzoic acid	<b>-1.077</b>
37	Aflatoxin B <sub>1</sub>	<b>-1.783</b>
68	2,4-Dinitrophenol	<b>-1.128</b>
97	Phenacetin	0.292
109	Frusemide	0.109
113	Acetaminophen	0.386
116	Cyclophosphamide * H <sub>2</sub> O	<b>-1.310</b>
123	Isoniazid	-0.332
125	Carbon tetrachloride	0.229
192	1,3-Bis(2-chloroethyl)-1-nitrosourea	<b>-1.176</b>
260	Coumarin	-0.427
273	Bromobenzene	0.374
279	Thioacetamide	-0.294
281	1,2-Dibromomethane	<b>-1.106</b>
292	Allyl alcohol	<b>-0.952</b>

<sup>a</sup> defined as observed log LD<sub>50</sub> (mmol/kg) - predicted log LD<sub>50</sub> (mmol/kg)

<sup>b</sup> modified from Table 12 of Halle (1998)

**bold numbers:** outliers (i.e., observed log LD<sub>50</sub> (mmol/kg) - predicted log LD<sub>50</sub> (mmol/kg) > 0.699)

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