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Inter-Species Toxicity Correlations between Rat, Mouse, and Photobacterium phophoreum

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INTER-SPECIES TOXICITY CORRELATIONS BETWEEN RAT, MOUSE AND *Photobacterium phosphoreum*

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Abstract - This study investigates quantitatively the inter-species relationships of the acute toxicity of 684 organic chemicals to the rat, the mouse and the luminescent marine bacterium *Photobacterium phosphoreum*, commonly known as Microtox™ [3] test.

The results indicate significant relationships between the Microtox EC50 and rat and mouse LD50 values. The goodness of fit increases strongly from the oral to the intraperitoneal to the intravenous route of administration for each of the mouse and rat. Standard errors of the estimated rat values range from 0.52 to 0.72 log units of toxicity (after and before outlier removal, respectively) over a toxicity range of 4.6 (intraperitoneal) to 5.0 (oral) log units (mmol/kg b.w.) of toxicity. For each of the three routes of administration, rat and mouse data are also highly correlated. This allows the computation of rat toxicities from mouse data and vice versa with standard errors of the estimates of 0.28 (intraperitoneal) to 0.30 (oral) log units.

Keywords - Acute toxicity, correlation, rat, mouse, oral, intravenous, intraperitoneal, Microtox, *Photobacterium phosphoreum*.

[3] Microtox is a registered trademark of Microbics Corp., Carlsbad, California.

MANAGEMENT PERSPECTIVE

Title: Inter-species toxicity correlations between rat, mouse and *Photobacterium phosphoreum*

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Perspective:

This work presents analyses of the linear correlations between the acute toxicity of 684 organic chemicals in the Microtox test, which uses the luminescent bacterium *Photobacterium phosphoreum* and the acute lethal doses to the rat and the mouse, for three routes of administration, i.e. oral, intraperitoneal and intravenous.

The results show:

(i) The goodness of fit of the Microtox EC50 values with the mammal LD50 values increases strongly from the oral to the *intraperitoneal* to the *intravenous* route of administration for each of the mouse and rat.

(ii) For each of the three routes of administration, rat and mouse data are also highly correlated. This allows the computation of rat toxicities from mouse data and vice versa with standard errors of the estimates of 0.28 (*intraperitoneal*) to 0.30 (oral) log units.

(iii) The usefulness of the comparatively quick and inexpensive *Photobacterium phosphoreum* (Microtox) test for the approximation of the acute toxicity of individual chemicals to the rat and mouse.

(iv) The usefulness of the large database on rat and mouse LD50 values to estimate toxicity of chemicals to aquatic organisms.

INTRODUCTION

Recent studies into the inter-species correlations of Microtox data with the acute and subacute to chronic toxicities of organic compounds to a variety of aquatic organisms have shown high degrees of collinearity. In particular, this has been demonstrated for the acute effects on several freshwater species, especially the fathead minnow (*Pimephales promelas)* [1], guppy (*Poecilia reticulata*), goldorfe (*Leuciscus idus melanotus*) and *Daphnia magna* [1], the marine fish sheepshead minnow (*Cyprinodon variegatus*) [2] as well as subchronic effects on the fathead minnow [2].

Parallel to these investigations, the availability of large data sets on the toxicity of organic substances to the mouse [3] and to *Photobacterium phosphoreum* [4,5] as well as the identified need to develop faster and less expensive product screening tests [6] resulted in recently published correlations of acute oral and i.v. LD50 values for mouse with the corresponding Microtox EC50 values [6,7]. These regressions showed statistically significant relationships with correlation coefficients of r= 0.29 (n= 123, P= 0.0012) for the oral mouse LD50 and r= 0.73 (n= 51, P< 0.0001) for the i.v. mouse LD50 data [6].

The availability of a large, multispecies database with all data in a common format [8] is now being utilized to expand these relationships to a wider set of chemicals with numerous chemical classes as well as to include oral, intravenous and intraperitoneal LD50 data for both the mouse and rat. With this dataset, covering approximately 600 individual chemicals, the previously found relationships can be investigated in more detail, possibly also leading to the identification of a common structural feature of highly toxic outliers and strengthen the statistical values of the underlying relationships, such as shown for the fathead minnow [1].

DATA SOURCES AND CONVERSIONS

Sources of the data were the COMPUTOX™ Database module [8] augmented with new on-line data from the *Registry of Toxic Effects of Chemicals* [3] database. The COMPUTOX Database was developed from data published in the primary scientific literature, data in certain other data compilations, such as the Merck Index [9], the *Photobacterium phosphoreum* Toxicity Data Index [5], the log K_{ow} compilations and predictions by Hansch and Leo [10] and Sangster [11], as well as from private

and unpublished data. The main focus in the development of this database has been on aquatic species. Table 1 lists some of the aquatic and terrestrial species and endpoints, as well as some of the physico-chemical descriptors given in the database. One overriding criterion is the compatibility of the data; this was achieved by rigorous standardization as to the units, coupled with reference to the original publications where thought to be necessary. For example, the octanol/water partition coefficients are presented in four columns to distinguish between measured, CLOGP calculated [10], LOGKOW derived [11] and "final" values. Where thought necessary, data taken from other compilations were checked for accuracy against the values found in the primary literature. Experience shows though, that errors occur even in the primary literature, some of which may be difficult to spot and to eliminate at this time.

The COMPUTOX database contains published and unpublished data in a common format [-log(BE)] = [log(1/BE)], where BE is the concentration needed to reach the toxicological endpoint, such as LC50, EC50, LD50, and soforth. At present, the majority of data are acute toxicity values for aquatic species, i.e. 30-min Microtox EC50, 48-hr *Daphnia magna* LC50, and 96-hr fathead minnow (*Pimephales promelas*) LC50 concentrations and a few subacute to chronic effects, such as 32-day fathead minnow LC50 values. For the terrestrial species, LD50 values for oral, intravenous, and intraperitoneal exposure routes for rat and mouse are given. At present, 35 toxicity endpoints are covered for 30 species.

In order to be able to undertake qualitative and quantitative comparisons between the data it is imperative that they are presented in a common format. In general, biological data are found in the literature in the form of mg/L or mg/kg body weight. This form of data cannot be compared and must be transformed to molar units. For practical purposes, we use millimolar (mmol/L and mmol/kg body weight) units. Due to the large span of toxicity and physico-chemical values for each parameter (commonly up to ten orders of magnitude), it is also of great advantage to transform all data into a logarithmic notation (base ten). This transformation is accompanied by an inversion to result in all toxicity data being given in the format log(L/mmol) for LC and EC type data and log(kg/mmol) for LD type data. Examples have been given in the *Photobacterium phosphoreum* toxicity data index [5]. To a large measure, the database used here is a horizontal extension of the data given in that index.

This spreadsheet presently contains approximately 1500 lines (after separation of multiple entries into a separate spreadsheet), each of which represents a unique chemical. The data are located in, at present, approximately 75 columns, where each column is a unique measured or computed

toxicological or physico-chemical parameter. With the computing power of modern personal computers, such as 486 microprocessor-based machines running at 50 Mhz or faster, sorting of a spreadsheet of that size takes about 10 to 20 seconds. Sorting can be done by any of the columns, i.e. numerical or label entries, or combinations thereof, in either ascending or descending order. In theory, this gives presently access to $2x\{75!-(75-5)!\} \approx 4{,}000{,}000{,}000$ ways of sorting these data. Of course, sorting is just one of several key features which make spreadsheets so useful. Other such features include (i) the ability to generate formula which can virtually perform any mathematical and statistical operation on any number of columns, (ii) plotting and graphing techniques which include the preparation of colour prints and slides, (iii) the interactive on-screen presence of large sections of data.

All correlations were run on a desktop computer with a 486 processor, using the Quattro® Pro (Borland International) spreadsheet, the Harvard Graphics™ (SPC Software Publishing Corp.) graphing, and the Statgraphics® (Statistical Graphics Corp.) and the SPSS® (SPSS Inc.) statistics programs.

RESULTS

Linear Regressions of Rat and Mouse LD50 versus Microtox EC50

Investigation of the LD50 data for the same route of exposure each for the rat and mouse show almost identical results when linearly regressed against the Microtox data. As given in Table 2, the oral route entry regressions for the rat and mouse have the same slope, correlation coefficient and virtually the same intercept and standard error. For the intraperitoneal and intravenous routes of entry, high degrees of collinearity and commonality also exist between the rat and mouse. Figure 1 shows an example of these results in a plot of mouse *i.v.* LD50 data versus the corresponding Microtox data for 158 organic chemicals.

The high degree of similarity between the corresponding regressions for the same route of exposure for rat and mouse is also evident from Figure 2. It shows three distinct pairs of regressions (one each for mouse and rat) for each of the exposure routes. It is apparent from the visual inspection

of Figure 2, that the significant correlations of mouse and rat LD50 data allow their combination, for a given route of administration, as the variation between the species is much smaller than the variation of the regression slopes between the three exposure routes for either species.

Combination of Rat and Mouse Data

The similarity of the regressions between mouse and rat for each of the exposure routes allows the development of inter-species regressions (specific for each route of exposure) as shown in Table 3. These equations can then be used for the prediction of missing rat (or mouse) data for substances for which only the corresponding mouse (or rat) data are available. This combination of mouse and rat data provides for much larger extended data sets of the oral rat (n= 531 versus 471), the intraperitoneal rat (n= 427 versus 195), and the intravenous rat (n= 180 versus 54) LD50 values. In total, these inter-species correlations provide for a substantial increase of the available "rat" data from 720 to 1138, thus allowing for more degrees of freedom in the statistical procedures, hence increased significance.

Linear Regressions of Microtox Data versus Extended Rat Data Sets

Using the extended sets for each of the oral, intraperitoneal and intravenous rat data, linear regressions were determined for each route of entry versus the corresponding Microtox data. The statistics and regression results for the extended rat data sets versus the corresponding Microtox EC50 values are given in Table 4. In total, these regressions use data on 684 different chemicals. These chemicals have not been preselected in any way, therefore cover a multitude of physico-chemical characteristics, chemical and biological properties and, presumably, also varying mechanisms of toxic action.

It was found that the extended oral rat data had the lowest coefficient of determination with r= 0.33 (n= 531, SE= 0.72) before, and r= 0.41 (n= 506, SE= 0.59) after outlier rejection. (Table 4). For the intraperitoneal rat data, the correlation coefficients are higher with r= 0.43 (n= 427, SE= 0.70) before, and r= 0.51 (n= 406, SE= 0.59) after outlier rejection. The highest correlations are found for the intravenous rat data with r= 0.66 (n= 180, SE= 0.65) before and r= 0.75 (n= 171, SE= 0.52) after outlier rejection. Plots showing the regression slopes for the extended data sets are shown in Figures 3 to 5.

Significance of Results

An analysis of variance (ANOVA) was performed on each of the extended data sets using the hypothesis that a linear relationship exists between the various routes of entry for the extended rat sets and the Microtox test. The results were highly significant, i.e. the variates y and x are probably connected by a genuine relationship.

The significance point for the correlation coefficient (r) is related to the corresponding (double-sided) critical levels of t by the formula

$$r = t/\sqrt{(\phi + t^2)},$$

where ϕ= degrees of freedom. The deviate of the t-curve which cuts off a double tail equivalent to P= 0.01 (99% significance) is given by t= 2.58 for $\phi = \infty$. As seen in Table 5, the F ratios obtained for the three relationships are much larger than the criterion at the 99.9% confidence level (F_{∞}= 10.83). This result indicates that the regressions of the extended rat data versus the Microtox data are highly significant and strengthen the hypothesis that a genuine linear relationship exists between the terrestrial and aquatic toxicity data.

DISCUSSION AND CONCLUSIONS

In terms of inter-species toxicity correlations, our results clearly show that there is a high degree of collinearity of the LD50 values for rat and mouse. These collinearities exist for several hundred chemicals with few outliers for each of three administration routes investigated, namely oral, intraperitoneal and intravenous exposure and were proven by both linear regression and ANOVA analysis. These correlations allow the computation of extended data sets of rat values from mouse data or vice versa.

This work also confirms earlier results on smaller data sets which demonstrated various degrees of relationships of the aquatic Microtox test data with oral and intravenous LD50 values for

the mouse [6]. These relationships are also shown to exist for the intraperitoneal route of administration for both the mouse and rat. The slopes of rat and mouse LD50 regressions on the corresponding Microtox EC50 values are practically identical between the two species, but vary strongly with the route of exposure and increase in the order oral < intraperitoneal < intravenous. This change in regression slopes has also been observed on much smaller data sets [6,14] and can be expected from the relative lack of metabolization and quicker transport of a chemical when administered intravenously relative to oral administration.

Earlier attempts to correlate aquatic and non-aquatic toxicity endpoints, such as mouse or rat LD50 values with acute lethal concentrations of chemicals to fish [12], *Daphnia magna* [13] and *Photobacterium phosphoreum* [6,7] have met with varying degrees of success. Frequently, small sets of data resulted in statistical limitations, small ranges of the observed effects, and lack of statistical significance. Our work demonstrates highly significant relationships over large toxicity ranges, even without selection as to the type of chemical class or mechanism of toxic action. The percentage of statistical outliers for the regression of the rat LD50 values against the Microtox values remains the same at 5% when going from the "pure" rat data to the extended set which incorporate rat values predicted from the mouse data. This is the case even for the intraperitoneal and intravenous route LD50 values where the number of extended data is more than double of pure data and, obviously provides another indication of the validity of both the mouse-rat and rat-Microtox relationships.

Without any preselection of the chemicals represented or, alternatively inclusion of additional variables into multilinear regressions, the standard errors of the estimated (rat) values from the Microtox values are not likely to decrease much below the observed values of approximately 0.7 log units (Table 2). However, as has been shown for rat - *Daphnia magna* correlations [13], for fathead minnow (*Pimephales promelas*) - Microtox correlations [1,2], and for numerous correlations of acute and sublethal effects of narrowly defined groups of chemicals with the octanol/water partition coefficient [14,15], the incorporation of indicators for specific functional groups, such as for nitro, amino, hydroxy, or ether groups, can substantially decrease the standard errors and increase the correlation coefficients. The toxicity database we are using presently contains such indicators for 27 functional groups or structural elements. Further investigations into the effects of particular structure elements on the rat - *Photobacterium* relationships are in progress and will be reported on later.

Further, it is apparent that investigations with larger data sets of several hundred compounds and a multitude of parameters are most effectively dealt with by using modern desktop computers

with advanced spreadsheet software. The advantages of the spreadsheet type databases over the "file card" principle are significant and have only partially been explored here. Apart from the powerful sorting and query routines, which one can also label as typical "database" functions, the spreadsheet macro command functions allow the development of semi-automatic applications, including search and replace functions, worksheet linkages and other operations. In addition, simple graphing and statistical tools are very helpful in generating charts, plots and correlations of the data. For example, the built-in two-dimensional plotting and linear regression functions were used to generate all of the shown plots and regression functions. For more advanced statistical procedures, the spreadsheet data can easily be exported into specialized statistical software programs. Of course, a basic premise of the spreadsheet type database is the presentation of all values in a standard form, e.g. the base ten logarithms of the inverse millimolar concentration or dosage values.

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LIST OF FIGURES

Fig. 1. Scatterplot and regression of mouse intravenous LD50 versus Microtox EC50 values for 158 chemicals.

Fig. 2. Graph of linear regressions of oral, intraperitoneal and intravenous LD50 data for each rat and mouse versus the Microtox EC50 values for the same chemicals.

Fig. 3. Scatterplot and regression of extended rat oral LD50 versus Microtox EC50 values for 506 chemicals.

Fig. 4. Scatterplot and regression of extended rat intraperitoneal LD50 versus Microtox EC50 values for 406 chemicals.

Fig. 5. Scatterplot and regression of extended intravenous rat LD50 versus Microtox EC50 values for 171 chemicals after removal of outliers. Outliers removed are: Name (Microtox value, Rat value): Mitomycin-C (1.39, 2.05), isonicotinic acid (0.01, -1.50), Parathion (1.54, 1.88), tetramethylammonium bromide (-0.68, 2.02), hydrogen cyanide (0.50, 1.52), toluene (0.67, -1.33), 1,6-diisocyanatohexane (1.03, 1.57), Carbofuran (1.03, 2.77), and Paraquat [cation] (-0.71, 1.09).

Table 1. A select list of the number of chemicals for which bioassay endpoints and physico-chemical parameters are given in the COMPUTOX™ database.

Species	Endpoint	Chemicals
Microtox	**5 to 30-min EC50**	**1500***
Mouse	**oral LD50**	**425**
Mouse	**intraperitoneal LD50**	**432**
Mouse	**intravenous LD50**	**190**
Rat	**oral LD50**	**592**
Rat	**intraperitoneal LD50**	**221**
Rat	**intravenous LD50**	**66**
Partition coefficient	octanol/water	1000*
Molar refractivity	cm^3/g	200*
Solubility	aqueous; (1/mM)	300*
Henry constant	dimensionless	150*
Topology descriptors	up to 27 variables	1500*

* approximate number.

Table 2. Statistics and results of linear regressions of oral, intraperitoneal and intravenous rat and mouse LD50 basic data versus the corresponding Microtox EC50 values, before outlier rejection.

	Count	Minimum	Maximum	Range	Slope	Intercept	r	r^2	S.E.
Independent variable:									
Microtox	675	-4.00	5.73	9.73	n/a	n/a	n/a	n/a	n/a
Dependent variables:									
Oral rat	471	-2.66	2.28	4.94	0.20	-0.96	0.35	0.12	0.74
Oral mouse	344	-2.46	2.15	4.61	0.20	-0.86	0.35	0.13	0.72
Intraperitoneal rat	195	-2.37	2.22	4.59	0.29	-0.48	0.48	0.23	0.82
Intraperitoneal mouse	378	-2.83	3.28	6.11	0.25	-0.49	0.43	0.18	0.70
Intravenous rat	54	-1.98	2.05	4.03	0.40	-0.25	0.73	0.53	0.79
Intravenous mouse	165	-2.17	1.93	4.10	0.35	-0.30	0.68	0.46	0.61

Table 3. Inter-species regressions (rat= a + b×mouse) between mouse and rat LD50 values for oral, intraperitoneal and intravenous routes of exposure.

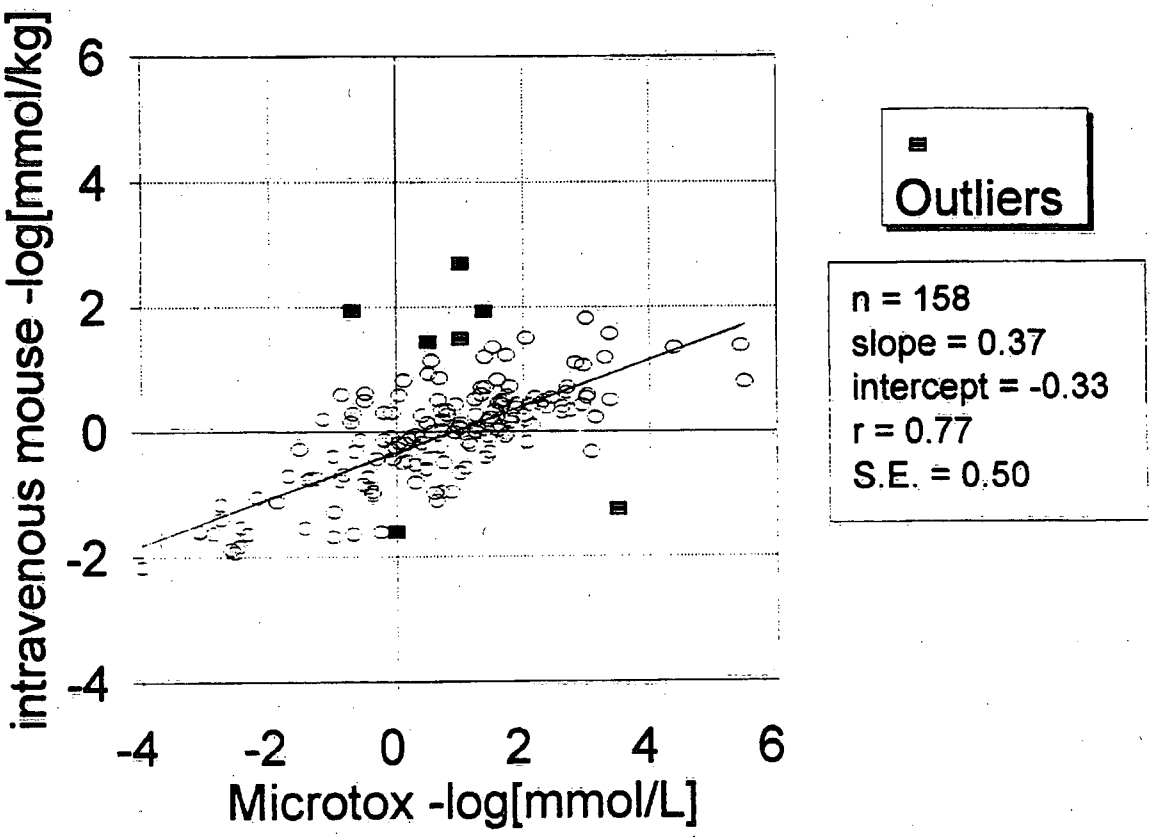
Dependent	Independent	n	r	r^2	slope	intercept	S.E
oral rat	oral mouse	330	0.94	0.88	0.97	-0.04	0.30
intraperitoneal rat	intraperitoneal mouse	162	0.96	0.92	1.02	-0.02	0.28
intravenous rat	intravenous mouse	41	0.97	0.94	0.99	0.10	0.29

Table 4. Statistics and regression results for extended rat oral, intraperitoneal and intravenous LD50 data versus Microtox EC50 values, before and after outlier rejection.

	Count	Minimum	Maximum	Range	Slope	Intercept	r	r^2	S.E.	F-ratio
Independent variable:										
Microtox	675	-4.00	5.73	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Dependent variables:										
Oral rat										
Before outlier rej.	531	-2.52	2.47	4.99	0.19	-0.95	0.33	0.11	0.72	63.4
After outlier rej.	506	-2.52	2.47	4.99	0.20	-1.03	0.41	0.17	0.59	102.2
Intraperitoneal rat										
Before outlier rej.	427	-2.37	2.22	4.59	0.25	-0.50	0.43	0.18	0.70	95.7
After outlier rej.	406	-2.37	2.22	4.59	0.26	-0.57	0.51	0.26	0.59	141.5
Intravenous rat										
Before outlier rej.	180	-1.98	2.77	4.75	0.35	-0.20	0.66	0.44	0.65	139.7
After outlier rej.	171	-1.98	2.77	4.75	0.36	-0.26	0.75	0.57	0.52	219.3

Table 5. ANOVA results and correlation coefficient significance for linear relationships between the rat and Microtox bioassay.

Species	Df N,D	F P=0.001	F-ratio	Sig. of F	r-criteria	r-meausred
oral rat (extended)	1,504	10.83	102.2	0.000	0.114	0.411
intraperitoneal rat (extended)	1,404	10.83	141.5	0.000	0.125	0.509
intravenous rat (extended)	1,169	10.83	219.3	0.000	0.191	0.751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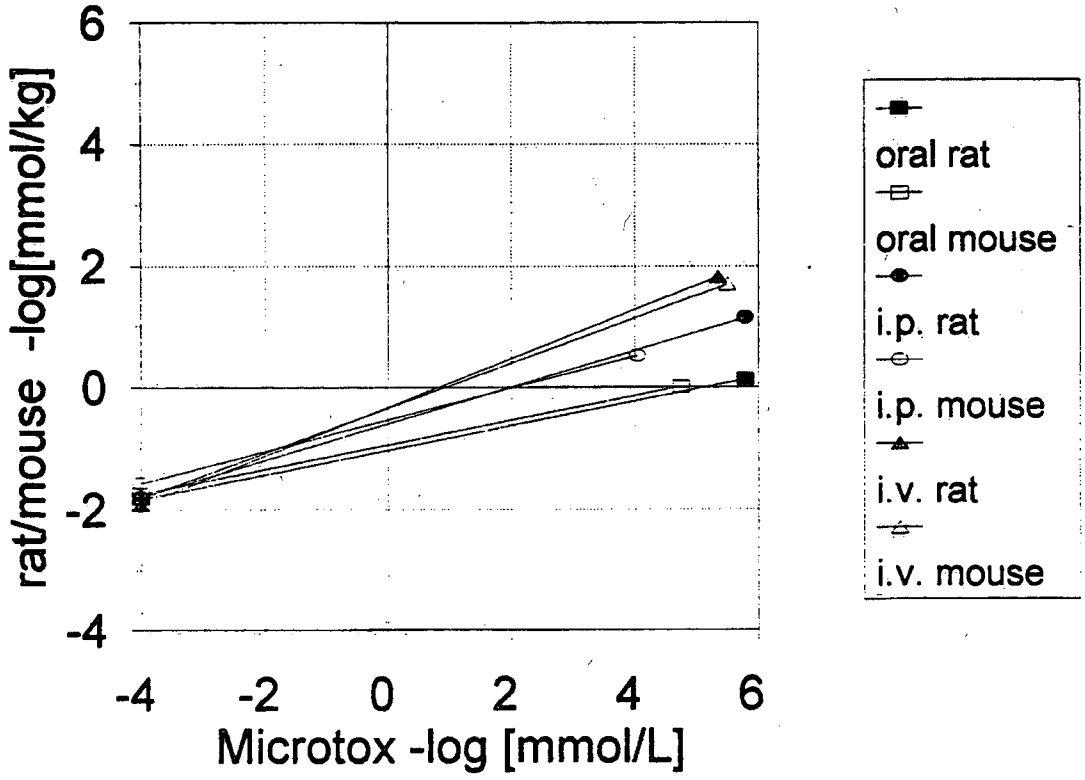
6
4
2
0
-2
-4
-4
-2
0
2
4
6
Microtox -log[mmol/L]
Outliers
n = 158
slope = 0.37
intercept = -0.33
r = 0.77
S.E. = 0.50

Fig. 1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4
2
0
-2
-4
rat/mouse -log[mmol/kg]
-4
-2
0
2
4
6
Microtox -log [mmol/L]
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oral mouse
i.p. rat
i.p. mouse
i.v. rat
i.v. mouse

Fig. 2

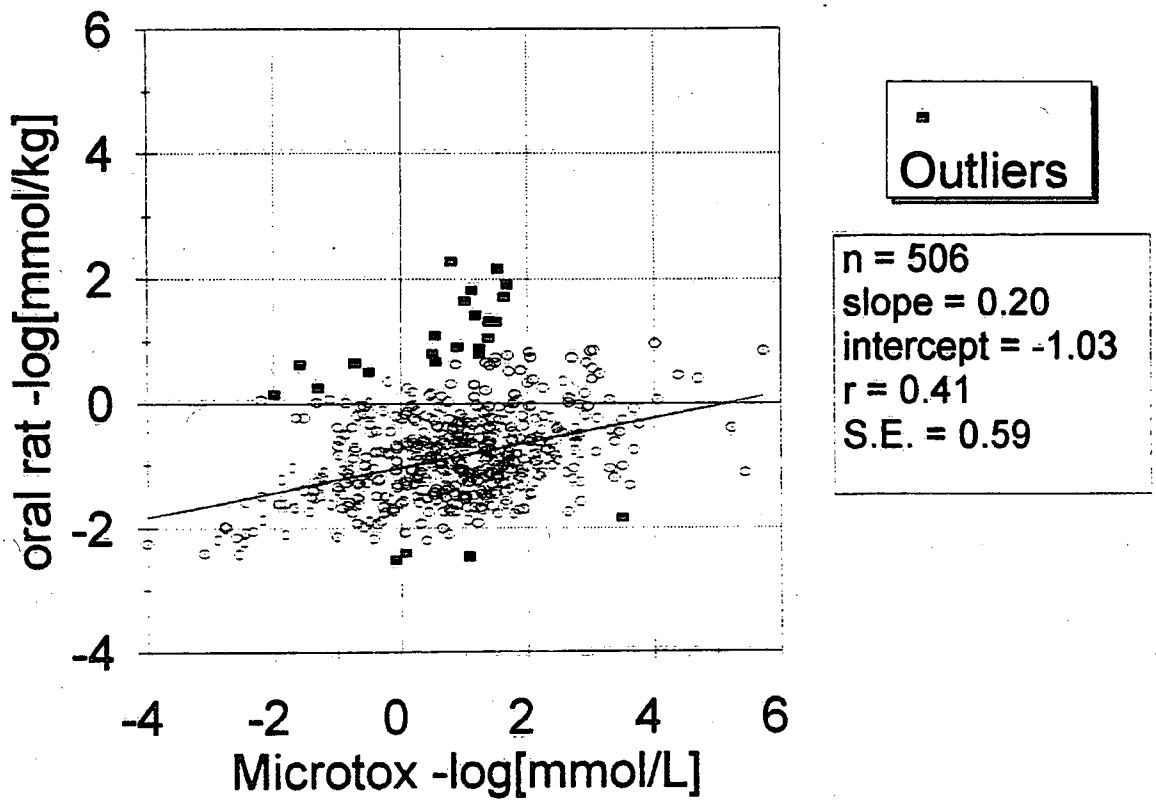
oral rat -log[mmol/kg]
6
4
2
0
-2
-4
-4
-2
0
2
4
6
Microtox -log[mmol/L]
Outliers
n = 506
slope = 0.20
intercept = -1.03
r = 0.41
S.E. = 0.59

Fig. 3

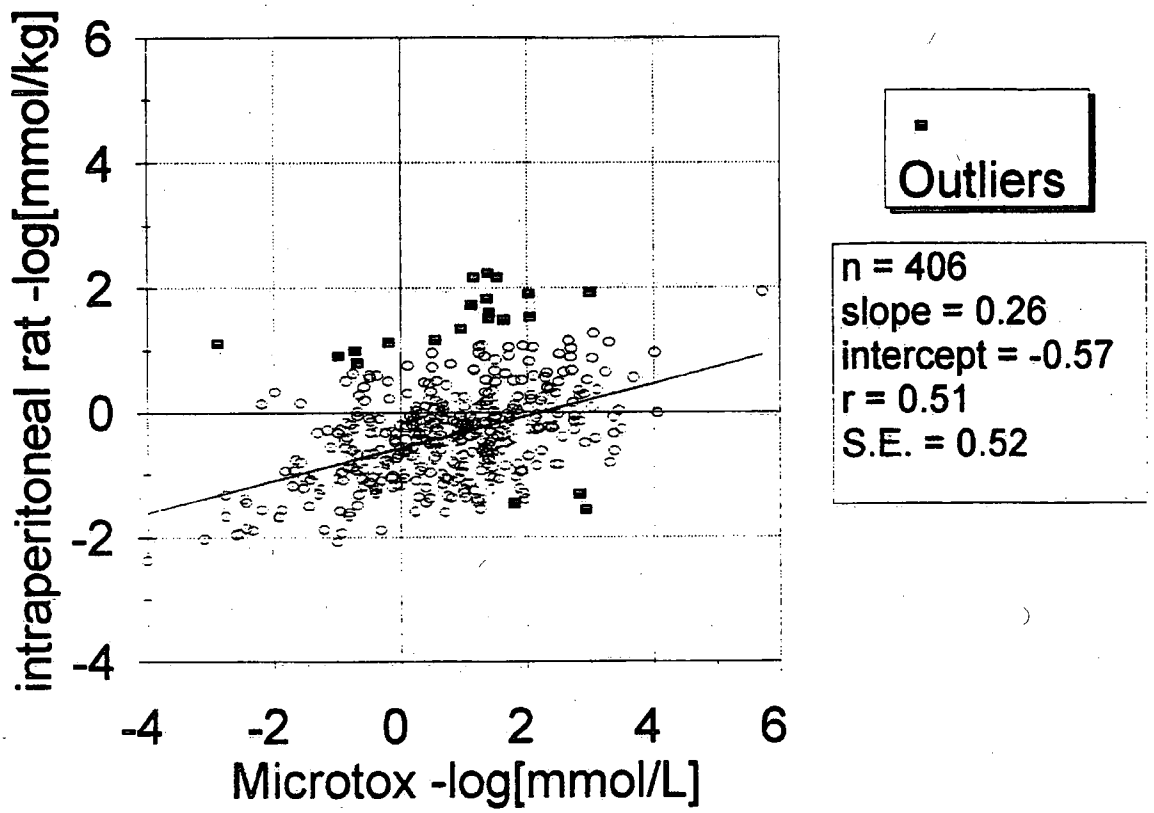
intraperitoneal rat -log[mmol/kg]
6
4
2
0
-2
-4
-4
-2
0
2
4
6
Microtox -log[mmol/L]
Outliers
n = 406
slope = 0.26
intercept = -0.57
r = 0.51
S.E. = 0.52

Fig. 4.

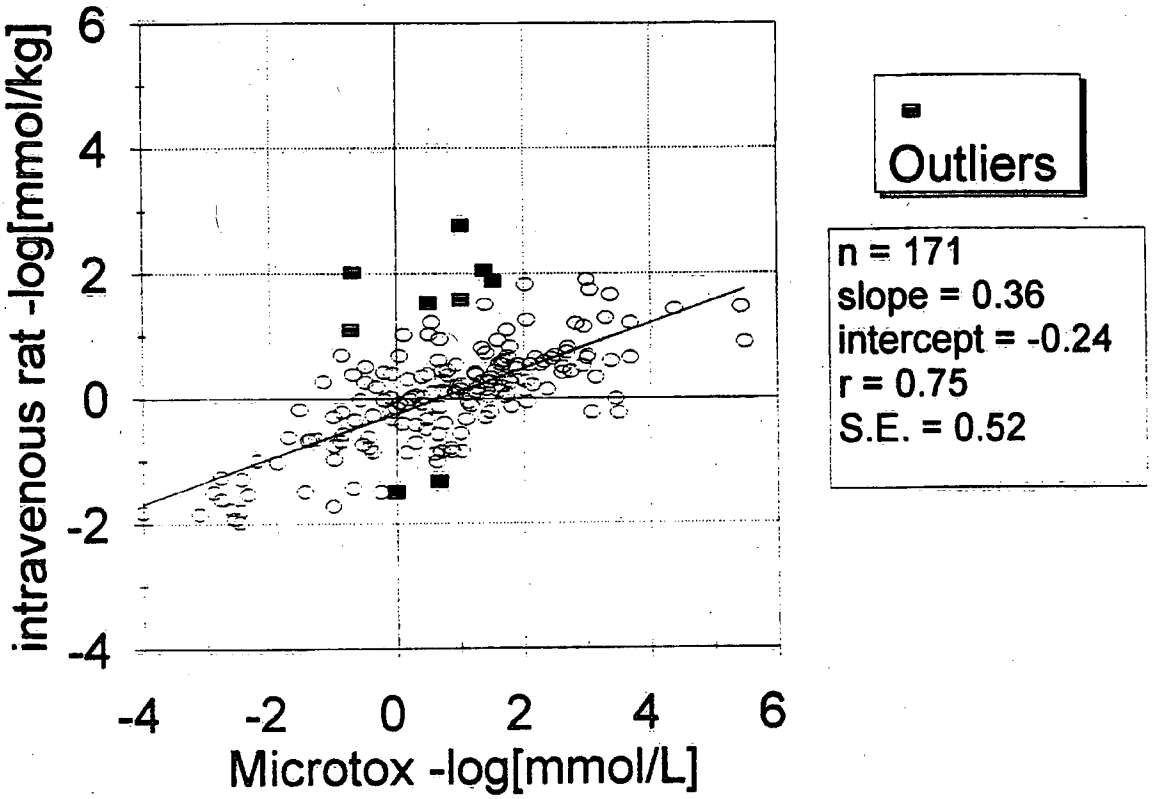
intravenous rat -log[mmol/kg]
6
4
2
0
-2
-4
-4
-2
0
2
4
6
Microtox -log[mmol/L]
Outliers
n = 171
slope = 0.36
intercept = -0.24
r = 0.75
S.E. = 0.52

Fig. 5

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