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Modeling Joint Effects of Mixtures of Chemicals on Microorganisms Using Quantitative Structure Activity Relationships

Grant Nº AFOSR-91-0394

Interim Progress Report
Phase II
August 1992 - October 1993

By
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- Phase II: Study Using Activated Sludge Cultures -

ABSTRACT

Toxicity of 50 organic chemicals to activated sludge microorganisms was determined using the respirometeric technique. Using this experimental database, models for predicting toxicity (IC50 values) were developed using QSAR techniques. Toxicity measurements were also made for sixteen multi-component mixtures. The joint effects of organic chemicals in these mixtures were analyzed by three different approaches. Using the QSAR models developed from single chemical studies, an approach was developed to analyze and predict joint effects of chemicals in mixtures. The results of this study indicated that the joint effects could be considered simply additive for the different classes of chemicals tested. Using the results obtained during the first phase of this project for a surrogate test microorganism- Polytox, toxicity correlations were established between activated sludge and the test cultures.

INTRODUCTION

Acute and chronic toxicity testing is a major component in the NPDES permitting process. The concept of whole effluent toxicity testing has been introduced into this program due to the realization that a mixture of several chemicals may exhibit greater toxicity than they would individually. While current Water Quality Standards are based on single chemical toxicity assays, in future, controls may be set based on the joint effects of mixtures of two or more chemicals. Non-point sources, industrial effluents, leachates and contaminated groundwaters are all known to contain several chemicals in mixtures. Thus, an ability to analyze and predict joint effects of mixtures of chemicals on microorganisms and other aquatic life forms will be of considerable benefit in managing the environmental hazards of synthetic chemicals.

Several ecological researchers, notably from Europe, have studied the effects of mixtures of chemicals on fish (Ref 1 - 20). Hardly any studies have been reported on the joint effects of multiple chemicals on microorganisms. As microorganisms are employed in municipal waste treatment by environmental engineers, and are also present in the natural environment, it would be of interest to be able predict such effects on microorganisms. This research was undertaken in our laboratories to determine and predict joint effects of binary and multiple chemical mixtures on three classes of organisms of interest to environmental engineers. This interim report covers the results of the Phase II study during the second year, and compares the results obtained during the first two years.

OBJECTIVES OF PHASE II STUDY

The ultimate objective of this 3-year research is to develop an approach to predict the joint toxic effects of mixtures of organic chemicals to microorganisms. Towards this end, the following tasks were identified for Phase II during the second year:

a) measurement of single chemical toxicity to activated sludge (A/S) microorganisms and establishing the reproducibility of the respirometeric test procedure;
b) development of QSAR models to predict single chemical toxicity to A/S microorganisms;
c) establishing correlations between the surrogate test culture- Polytox, (assayed during the first year) and A/S microorganisms;
d) measurement and analysis of toxicity of multi-component mixtures to A/S microorganisms and verifying simple additivity;
e) developing an approach to predict joint effects of mixtures of organic chemicals to A/S culture based on the molecular structures of the components of the mixtures.

Work during the third year will focus on the effects of mixtures on anaerobic cultures to predict joint effects on them using molecular structures of the components and the surrogate test culture results.

EXPERIMENTAL APPROACH

Respirometeric test procedure-

All tests were conducted using research grade chemicals as supplied by the manufacturers without any further purification. The toxicity tests were run on a 12-reactor computer interfaced N-Con Respirometer as detailed in Appendix I. The test procedure is detailed in Appendix II. The percent inhibition caused by a toxicant at a given concentration was determined by comparing the oxygen uptake rate of a toxicant-free control reactor against the rates of eight other reactors spiked with different concentrations of the toxicant. This rate was in turn obtained from the slopes of the linear portion of the oxygen uptake curves generated by the respirometer for each reactor. The % inhibition values were plotted against the respective concentrations, and from these plots, the IC50 values were then determined [IC50 is the concentration of the toxicant at which a 50% inhibition is caused]. The above procedure is illustrated schematically in Fig 1.

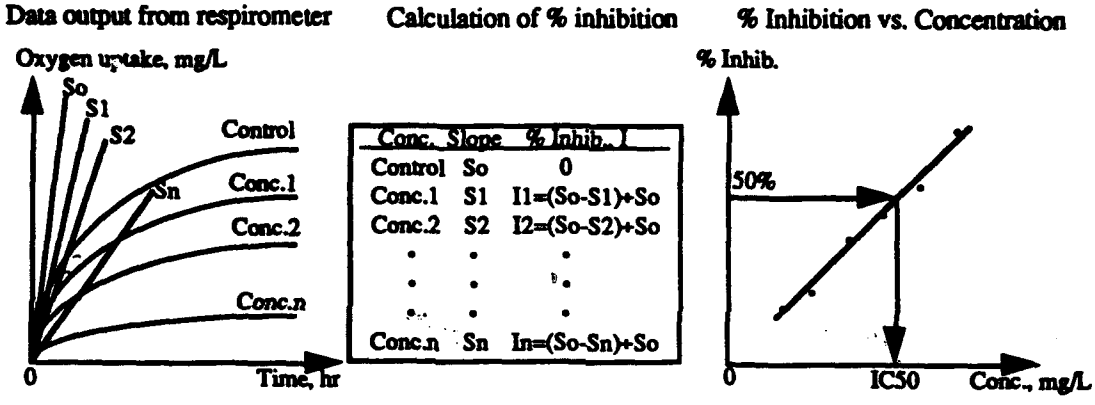


Figure 1. Schematic Illustration of Procedure to Find IC50

Test Chemicals-

A total of 50 organic chemicals (see Table II) chosen from the list of "chemicals of concern to the US Air Force" (Proposer's Guide to AFOSR Research Program, US Air Force, 1986) were assayed. Of those chosen, 17 are listed as priority pollutants by the US EPA (Federal Register, 46 CRF, 2264 1981). The selected chemicals included simple and halo-substituted alkanes and aromatics, alcohols, esters, ketones, amines etc. These chemicals spanned a wide range of aqueous solubility, Henry's Constant and octanol-water partition coefficient. Such a variety of chemicals would enhance the robustness and the utility of the QSAR models. The same set of chemicals had been assayed during the first year using the surrogate test cultures.

From this list of 50 chemicals, 40 were used as "training chemicals" to develop the models and the remaining 10 were reserved in a "testing set" to test the predictive ability of the models. The ten testing set members (ID # 10, 13, 17, 20, 23, 30, 34, 37, 40 and 43 in Table II) were selected as far as possible to include "new types" of chemicals such as 2,4 dimethyl phenol and cyclohexanone, which were not represented in the training set. These chemicals contained combination of multiple molecular features (e.g., by the aromatic alcohol, 2,4 dimethyl phenol) that were represented in the training set individually (e.g., by the aromatics and the alcohols).

In the multi-component mixture tests, ten mixtures each containing 10 different chemicals mixed in equitoxic proportions were assayed. For each mixture, one control reactor and six spiked reactors were run. The six reactors received 0.04, 0.06, 0.08, 0.1, 0.12 and 0.14 Toxic Units of each of the ten components. Additional six testing mixtures, each containing 8 different chemicals, were also assayed. In this case, the six spiked reactors received 0.05, 0.075, 0.1, 0.125, 0.150 and 0.175 Toxic Units of each of the components.

Activated sludge test cultures-
The A/S test cultures were obtained daily from the aeration tank of the nearby Las Cruces Wastewater Treatment Plant that receives mainly municipal sewage. The MLSS and MLVSS of activated sludge varied from 1,200 to 2,600 [mg/L] and 1,020 to 1,970, [mg/L] respectively. The reactors received 10 mL of activated sludge each.

MODELING APPROACH

The single chemical toxicity results from the 40 chemicals placed in the testing set were used to develop QSAR models. Molecular connectivity indexes were calculated for the chemicals following the algorithms developed by Kier and Hall and modified by Nirmalakhandan (1988). Simple and multiple step-wise regression analysis procedures were used to derive the QSAR model with IC50 values as the dependent variable. The IC50 values calculated from the QSAR models were then compared with the experimentally measured values.

In the multi-component mixture studies, the joint effects were analyzed using three concepts: Toxic Unit, Additivity Index, and Mixture Toxicity Index. The validity of these concepts was further verified using the results of the 8-component testing set. Finally, the QSAR models developed from single chemical tests were used to predict the concentrations of the components in the 8-component mixture that would cause 50% inhibition. These predicted concentrations were then compared with the experimentally measured concentrations.

RESULTS AND DISCUSSION

Reproducibility Studies-

Preliminary studies were conducted to establish the reproducibility and the variability in the experimental IC50 results and compare with similar results obtained in Phase I for Polytox surrogate cultures. Four "cold start" runs were made for four selected chemicals to evaluate the variations in the final IC50 values. From the results summarized in Table 1, it can be seen that the Polytox testing procedure is more consistent yielding IC50 values with an average standard deviation of 16.4 while the A/S testing procedure yielded slightly higher variations with standard deviation of 22.6. These variations are comparable to those reported by Blum (1989) for activated sludge cultures and Microtox, and may be considered acceptable for microbial toxicity work.

Table I. Reproducibility of IC_{50} Values from Polytox and Activated Sludge Tests

Chemical		Test results				Statistics	
		Run 1	Run 2	Run 3	Run 4	Mean	SD
Polytox tests:							
Toluene	r^2	0.870	0.990	0.872	0.940	0.918	0.058
	IC50 [mg/L]	207	176	186	186	188.8	13.0
2,4 Dimethyl-	r^2	0.953	0.800	0.907	0.871	0.883	0.065
phenol	IC50 [mg/L]	240	207	228	260	233.8	22.2
Cyclohexane	r^2	0.964	0.968	0.902	0.900	0.934	0.038
	IC50 [mg/L]	74	68	62	58	65.5	7.0
Ethanolamine	r^2	0.955	0.981	0.916	0.928	0.945	0.029
	IC50 [mg/L]	160	105	120	132	129.3	23.3
					Mean SD of IC50 values		16.4
Activated sludge tests:							
Toluene	r^2	0.839	1.000	0.969	0.963	0.943	0.071
	IC50 [mg/L]	292	322	328	322	315.9	16.5
2,4 Dimethyl-	r^2	0.964	0.765	0.957	0.987	0.918	0.103
phenol	IC50 [mg/L]	224	259	199	286	242.2	38.2
Cyclohexane	r^2	0.917	0.980	0.842	0.942	0.920	0.058
	IC50 [mg/L]	133	146	167	150	148.9	13.7
Ethanolamine	r^2	0.897	0.987	0.905	0.986	0.944	0.049
	IC50 [mg/L]	115	154	146	167	145.5	22.2
					Mean SD of IC50 values		22.6

IC50 Results-

Experimentally determined IC50 values for the 50 chemicals for the surrogate test culture, Polytox, (in Phase I) and for A/S cultures (in Phase II) are shown in Table II.

TABLE II. Comparison of IC_{50} Values for Polytox and Activated Sludge

ID #	Chemical	Type*	IC50 Values [mg/L]	
			Polytox	Act. sludge
1	Benzene	Aro	685	993
2	Toluene	Aro	207	292
3	Xylene	Aro	140	166
4	Ethylbenzene	Aro	220	222
5	Chlorobenzene	Aro	350	155
6	1,2 Dichlorobenzene	Aro	135	49
7	1,3 Dichlorobenzene	Aro	40	63
8	1,4 Dichlorobenzene	Aro	6	14
9	1,2,4 Trichlorobenzene	Aro	23	35
10	2,4 Dimethyl phenol	Aro	240	224
11	Methylene chloride	Hal	1,750	1,994
12	Dibromomethane	Hal	1,110	1,572
13	Carbon tetrachloride	Hal	325	432
14	1,2 Dichloroethane	Hal	685	1,385
15	1,1,1 Trichloroethane	Hal	415	659
16	1,1,2,2 Tetrachloroethane	Hal	180	197
17	1,2 Dichloropropane	Hal	500	861
18	Bromochloromethane	Hal	1,800	2,672
19	Bromodichloromethane	Hal	90	249
20	Chlorodibromomethane	Hal	425	206
21	Ethylene dibromide	Hal	520	1,271
22	1,2 Dichloroethylene	Hal	350	1,249
23	Trichloroethylene	Hal	500	770
24	Tetrachloroethylene	Hal	175	299
25	Cyclohexane	Alk	74	133
26	Pentane	Alk	70	150
27	Hexane	Alk	38	47
28	Heptane	Alk	18	58
29	Octane	Alk	8	60
30	Bis (2-chloroethyl) ether	Alc	1,600	3,025
31	Ethanol	Alc	40,000	26,311
32	Propanol	Alc	7,200	10,875
33	Pentanol	Alc	2,325	3,528
34	Octanol	Alc	126	194
35	n-Butyl acetate	Alc	3,750	1,649
36	Isobutyl acetate	Alc	1,600	2,156
37	n-Amyl acetate	Alc	440	1,031
38	Ethyl acetate	Alc	5,400	5,427
39	Acetone	Alc	48,000	48,619
40	Methyl ethyl ketone	Alc	1,900	1,873
41	Methyl isobutyl ketone	Alc	2,600	2,811
42	Methyl n-propyl ketone	Alc	4,500	4,267
43	Cyclohexanone	Alc	3,750	5,452
44	n-Butyl amine	Ami	90	111
45	t-Butyl amine	Ami	85	90
46	Diethylamine	Ami	104	100
47	Acetic acid	Ami	287	299
48	Cyclohexylamine	Ami	60	103
49	Ethanolamine	Ami	160	115
50	Triethanolamine	Ami	900	741

* Aro- aromatic; Hal- halogenated aliphatic; Alk- alkanes; Alc- alcohols, esters, ketones and ethers; Ami- amines.

Correlation of IC50 Between Polytox and A/S-
Very good correlation was found between Polytox IC50 values and A/S IC50 values. Figure 2 shows the correlation between the two cultures, split by the 5 different families of chemicals assayed. The IC50 [unit: mg/L] relationship between the two cultures is given by:

$$\log IC50_{A/S} = 0.412 + 0.889 \log IC50_{Polytox} \quad (1)$$

$n = 50$; $r = 0.960$; $r^2 = 0.922$; $SE = 0.225$.

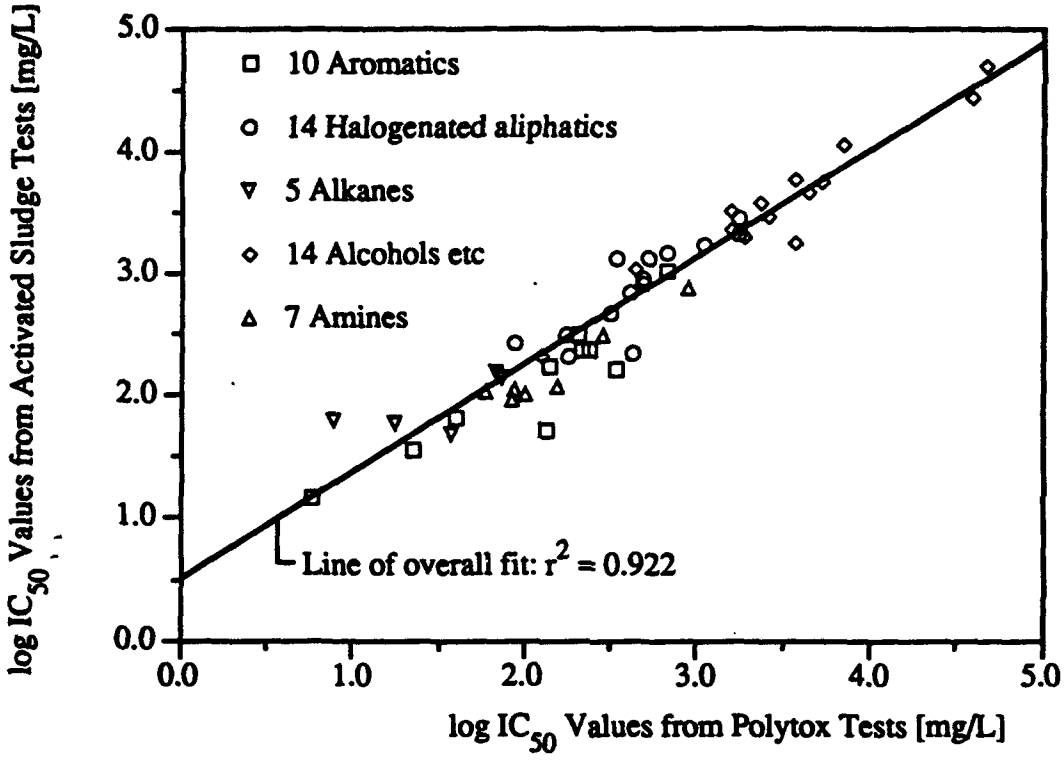


FIGURE 2. Comparison Between Polytox and Activated Sludge IC50 Values

Similar comparison has been done between IC50 values from the Microtox Test, and from A/S cultures by Blum (1989) for 34 chemicals similar to those tested here. The correlation between the two was reported as "fair" with $r^2 = 0.69$ and $SE = 0.48$. In comparison, the Polytox results appear to correlate with A/S results more closely than the Microtox Test results with higher r^2 and lower SE. This is as expected because, Polytox culture is formulated from selected streams from A/S whereas, the Microtox Test organisms are of marine origin.

The relationship given by Eq. 1 shows that, in general, the Polytox culture is more sensitive than the A/S culture. When a paired t-test was done to test if the difference of the means of the IC50 values from the two tests was significant, the A/S logIC50 values were found to be greater than Polytox logIC50 values by 0.129 at 95% confidence interval. Thus, the toxicity predicted by Polytox could be a conservative estimation.

Based on these findings, for chemicals belonging to congeneric classes similar to those tested here, Polytox may be used as a good surrogate test culture for rapid estimation of toxicity to A/S culture.

Single Chemical QSAR Models-

The results from the training set of 40 chemicals were used to develop QSAR models for IC50 [unit: mM/L] for A/S cultures using the three common approaches: the molecular connectivity (MCI) approach; the Linear Solvation Energy Relationships (LSER) approach; and, the logP approach.

The MCI Approach:

In the MCI approach, a different QSAR model was developed for each family:

Aromatic Family:

$$\log IC50 = 3.364 - 1.191\ {}^{1}\chi^{v} \quad (2)$$

$n = 8$; $r = 0.887$; $r^2 = 0.787$; $SE = 0.294$.

Halogenated Aliphatics Family:

$$\log IC50 = 2.781 - 0.446\ {}^{0}\chi^{v} \quad (3)$$

$n = 10$; $r = 0.868$; $r^2 = 0.753$; $SE = 0.252$.

Alkanes Family:

$$\log IC50 = 1.103 - 0.381\ {}^{1}\chi^{v} \quad (4)$$

$n = 5$; $r = 0.839$; $r^2 = 0.703$; $SE = 0.181$.

Alcohols, Ketones and Esters Family:

$$\log IC50 = 3.663 - 0.892\ {}^{1}\chi^{v} \quad (5)$$

$n = 9$; $r = 0.934$; $r^2 = 0.872$; $SE = 0.243$.

Amines and Acids Family:

$$\log IC50 = 0.819 - 0.327\ {}^{1}\chi^{v} \quad (6)$$

$n = 6$; $r = 0.852$; $r^2 = 0.726$; $SE = 0.142$.

Statistical details of Eq. (2) to (6) are shown in Appendix III, Table A-III-1 to A-III-5 and Figure A-III-1 to A-III-5 respectively for the 5 families. The fitted values are plotted against the experimental values as illustrated below in Figure 3, showing an overall r^2 of 0.869.

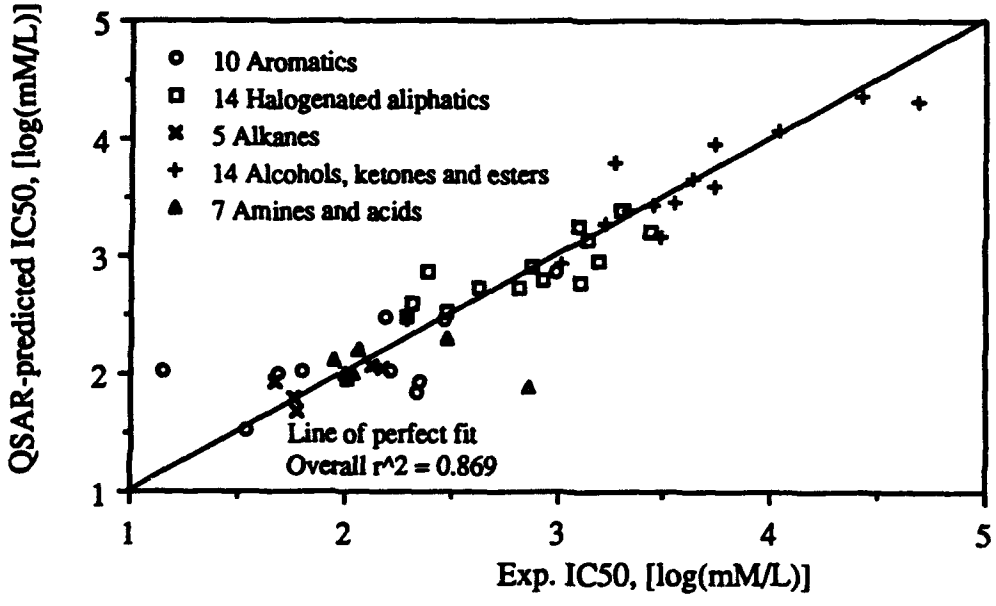


FIGURE 3. Comparison Between Fitted and Experimental IC50 Values for A/S.

The LSER Approach:

In the LSER approach, a single QSAR model was first developed to cover the entire data set. Solvatochromic parameters used in this analysis are listed in Appendix IV, Table A-IV-1. The QSAR model resulted in the following:

$$\log IC50_{A/S} = 1.721 - 2.578\ Vi/100 + 0.372\ \pi^* + 1.138\ \beta - 0.067\ \alpha \qquad (7)$$

$n = 47$; $r = 0.605$; $r^2 = 0.366$; $SE = 0.706$.

Statistical details of the above equation are shown in Table A-IV-2. Since the quality of the fit was not acceptable, casewise regression was done as in the case of the MCI approach. The different LSER models for the different families are summarized in Appendix IV (Table A-IV-3 to A-IV-7). While in some cases, the LSER models are superior to the MCI models, this approach is limited by the nonavailability of the solvatochromic parameters for many of the common chemicals. When LSER model parameters are not readily available in the literature, they have to be experimentally determined or estimated using groundrules proposed by Kamlet and coworkers (1983). The estimated values are however error-prone and the groundrules are not well established. This is a serious drawback of the LSER approach, whereas, in the MCI approach, the MCI values may be readily calculated using a rigid set of algorithms for all families of chemicals without any error.

The logP Approach:

In the logP approach, the entire data set was first used to develop a QSAR model, resulting in the following:

$$\log IC50_{A/S} = 1.469 - 0.391\ \log P \qquad (8)$$

$n = 48$; $r = 0.641$; $r^2 = 0.411$; $SE = 0.654$.

Statistical details of the above equation are shown in the Appendix V, Table A-V-1. Again, casewise models were developed as before, which are summarized in the Appendix V (Table A-V-2 to A-V-6 and Figure A-V-2 to A-V-6).

Comparison of the 3 QSAR Approaches

The quality and utility of the three QSAR models were compared on the basis of the adjusted r^2. The reason for the use of adjusted r^2 is that different data sets were modeled using different numbers of independent variables. It is calculated as follows:

$$\text{Adjusted } r^2 = \frac{((n-1)\,r^2 - p)}{(n - p - 1)}$$

where,

n = Nº of cases used in analysis for r^2, and
p = Nº of independent variables.

The adjusted r^2 for the three QSAR models for the 5 families are tabulated in Appendix VI, Table A-VI-1. As can be seen from this Table, for the aromatic and the alkane families, adjusted r^2 of LSER and logP models are higher than that of the MCI model; but, for all the other families, adjusted r^2 of the MCI models are higher than those of the LSER and the logP models. However, based on the limited availability and the uncertainty of the solvatochromic parameters in the LSER approach and the logP values on one hand, and the ease of calculation and the error-free nature of the connectivity indices on the other hand, it is recommended that the MCI models have a better utility value to the practicing engineer. Thus, further modeling in this research utilized only the MCI approach.

Comparison Between MCI Models for A/S and Polytox

The same chemicals used in the QSAR model development for A/S had also been assayed in Phase I using the surrogate test culture-Polytox. The IC50 results from that study yielded MCI QSAR models for Polytox, which are very similar to those obtained for A/S in Phase II:

Aromatic Family:

$$\log IC50 = 3.258 - 1.133\ {}^{1}\chi^{v} \quad (9)$$

$n = 9$; $r = 0.852$; $r^2 = 0.726$; $SE = 0.311$.

Halogenated Aliphatics Family:

$$\log IC50 = 2.670 - 0.448\ {}^{0}\chi^{v} \quad (10)$$

$n = 12$; $r = 0.942$; $r^2 = 0.887$; $SE = 0.141$.

Alkanes Family:

$$\log IC50 = 1.851 - 0.765\ {}^{1}\chi^{v} \quad (11)$$

$n = 5$; $r = 0.999$; $r^2 = 0.999$; $SE = 0.018$.

Alcohols, Ketones and Esters Family:

$$\log IC50 = 3.690 - 0.896\ {}^{1}\chi^{v} \quad (12)$$

$n = 14$; $r = 0.954$; $r^2 = 0.910$; $SE = 0.246$.

Amines and Acids Family:

$$\log IC50 = 1.045 - 0.470\ {}^{1}\chi^{v} \quad (13)$$

$n = 6$; $r = 0.957$; $r^2 = 0.915$; $SE = 0.101$.

The MCI QSAR models for the two cultures are remarkably similar in form, quality, and significance. While supporting the earlier finding of good correlation between the two cultures, the similarity of the QSAR models also suggests that the chemicals act on these two organisms by very similar mechanisms. This finding may be of significant value in analyzing joint effects of mixtures of several chemicals.

Prediction of IC50 Values for Testing Set

The 10 chemicals reserved in the "testing set" were used to compare the predicted IC50 values by two approaches. In the first approach, Equation (1) was used to predict IC50 values for A/S based on surrogate test culture- Polytox IC50 values. These results are presented in the Appendix, Table A-V-II 1 and A-VII-2. In the second approach, Equations (2) to (6) were used to predict IC50 values for A/S based on the QSAR models.

These predicted IC50 values are then compared, in turn, against the respective experimentally measured values for A/S. The agreement between the experimental and the predicted values was found to be very good as shown in Fig 4: in the first approach, $r^2 = 0.901$, $SE = 0.154$; and in the second approach, $r^2 = 0.844$, $SE = 0.217$.

However, as shown in Figure 4, the QSAR-predicted line is not significantly different from the line of perfect prediction, but the Polytox-predicted line is significantly different. This implies that the predictions of QSAR models are almost numerically identical to the experimental values, and are better than the predictions of the Polytox model. In evaluating these comparisons, it should also be noted that the QSAR approach does not require any experimental inputs whatsoever. In addition, the r^2 and SE of this agreement are comparable to those found in the experimental inhibition percentage vs. concentration plots suggesting that the uncertainty of these predictions are comparable to those of the experimental data themselves. Thus, for organic chemicals belonging to similar congeneric classes as those tested here, the above QSAR models can be expected to predict satisfactory IC50 values for A/S.

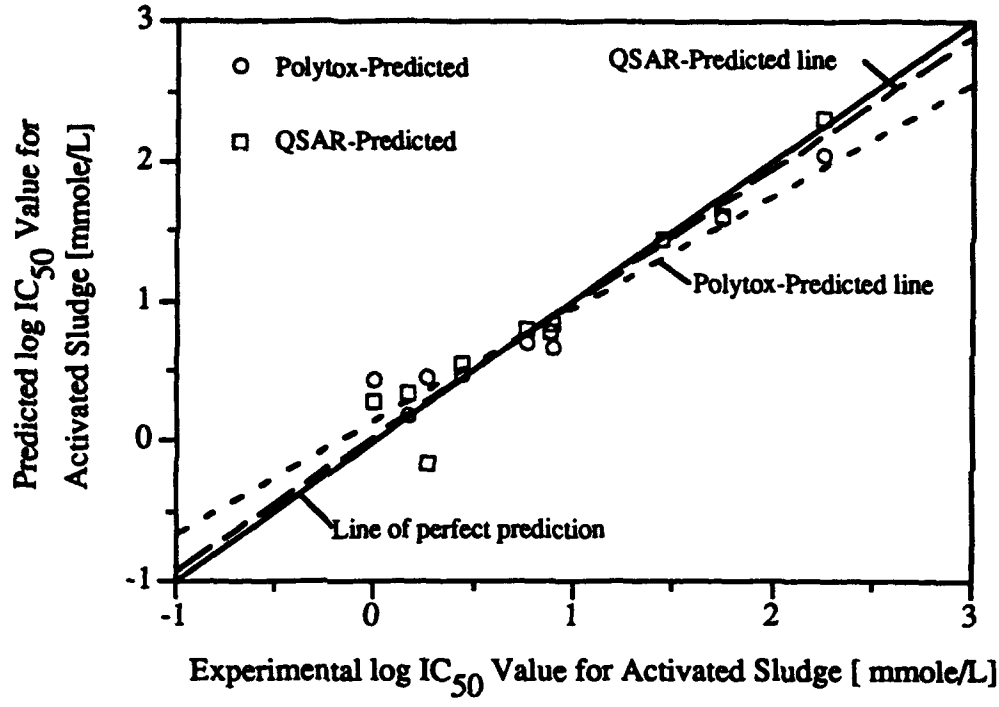


FIGURE 4. Comparison Between Predicted and Measured IC50 Values for A/S.

Applicability of QSAR Models

It is commonly thought that the composition and characteristics of A/S cultures vary considerably from plant to plant and from time to time. If that is the case, the utility value of QSAR models in rapid estimation of toxicity would be very difficult and questionable, or even impossible. To investigate the variability in A/S IC50 values, the limited toxicity data of A/S cultures reported in the literature by different workers at different times and locations were compiled and compared with predictions of QSAR models developed in this study.

The dataset reported by Blum (1989) contained 47 chemicals all of which belonged to the 5 families assayed in this study. Out of 47 tested chemicals reported by Blum (1989), 15 had been assayed by us, and the other 32 were "new" chemicals. Volskay and Grady (1988) had reported toxicity of 15 chemicals using laboratory grown activated sludge by synthetic feed. They adapted the OECD Method 209, using inhibition of oxygen uptake rate as the measure of toxicity. Out of 15 chemicals assayed by Volskay and Grady (1988), 11 were similar in molecular structure to ours. Of these 11 chemicals, 7 had been tested in this study, and thus 4 were "new" chemicals. In all therefore, including 47 chemicals from this study, a total 108 data points were available for comparison, representing IC50 values of 83 different chemicals, 36 of them not used in the QSAR model development. The QSAR models, Eq. (2) to (6), were used to predict the IC50 values for these 83 chemicals. Table A-VIII-1 in Appendix VIII shows these predictions and the experimental IC50 values. Figure 5 illustrates excellent agreement between the two, spanning over 4 orders of magnitude, with an r^2 = 0.798 at P = 0.0001.

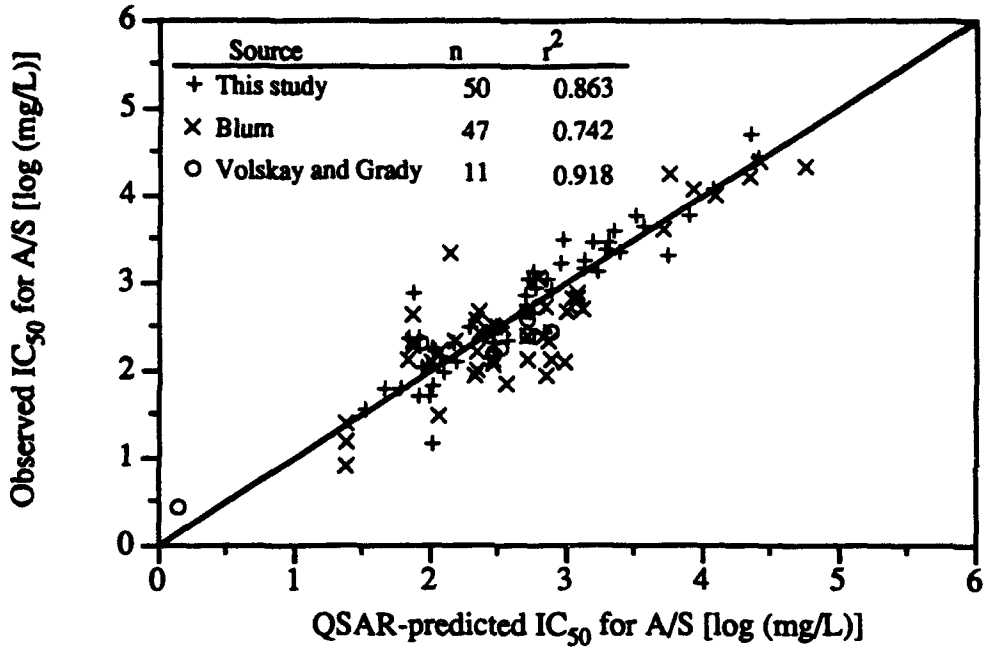


FIGURE 5. Comparison Between Predicted and Observed IC50 Values for A/S.

This finding is highly significant in two aspects: 1) the "variation in activated sludge" has minimal effect on toxicity response to common organic chemicals, and 2) the QSAR models can satisfactorily predict toxicity to A/S. In addition, it has to be mentioned that even though the QSAR models were derived from training set of 40 chemicals, their predictions for the total 43 "new" testing chemicals appears quite satisfactory. In these 43 "new" testing chemicals, some contained combined molecular features that were represented in the training set individually. For example, the testing set contained 10 halogenated phenols (Blum, 1989) whereas the training set did not contain any phenols at all, but only aromatic structures and alcoholic structures separately. However, these independently represented molecular features are well encoded by the connectivity indexes and the QSAR models enabling satisfactory predictions for chemicals containing combined fragments. This ability of the QSAR models also adds further credence to the predictive approach.

Multi-Component Mixture Toxicity

The multi-component mixture studies were similar to those done for Polytox during Phase I. These results are summarized in Table III. Details of these experimental results are presented in Appendix IX and X. The hypothesis of simple additivity in these mixtures was tested using three concepts: the Toxic Unit (TU) concept; the Additivity Index (AI) concept; and, the Mixture Toxicity Index, (MTI). While the average TU, AI and MTI values deviate from the expected values of 1, 0 and 1, this anomaly is believed to be due to the variability of the activated sludge cultures. While the simple additivity was verified for the Polytox microbial test cultures in Phase I, that for activated sludge appears somewhat questionable. This finding will be further analyzed and resolved during the third year of the project. Nevertheless, these findings are similar to those reported in the literature for mixture toxicity studies on fish (Konemann 1981 a, b, c)

TABLE III. Summary of Analysis for Simple Additivity for 10- and 8-component Mixtures.

Mixture	Chemicals in Mixture		Joint Effects Analyzed by		
Nº	ID # of components	n	TU TU = Σ TU	AI AI = M - 1	MTI MTI=1-logM/logn
10C-1	4,5,10,36,32,33,12,18,1,2	10	1.57	0.57	0.80
10C-2	4,5,10,36,32,33,12,18,22,23	10	1.60	0.60	0.79
10C-3	40,41,35,36,32,33,4,5,10,17	10	1.70	0.70	0.77
10C-4	40,41,35,36,32,33,4,5,10,2	10	1.50	0.50	0.83
10C-5	40,41,35,36,32,33,31,43,34,17	10	1.84	0.84	0.73
10C-6	40,41,35,36,31,43,12,18,1,2	10	1.54	0.54	0.81
10C-7	40,41,43,31,32,33,12,18,22,23	10	1.91	0.91	0.72
10C-8	40,41,35,36,4,5,17,43,34,17	10	1.83	0.83	0.74
10C-9	40,41,35,36,4,5,17,18,1,2	10	1.57	0.57	0.80
10C-10	40,41,43,4,5,17,12,18,22,23	10	1.31	0.31	0.88
8C-1	40,41,35,36,32,33,12,30	8	5.17	4.17	0.21
8C-2	40,41,35,36,12,18,1,2	8	1.59	0.59	0.78
8C-3	40,41,35,36,32,33,22,23	8	1.47	0.47	0.81
8C-4	4,36,32,33,12,18,34,17	8	1.53	0.53	0.80
8C-5	4,10,36,32,33,18,22,23	8	1.37	0.37	0.85
8C-6	40,35,21,15,4,5,10,2	8	1.48	0.48	0.81
	Average		1.81	0.81	0.76

ID #s are same as in Table II.

Prediction of mixture concentrations

Assuming that the joint effects of the chemicals being tested are according to simple additivity, the approach proposed under the modeling section was applied to verify the predicted concentrations of the components in the mixtures. To predict the concentrations, perfect additivity, i.e $\Sigma TU = 1$ was assumed. In addition, the individual IC_{50} values for the two cultures were estimated using the QSAR models reported above for the two cultures. The concentration of chemical i in the N-component mixture is then predicted as = $(\Sigma TU/N) \times IC_{50,i}$, N being 8 in this study. These predicted concentrations are compared against the experimentally determined concentrations in Figure 6. The overall agreement between the predicted and experimental concentrations for the two cultures (48 pairs of data points) is fair with $r^2 = 0.76$, SE = 0.31 for Polytox and $r^2 = 0.81$, SE = 0.26 for the A/S cultures. The minor deviations from ideal predictions are due to: the slight inadequacies of the QSAR models; slight deviations from simple additivity; and, experimental uncertainties. Nevertheless, this degree of agreement may be acceptable in toxicity work considering that the predictions are made without any experimental inputs.

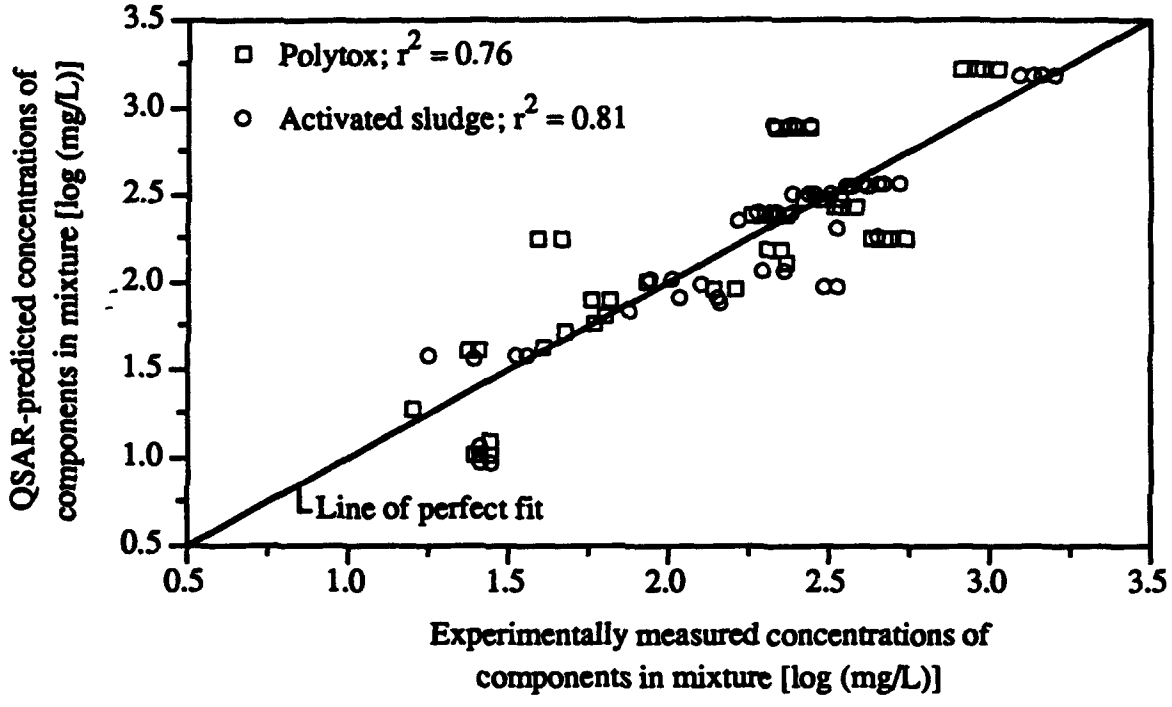


FIGURE 6 Comparison between predicted and experimental concentrations in mixtures.

Conclusions

The basic research completed during the first two years of this project has generated a large microbial toxicity data base for chemicals that are of concern to US Air Force. The findings of this research will be of considerable benefit to practicing engineers, regulators and utilities. The following is a summary of our findings:

1. The test culture evaluated in this research, Polytox, can be used as a good surrogate for testing toxicity to activated sludge microorganisms.
2. The respirometeric technique developed in this research has been demonstrated to be a simple, rapid, and reproducible toxicity testing technique.
3. The molecular connectivity index, MCI, approach is a simple and powerful one in developing QSAR models to predict microbial toxicity.
4. The QSAR models developed in this research can be confidently used to predict toxicity of new chemicals to activated sludge microorganisms.
5. The joint toxic effects of mixtures of chemicals assayed in this research can be considered to be simply additive.
6. The QSAR approach developed in this research can be used confidently to predict joint toxic effects of chemicals similar to those assayed here, based solely on molecular structural information, without any experimental inputs whatsoever.

Publications

Based on the research completed during the first two years, two MS thesis have been successfully defended and several publications have been generated. These are listed below:

MS Thesis:

1. "Modeling Joint Effects of Mixtures of Organic Chemicals on Microorganisms" Mohsin, M., MS Thesis, New Mexico State University, May 1993.
2. "Comparison of Interspecies Toxicity of Organic Chemicals Using QSAR Methods" Sun, B., MS Thesis, New Mexico State University, Nov. 1993.

Conference Presentations:

1. "Modeling and Analysis of Microbial Toxicity of Mixtures of Organic Chemicals" Khandan, N. N., Eckenfelder Seminar Series, Vanderbilt University, Nashville, April 1993.
2. "Predicting of Toxicity of Mixtures of Chemicals Using Models Based on Molecular Structures" Khandan, N. N., International Congress on Modeling and Simulation, Perth, Australia , Dec. 1993. [In Proceedings]

Refereed Journal Publications:

1. "Toxicity of Mixtures of Organic Chemicals to Microorganisms" Khandan, N. N., et al; To appear in *Water Research*, Feb 1994.
2. "Estimating Toxicity of Organic Chemicals to Activated Sludge Microorganisms" Sun, B., Khandan, N. N., Hall, E., Wang, X. H., Prakash, J., and Maynes, R.; Submitted to *Jour. Env. Engrg. Div. ASCE*, July 1993.
3. "Analyzing and Modeling Toxicity of Mixtures of Organic Chemicals to Microorganisms" Khandan, N. N., Sun, B., Arulgnanendran, V. J., Mohsin, M., Wang, X. H., Prakash, J., and Hall, N., Submitted to *Water Sci. & Tech.*, July 1993.

Bibliography

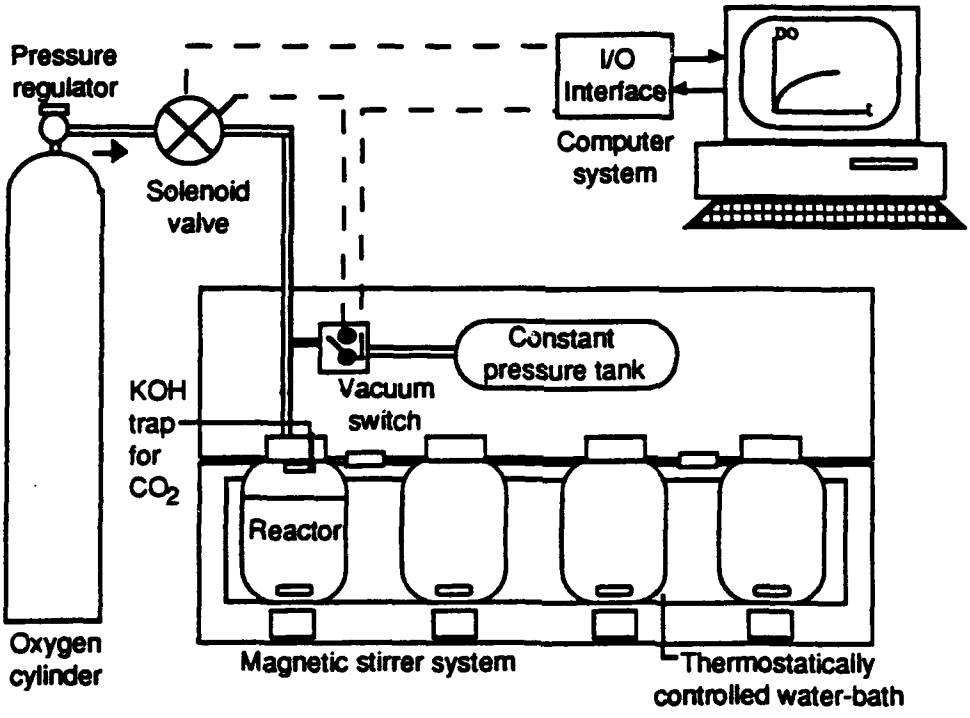
1. Abernathy et al (1986); Aquatic Toxic., 8, 163-172.
2 Anderson P D and Webber L J (1975); Proc. Int Conf. on Heavy Metals, Ontario, Canada.
3. Ashford J R (1958); Biometrika; 45, 74-88.
4. Ashford J R and Smith C S (1964); Biometrika; 51, 413-428.
5. Ashford J R and Smith C S (1965); Biometrics; 21, 182-189.
6 Hall, L H and Kier, B K (1984); Bull. Environ. Contam. Toxicol., 32, 354-362.
7. Hermens et al (1985); Aquatic Toxicology; 6, 209-217.
8. Hermens et al (1985); Ecotoxicology and Env Safety; 9, 17-25.
9. Hermens et al (1985); Env Toxicology and Chem.; 4, 273-279.
10. Konemann H (1981); Toxicology; 19, 209-221.
11. Konemann H (1981); Toxicology; 19, 223-228.
12. Konemann H (1981); Toxicology; 19, 229-238.
13. Marking L L (1977); ASTM STP Publication 634, 99-108.
14. Plackett R L and Hewlett P S (1948); Ann Appl. Biol.; 35, 347-358.
15. Plackett R L and Hewlett P S (1952); J . R. Statis. Sco.; B 14, 141-154.
16. Plackett R L and Hewlett P S (1963); Biometrics; 19, 517-531.
17. Plackett R L and Hewlett P S (1967); Biometrics; 23, 27-44.
18. Sprague J B (1964); J Fish Res Ca.; 21, 17-26.
19. Sprague J B and Ramsay, B A (1965); J. Fish. Res. Bd Can.; 22, 425-432.
20. Sprague J B (1970); Water Res; 4, 3-32.
21. Nirmalakhandan N. (1988); PhD Dissertation, Drexel University, PA, 1988.
22. Blum D. J. W. (1989); PhD Dissertation, Drexel University, PA.
23.
24. Volskay, V. T. and Grady C. P. L. (1988); Jour. WPCF, 60, 10, 1850-1856.

APPENDIX I

Appendix I:
Details of Respirometer System

The respirometer being used in our research was developed here at New Mexico State University, and is commercially marketed by N-CON Corporation, Inc., NY. The system has been recently modified in our laboratory to work either in the aerobic or anaerobic mode. The reactors in this system are maintained at constant temperature and pressure. Changes in headspace pressure, due to gas production (or consumption), are sensed by a pressure (or vacuum) switch and are converted to gas volume using Ideal Gas Laws, reactor volume, temperature and type of gas being exchanged, and, monitored on a real time basis. These volumes can then be easily related to biological activity in the reactor. A brief description of the system in the aerobic mode is as follows.

In this mode, the CO_2 produced is absorbed by KOH pellets placed in the headspace. Thus, consumption of O_2 results in a vacuum in the headspace. A vacuum switch has its vacuum side connected to the headspace. The pressure side of the switch is connected to a closed, constant pressure tank, thus providing a steady reference pressure, eliminating any fluctuations due to barometric/atmospheric variations. When the pressure differential across the switch exceeds 2.5 mm H_2O, a signal is sent through the data acquisition system to the computer and, a precise pulse of oxygen from an oxygen cylinder is injected into the headspace. The computer keeps track of the number of pulses (or the amount) of oxygen supply as a function of time. From this data, oxygen utilization rate can then be established. A schematic arrangement of this system is shown below:



APPENDIX II

Appendix II

Activated sludge test procedure

The A/S test cultures were obtained daily from the aeration tank of the nearby Las Cruces Wastewater Treatment Plant that receives mainly municipal sewage. The MLSS and MLVSS of activated sludge varied from 1,200 to 2,600 [mg/L] and 1,020 to 1,970, [mg/L] respectively. The reactors received 10 mL of activated sludge each. The test reactors were topped with tap water to bring up to final volume of 60 mL while the control reactors were topped up to 62 mL. The test reactors were dosed with the toxicants dissolved in 2mL of acetone. The quantities of the test chemicals administered for each test were determined by trial and error to bring about inhibition in the range of 2 0 to 70%. All the reactors were then capped with potassium hydroxide pellets in holders attached to the caps. The contents of the reactors were kept mixed with magnetic stirrers.

The toxicity tests were run on a 12-reactor, computer-interfaced Comput-OX Respirometer (N-CON Corporation, NY). The capped reactors were placed in the respirometer water bath maintained at 25°C with continued supply of oxygen. The data acquisition system was then initiated to monitor and record the oxygen uptake of each reactor for the next 12 hours.

The toxicity was measured in terms IC_{50}, which is the concentration of the chemical that inhibited the microorganisms by 50% compared to the control reactor. The percent inhibition [%] at different concentrations of the toxicant was taken as the reductions in oxygen uptake rates of the spiked reactors compared to that of the control reactor. These % inhibition values were then plotted against the respective concentrations, and from these plots, the concentration causing 50% inhibition, IC_{50} was determined.

APPENDIX III

Table A-III-1: Correlation between 1xv and logIC50 for ARO family

Regression Summary
ARO,Exp. IC50, [log(mM/L)] vs. ARO,1xv

Count	8
Num. Missing	42
R	.887
R Squared	.787
Adjusted R Squared	.752
RMS Residual	.294

ANOVA Table
ARO,Exp. IC50, [log(mM/L)] vs. ARO,1xv

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.924	1.924	22.209	.0033
Residual	6	.520	.087		
Total	7	2.443			

Regression Coefficients
ARO,Exp. IC50, [log(mM/L)] vs. ARO,1xv

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	3.364	.703	3.364	4.784	.0030
ARO,1xv	-1.191	.253	-.887	-4.713	.0033

Confidence Intervals
ARO,Exp. IC50, [log(mM/L)] vs. ARO,1xv

	Coefficient	95% Lower	95% Upper
Intercept	3.364	1.643	5.084
ARO,1xv	-1.191	-1.809	-.573

Regression Plot

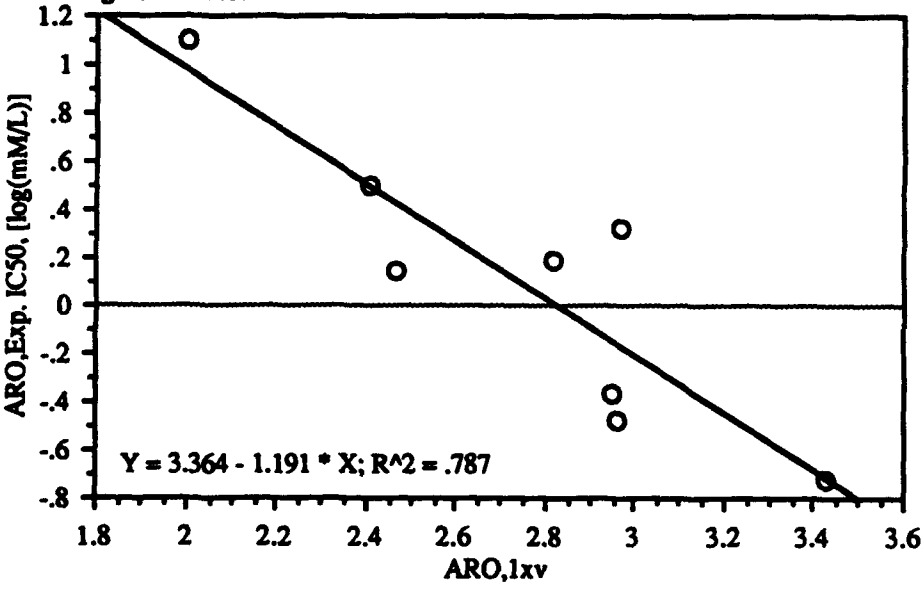


Figure A-III-1: Correlation between 1xv and logIC50 for ARO family

Table A-III-2: Correlation between 0xv and logIC50 for HAL family

Regression Summary
HAL,Exp. IC50, [log(mM/L)] vs. HAL,0xv

Count	10
Num. Missing	40
R	.868
R Squared	.753
Adjusted R Squared	.722
RMS Residual	.252

ANOVA Table
HAL,Exp. IC50, [log(mM/L)] vs. HAL,0xv

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.547	1.547	24.381	.0011
Residual	8	.507	.063		
Total	9	2.054			

Regression Coefficients
HAL,Exp. IC50, [log(mM/L)] vs. HAL,0xv

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	2.781	.410	2.781	6.776	.0001
HAL,0xv	-.446	.090	-.868	-4.938	.0011

Confidence Intervals
HAL,Exp. IC50, [log(mM/L)] vs. HAL,0xv

	Coefficient	95% Lower	95% Upper
Intercept	2.781	1.834	3.727
HAL,0xv	-.446	-.655	-.238

Regression Plot

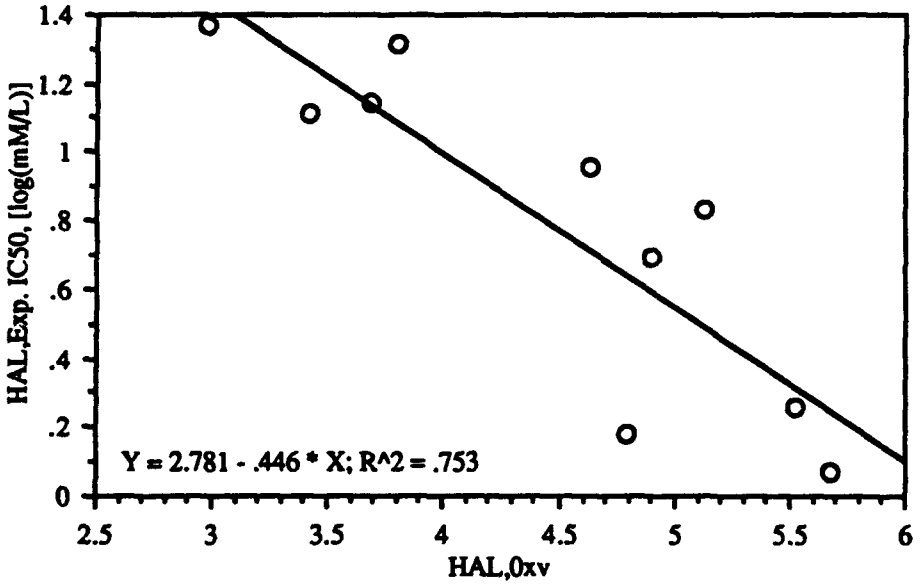


Figure A-III-2: Corelation between 0xv and logIC50 for HAL family

Table A-III-3: Correlation between 1xv and logIC50 for ALK family

Regression Summary
ALK,Exp. IC50, [log(mM/L)] vs. ALK,1xv

Count	5
Num. Missing	45
R	.839
R Squared	.703
Adjusted R Squared	.605
RMS Residual	.181

ANOVA Table
ALK,Exp. IC50, [log(mM/L)] vs. ALK,1xv

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.233	.233	7.117	.0758
Residual	3	.098	.033		
Total	4	.331			

Regression Coefficients
ALK,Exp. IC50, [log(mM/L)] vs. ALK,1xv

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.103	.440	1.103	2.504	.0874
ALK,1xv	-.381	.143	-.839	-2.668	.0758

Confidence Intervals
ALK,Exp. IC50, [log(mM/L)] vs. ALK,1xv

	Coefficient	95% Lower	95% Upper
Intercept	1.103	-.299	2.505
ALK,1xv	-.381	-.837	.074

Regression Plot

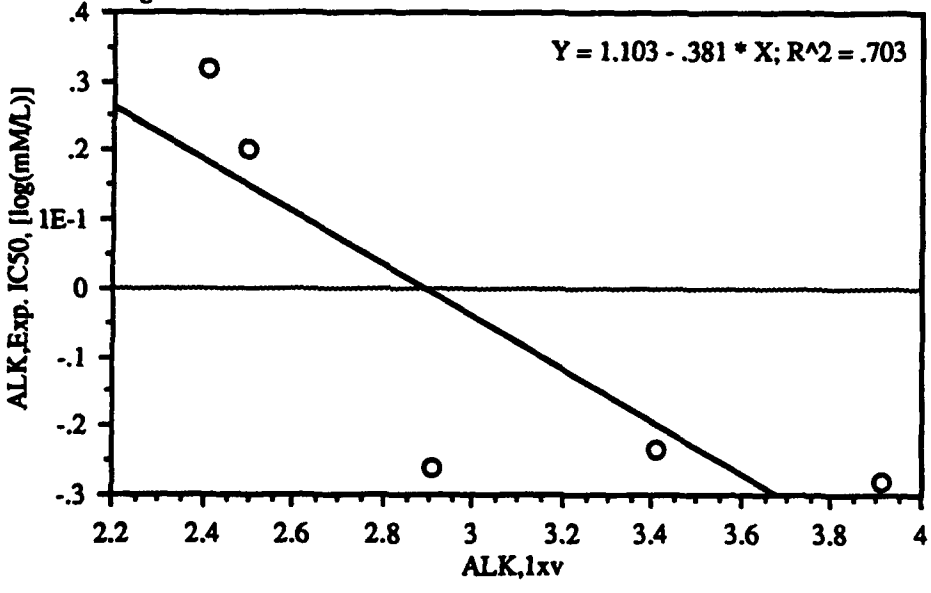


Figure A-III-3: Correlation between 1xv and logIC50 for ALK family

Table A-III-4: Correlation between 1xv and logIC50 for AKE family

Regression Summary
AKE,Exp. IC50, [log(mM/L)] vs. AKE,1xv

Count	9
Num. Missing	41
R	.934
R Squared	.872
Adjusted R Squared	.854
RMS Residual	.243

ANOVA Table
AKE,Exp. IC50, [log(mM/L)] vs. AKE,1xv

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	2.824	2.824	47.723	.0002
Residual	7	.414	.059		
Total	8	3.238			

Regression Coefficients
AKE,Exp. IC50, [log(mM/L)] vs. AKE,1xv

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	3.663	.271	3.663	13.494	<.0001
AKE,1xv	-.892	.129	-.934	-6.908	.0002

Confidence Intervals
AKE,Exp. IC50, [log(mM/L)] vs. AKE,1xv

	Coefficient	95% Lower	95% Upper
Intercept	3.663	3.021	4.304
AKE,1xv	-.892	-1.197	-.587

Regression Plot

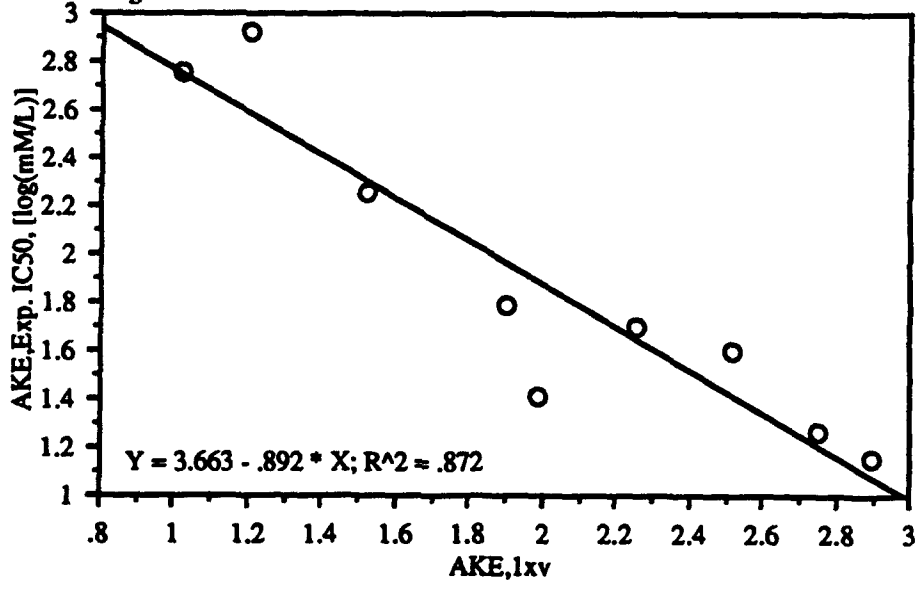


Figure A-III-4: Correlation between 1xv and logIC50 for AKE family

Table A-III-5: Correlation between 1xv and logIC50 for AMI family

Regression Summary
AMI,Exp. IC50, [log(mM/L)] vs. AMI,1xv

Count	6
Num. Missing	44
R	.852
R Squared	.726
Adjusted R Squared	.657
RMS Residual	.142

ANOVA Table
AMI,Exp. IC50, [log(mM/L)] vs. AMI,1xv

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.213	.213	10.595	.0312
Residual	4	.080	.020		
Total	5	.294			

Regression Coefficients
AMI,Exp. IC50, [log(mM/L)] vs. AMI,1xv

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	.819	.190	.819	4.319	.0125
AMI,1xv	-.327	.100	-.852	-3.255	.0312

Confidence Intervals
AMI,Exp. IC50, [log(mM/L)] vs. AMI,1xv

	Coefficient	95% Lower	95% Upper
Intercept	.819	.293	1.346
AMI,1xv	-.327	-.605	-.048

Regression Plot

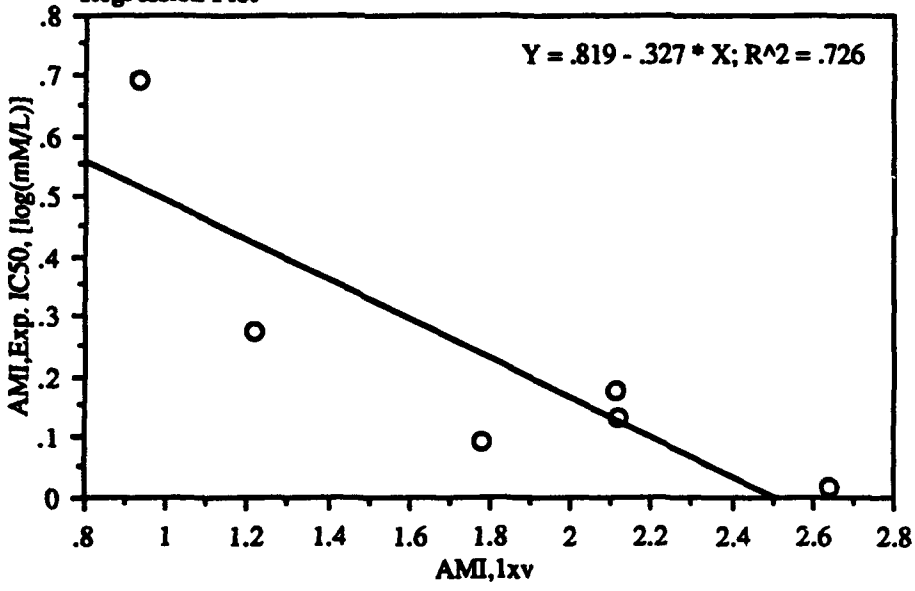


Figure A-III-5: Correlation between 1xv and logIC50 for AMI family

APPENDIX IV

Table A-IV-1: Comparison of Exp IC50 (A/S) vs. logP and LSER

Nº	Name	Type	Exp IC50	logP #	LSER #				
			log[mM/L]		Vi/100	π*	β	α	
1	Benzene	ARO	1.10	2.13	0.491	0.59	0.14	0.00	
2	Toluene	ARO	0.50	2.65	0.591	0.55	0.11	0.00	
3	Xylene	ARO	0.19	3.18	0.671	0.51	0.12	0.00	
4	Ethylbenzene	ARO	0.32	3.13	0.671	0.53	0.12	0.00	
5	Chlorobenzene	ARO	0.14	2.98	0.581	0.71	0.07	0.00	
6	1,2 Dichlorobenzene	ARO	-0.48	3.38	0.671	0.80	0.03	0.00	
7	1,3 Dichlorobenzene	ARO	-0.37	3.48	0.671	0.75	0.03	0.00	
8	1,4 Dichlorobenzene	ARO	-1.04	3.38	0.671	0.65	0.03	0.00	e
9	1,2,4 Trichlorobenzene	ARO	-0.72	3.98	0.761	0.75	0.03	0.00	
10	2,4 Dimethyl phenol	ARO	0.26	2.30	0.867	0.75	0.41	0.50	t
11	Methylene chloride	HAL	1.37	1.15	0.336	0.82	0.10	0.35	
12	Dibromomethane	HAL	0.96	0.83	0.374	0.92	0.10	0.05	
13	Carbon tetrachloride	HAL	0.45	2.64	0.514	0.28	0.10	0.00	t
14	1,2 Dichloroethane	HAL	1.15	1.32	0.442	0.81	0.10	0.00	
15	1,1,1 Trichloroethane	HAL	0.69	2.13	0.519	0.49	0.10	0.00	
16	1,1,2,2 Tetrachloroethane	HAL	0.07	2.04	0.617	0.95	0.10	0.13	e
17	1,2 Dichloropropane	HAL	0.88	1.87	0.548	0.81	0.10	0.00	t
18	Bromochloromethane	HAL	1.31	1.41					e
19	Bromodichloromethane	HAL	0.18						e
20	Chlorodibromomethane	HAL	0.00						t,e
21	Ethylene dibromide	HAL	0.83	2.02	0.528	0.75	0.05	0.00	
22	1,2 Dichloroethylene	HAL	1.11	1.63	0.406	0.44	0.10	0.00	
23	Trichloroethylene	HAL	0.77	2.29	0.897	0.53	0.10	0.00	t
24	Tetrachloroethylene	HAL	0.26	2.88	0.519	0.28	0.10	0.00	
25	Cyclohexane	ALK	0.20	3.44	0.598	0.00	0.00	0.00	

Note: t = testing chemicals
Note: e = excluded from analysis

Table A-IV-1 (cont'd)

Nº	Name	Type	Exp IC50	logP	LSER				
			log[mM/L]		Vi/100	π*	β	α	
26	Pentane	ALK	0.32	3.62	0.553	0.00	0.00	0.00	
27	Hexane	ALK	-0.26	4.11	0.648	0.00	0.00	0.00	
28	Heptane	ALK	-0.23	4.66	0.745	0.00	0.00	0.00	
29	Octane	ALK	-0.28	5.18	0.842	0.00	0.00	0.00	
30	Bis (2-chloroethyl) ether	AKE	1.33	2.77	0.654	0.81	0.10	0.00	
31	Ethanol	AKE	2.76	-0.25	0.305	0.40	0.45	0.33	
32	Propanol	AKE	2.26	0.28	0.402	0.40	0.45	0.33	t
33	Pentanol	AKE	1.60	1.53	0.593	0.40	0.45	0.33	
34	Octanol	AKE	0.17	2.97	0.882	0.40	0.45	0.33	t
35	n-Butyl acetate	AKE	1.15	1.73	0.716	0.46	0.45	0.00	
36	Isobutyl acetate	AKE	1.27	1.86	0.716	0.51	0.45	0.00	
37	n-Amyl acetate	AKE	0.90	2.39	0.813	0.49	0.45	0.00	t
38	Ethyl acetate	AKE	1.79	0.73	0.521	0.55	0.45	0.00	
39	Acetone	AKE	2.92	-0.24	0.380	0.71	0.48	0.00	
40	Methyl ethyl ketone	AKE	1.41	0.37	0.477	0.67	0.48	0.00	t
41	Methyl isobutyl ketone	AKE	1.45	1.36	0.670	0.63	0.48	0.00	
42	Methyl n-propyl ketone	AKE	1.69	0.84	0.574	0.65	0.48	0.00	
43	Cyclohexanone	AKE	1.74	0.81	0.571	0.75	0.53	0.00	t
44	n-Butylamine	AMI	0.18	0.32	0.535	0.31	0.69	0.00	
45	t-Butylamine	AMI	0.09	0.32	0.535	0.31	0.69	0.00	
46	Diethylamine	AMI	0.13	0.36	0.535	0.25	0.70	0.00	
47	Acetic acid	AMI	0.69	-0.32	0.325	0.64	0.45	1.12	
48	Cyclohexylamine	AMI	0.02	1.33	0.729	0.30	0.69	0.00	
49	Ethanolamine	AMI	0.27	-0.88	0.444	0.88	0.69	0.00	
50	Triethanolamine	AMI	0.70	1.37	0.709	0.14	0.71	0.00	

Data sources: 1) M. J. Kamlet et al, Jour. Phys. Chem., 1987, 91, 7, 1996-2004; 2) J. P. Hickey et al, Environ. Sci. Technol., 1991, 25, 1753-1760; 3) D. E. Leahy et al, Chromatographia, Aug. 1986, 21, 8, 473-477; 4) M. J. Kamlet et al, Environ. Sci. Technol., 1986, 20, 7, 690-695; 5) M. J. Kamlet et al, J. Am. Chem. Soc., 1984, 106, 2, 465-466; 6) R. W. Taft et al, J. Am. Chem. Soc., 1981, 103, 1080-1086; 7) R. W. Taft et al, J. Org. Chem., 1984, 49, 2001-2005; 8) M. J. Kamlet et al, J. Org. Chem., 1983, 48, 2877-2888.

Note: t = testing chemicals

Table A-IV-2: Correlation of experimental logIC50 values and LSER for the entire data set

Regression Summary
Exp IC50, log[mM/L] vs. 4 Independents

Count	47
Num. Missing	3
R	.605
R Squared	.366
Adjusted R Squared	.306
RMS Residual	.706

ANOVA Table
Exp IC50, log[mM/L] vs. 4 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	4	12.084	3.021	6.061	.0006
Residual	42	20.936	.498		
Total	46	33.020			

Regression Coefficients
Exp IC50, log[mM/L] vs. 4 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.721	.560	1.721	3.075	.0037
Vi/100	-2.578	.738	-.452	-3.495	.0011
π*	.372	.406	.116	.918	.3641
ß	1.138	.436	.327	2.608	.0125
á	-.067	.547	-.016	-.122	.9033

Confidence Intervals
Exp IC50, log[mM/L] vs. 4 Independents

	Coefficient	95% Lower	95% Upper
Intercept	1.721	.592	2.851
Vi/100	-2.578	-4.067	-1.089
π*	.372	-.447	1.192
ß	1.138	.258	2.019
á	-.067	-1.170	1.036

Table A-IV-3: Correlation of experimental logIC50 values and LSER for ARO family

Regression Summary
ARO,logIC50,mM/L vs. 3 Independents

Count	8
Num. Missing	6
R	.996
R Squared	.991
Adjusted R Squared	.985
RMS Residual	.073

ANOVA Table
ARO,logIC50,mM/L vs. 3 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	3	2.425	.808	152.162	.0001
Residual	4	.021	5.312E-3		
Total	7	2.446			

Regression Coefficients
ARO,logIC50,mM/L vs. 3 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.892	1.368	1.892	1.384	.2387
ARO,Vi/100	-3.618	.730	-.499	-4.957	.0077
ARO,π^*	-.149	1.080	-.029	-.138	.8970
ARO,β	7.379	3.204	.583	2.303	.0826

Confidence Intervals
ARO,logIC50,mM/L vs. 3 Independents

	Coefficient	95% Lower	95% Upper
Intercept	1.892	-1.905	5.690
ARO,Vi/100	-3.618	-5.645	-1.592
ARO,π^*	-.149	-3.148	2.850
ARO,β	7.379	-1.516	16.274

Table A-IV-4: Correlation of experimental logIC50 values and LSER for HAL family

Regression Summary
HAL,logIC50,mM/L vs. 4 Independents

Count	7
Num. Missing	7
R	.857
R Squared	.734
Adjusted R Squared	.203
RMS Residual	.324

ANOVA Table
HAL,logIC50,mM/L vs. 4 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	4	.579	.145	1.382	.4608
Residual	2	.210	.105		
Total	6	.789			

Regression Coefficients
HAL,logIC50,mM/L vs. 4 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	2.665	2.736	2.665	.974	.4329
HAL,Vi/100	-3.575	3.494	-.767	-1.023	.4138
HAL,π^*	.343	.829	.226	.413	.7195
HAL,β	-4.084	10.135	-.213	-.403	.7260
HAL,α	-.015	1.443	-5.399E-3	-.010	.9926

Confidence Intervals
HAL,logIC50,mM/L vs. 4 Independents

	Coefficient	95% Lower	95% Upper
Intercept	2.665	-9.109	14.439
HAL,Vi/100	-3.575	-18.607	11.458
HAL,π^*	.343	-3.225	3.910
HAL,β	-4.084	-47.693	39.525
HAL,α	-.015	-6.223	6.193

Table A-IV-5: Correlation of experimental logIC50 values and LSER for ALK family

Regression Summary
ALK,logIC50,mM/L vs. ALK,Vi/100

Count	5
Num. Missing	9
R	.821
R Squared	.674
Adjusted R Squared	.566
RMS Residual	.189

ANOVA Table
ALK,logIC50,mM/L vs. ALK,Vi/100

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.222	.222	6.211	.0883
Residual	3	.107	.036		
Total	4	.329			

Regression Coefficients
ALK,logIC50,mM/L vs. ALK,Vi/100

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.318	.555	1.318	2.373	.0982
ALK,Vi/100	-2.021	.811	-.821	-2.492	.0883

Confidence Intervals
ALK,logIC50,mM/L vs. ALK,Vi/100

	Coefficient	95% Lower	95% Upper
Intercept	1.318	-.449	3.086
ALK,Vi/100	-2.021	-4.601	.560

Table A-IV-6: Correlation of experimental logIC50 values and LSER for AKE family

Regression Summary
AKE,logIC50,mM/L vs. 4 Independents

Count	9
Num. Missing	5
R	.980
R Squared	.961
Adjusted R Squared	.922
RMS Residual	.178

ANOVA Table
AKE,logIC50,mM/L vs. 4 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	4	3.141	.785	24.702	.0044
Residual	4	.127	.032		
Total	8	3.269			

Regression Coefficients
AKE,logIC50,mM/L vs. 4 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	3.146	1.131	3.146	2.782	.0497
AKE,Vi/100	-4.084	.617	-.9[illegible]	-6.616	.0027
AKE,π*	.937	.955	.208	.982	.3818
AKE,β	.973	.800	.185	1.217	.2904
AKE,á	.168	.881	.038	.190	.8582

Confidence Intervals
AKE,logIC50,mM/L vs. 4 Independents

	Coefficient	95% Lower	95% Upper
Intercept	3.146	5.916E-3	6.285
AKE,Vi/100	-4.084	-5.798	-2.370
AKE,π*	.937	-1.713	3.588
AKE,β	.973	-1.247	3.194
AKE,á	.168	-2.278	2.614

Table A-IV-7: Correlation of experimental logIC50 values and LSER for AMI family

Regression Summary
AMI,logIC50,mM/L vs. 4 Independents

Count	7
Num. Missing	7
R	.927
R Squared	.859
Adjusted R Squared	.576
RMS Residual	.184

ANOVA Table
AMI,logIC50,mM/L vs. 4 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	4	.411	.103	3.036	.2628
Residual	2	.068	.034		
Total	6	.479			

Regression Coefficients
AMI,logIC50,mM/L vs. 4 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	-20.178	8.082	-20.178	-2.497	.1299
AMI,Vi/100	.272	.999	.136	.272	.8111
AMI,π*	.414	.452	.381	.917	.4561
AMI,ß	28.922	11.635	9.513	2.486	.1308
AMI,á	6.696	2.486	10.035	2.694	.1146

Confidence Intervals
AMI,logIC50,mM/L vs. 4 Independents

	Coefficient	95% Lower	95% Upper
Intercept	-20.178	-54.953	14.597
AMI,Vi/100	.272	-4.025	4.568
AMI,π*	.414	-1.531	2.360
AMI,ß	28.922	-21.138	78.981
AMI,á	6.696	-4.000	17.393

APPENDIX V

Table A-V-1: Correlation of experimental logIC50 values and logP for the entire data set

Regression Summary
Exp IC50, log[mM/L] vs. log P

Count	48
Num. Missing	2
R	.641
R Squared	.411
Adjusted R Squared	.398
RMS Residual	.654

ANOVA Table
Exp IC50, log[mM/L] vs. log P

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	13.721	13.721	32.090	<.0001
Residual	46	19.668	.428		
Total	47	33.389			

Regression Coefficients
Exp IC50, log[mM/L] vs. log P

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.469	.164	1.469	8.953	<.0001
log P	-.391	.069	-.641	-5.665	<.0001

Confidence Intervals
Exp IC50, log[mM/L] vs. log P

	Coefficient	95% Lower	95% Upper
Intercept	1.469	1.139	1.799
log P	-.391	-.529	-.252

Regression Plot
95% Confidence Bands

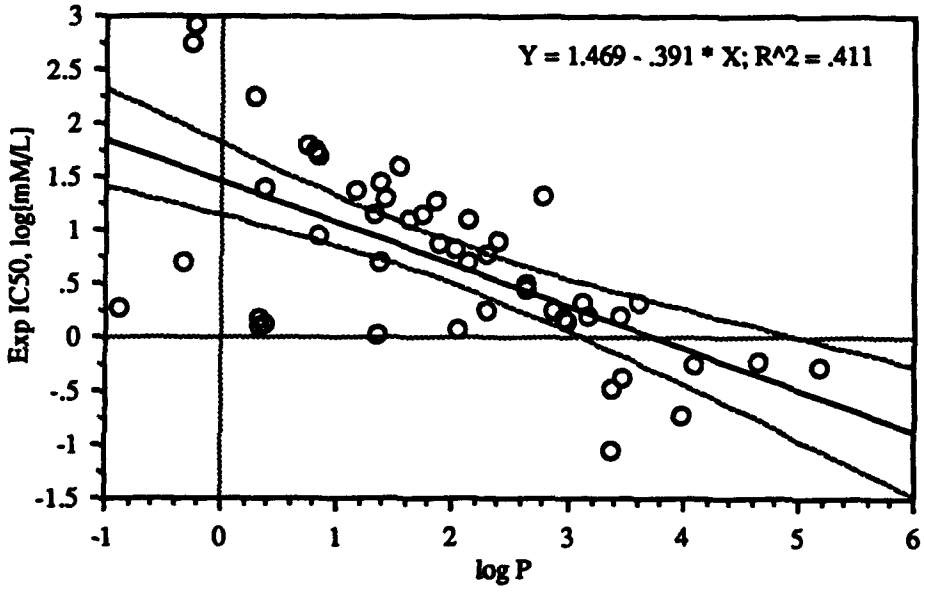


Figure A-V-1: Correlation of experimental logIC50 values and logP for the entire data set

Table A-V-2: Correlation of experimental logIC50 values and logP for ARO family

Regression Summary
ARO,logIC50,mM/L vs. ARO,logP

Count	8
Num. Missing	6
R	.958
R Squared	.917
Adjusted R Squared	.904
RMS Residual	.184

ANOVA Table
ARO,logIC50,mM/L vs. ARO,logP

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	2.244	2.244	66.616	.0002
Residual	6	.202	.034		
Total	7	2.446			

Regression Coefficients
ARO,logIC50,mM/L vs. ARO,logP

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	3.258	.394	3.258	8.266	.0002
ARO,logP	-1.019	.125	-.958	-8.162	.0002

Confidence Intervals
ARO,logIC50,mM/L vs. ARO,logP

	Coefficient	95% Lower	95% Upper
Intercept	3.258	2.294	4.223
ARO,logP	-1.019	-1.325	-.714

Regression Plot

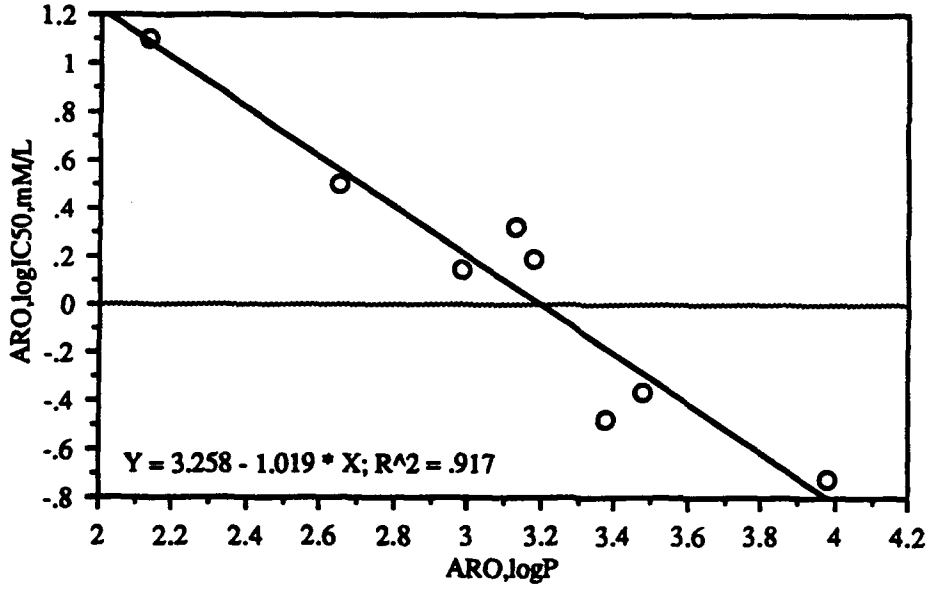


Figure A-V-2: Correlation of experimental logIC50 values and logP for ARO family

Table A-V-3: Correlation of experimental logIC50 values and logP for HAL family

Regression Summary
HAL,logIC50,mM/L vs. HAL,logP

Count	8
Num. Missing	6
R	.839
R Squared	.705
Adjusted R Squared	.655
RMS Residual	.214

ANOVA Table
HAL,logIC50,mM/L vs. HAL,logP

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.655	.655	14.316	.0091
Residual	6	.274	.046		
Total	7	.929			

Regression Coefficients
HAL,logIC50,mM/L vs. HAL,logP

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.745	.221	1.745	7.902	.0002
HAL,logP	-.470	.124	-.839	-3.784	.0091

Confidence Intervals
HAL,logIC50,mM/L vs. HAL,logP

	Coefficient	95% Lower	95% Upper
Intercept	1.745	1.205	2.286
HAL,logP	-.470	-.774	-.166

Regression Plot

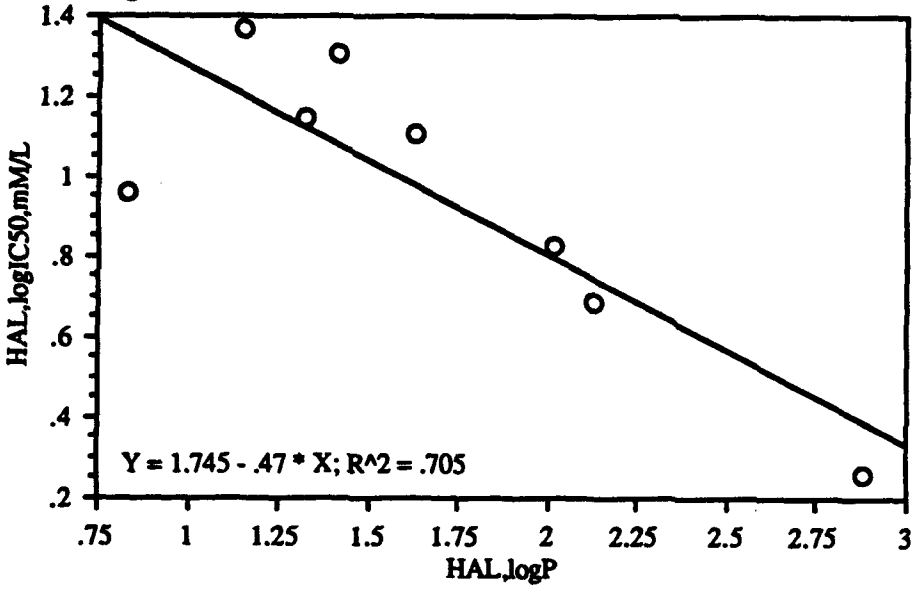


Figure A-V-3: Correlation of experimental logIC50 values and logP for HAL family

Table A-V-4: Correlation of experimental logIC50 values and logP for ALK family

Regression Summary
ALK,logIC50,mM/L vs. ALK,logP

Count	5
Num. Missing	9
R	.836
R Squared	.699
Adjusted R Squared	.599
RMS Residual	.182

ANOVA Table
ALK,logIC50,mM/L vs. ALK,logP

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.230	.230	6.977	.0776
Residual	3	.099	.033		
Total	4	.329			

Regression Coefficients
ALK,logIC50,mM/L vs. ALK,logP

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.342	.533	1.342	2.517	.0864
ALK,logP	-.331	.125	-.836	-2.641	.0776

Confidence Intervals
ALK,logIC50,mM/L vs. ALK,logP

	Coefficient	95% Lower	95% Upper
Intercept	1.342	-.355	3.040
ALK,logP	-.331	-.731	.068

Regression Plot

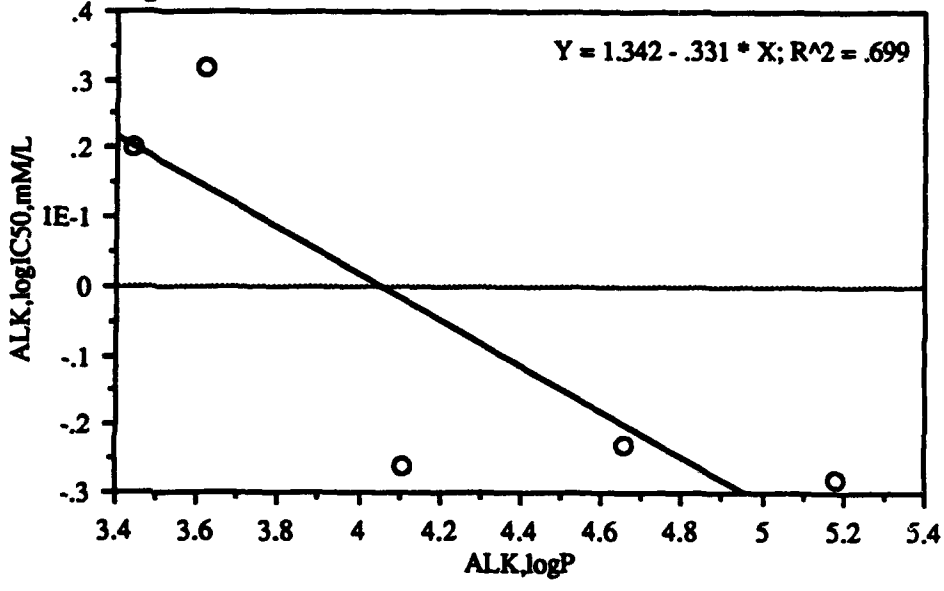


Figure A-V-4: Correlation of experimental logIC50 values and logP for ALK family

Table A-V-5: Correlation of experimental logIC50 values and logP for AKE family

Regression Summary
AKE,logIC50,mM/L vs. AKE,logP

Count	9
Num. Missing	5
R	.896
R Squared	.802
Adjusted R Squared	.774
RMS Residual	.304

ANOVA Table
AKE,logIC50,mM/L vs. AKE,logP

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	2.622	2.622	28.366	.0011
Residual	7	.647	.092		
Total	8	3.269			

Regression Coefficients
AKE,logIC50,mM/L vs. AKE,logP

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	2.438	.161	2.438	15.166	<.0001
AKE,logP	-.579	.109	-.896	-5.326	.0011

Confidence Intervals
AKE,logIC50,mM/L vs. AKE,logP

	Coefficient	95% Lower	95% Upper
Intercept	2.438	2.058	2.818
AKE,logP	-.579	-.836	-.322

Regression Plot

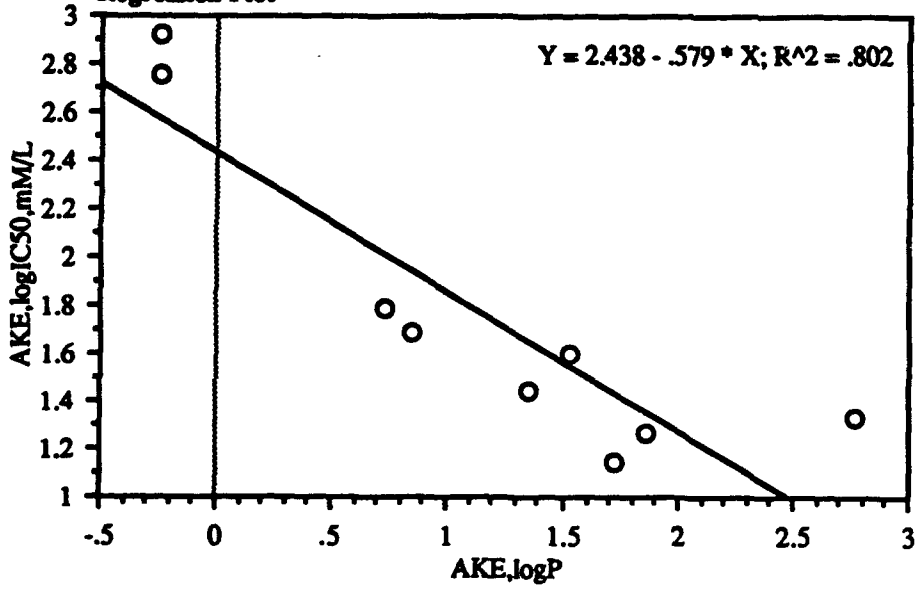


Figure A-V-5: Correlation of experimental logIC50 values and logP for AKE family

Table A-V-6: Correlation of experimental logIC50 values and logP for AMI family

Regression Summary
AMI,logIC50,mM/L vs. AMI,logP

Count	7
Num. Missing	7
R	.060
R Squared	3.583E-3
Adjusted R Squared	•
RMS Residual	.309

ANOVA Table
AMI,logIC50,mM/L vs. AMI,logP

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.715E-3	1.715E-3	.018	.8986
Residual	5	.477	.095		
Total	6	.479			

Regression Coefficients
AMI,logIC50,mM/L vs. AMI,logP

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	.305	.129	.305	2.357	.0650
AMI,logP	-.021	.155	-.060	-.134	.8986

Confidence Intervals
AMI,logIC50,mM/L vs. AMI,logP

	Coefficient	95% Lower	95% Upper
Intercept	.305	-.028	.637
AMI,logP	-.021	-.420	.378

Regression Plot

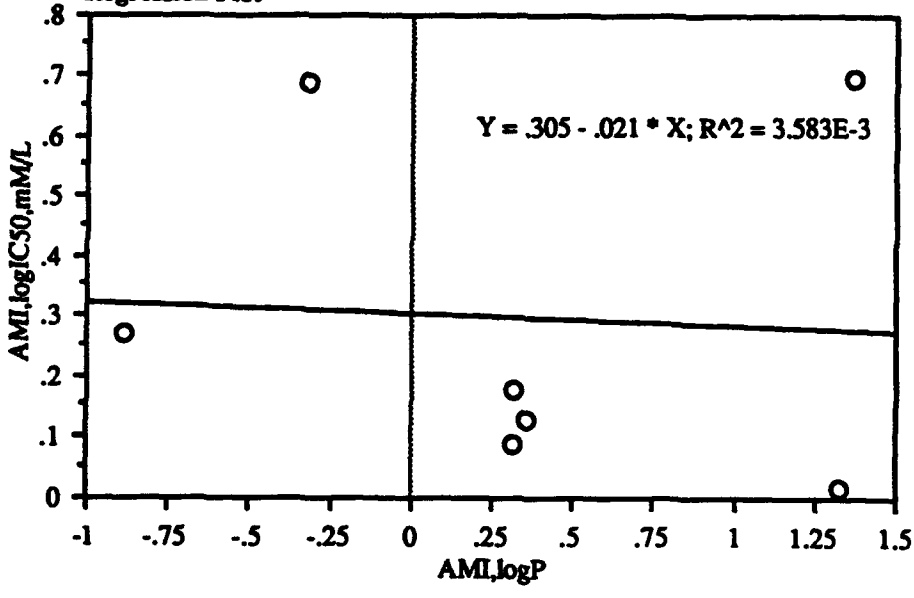


Figure A-V-6: Correlation of experimental logIC50 values and logP for AMI family

APPENDIX VI

Table A-VI-1: Comparison of three QASR models among MCI, LSER and logP

Type	Three QSAR models								
	MCI			LSER			logP		
ARO	n* = 8	r^2 =	0.787	n = 8	r^2 =	0.991	n =8	r^2 =	0.917
	p# = 1	adj.† r^2 =	0.752	p = 3	adj. r^2 =	0.985	p = 1	adj. r^2 =	0.904
		SE =	0.294		SE =	0.073		SE =	0.184
HAL	n =10	r^2 =	0.753	n = 7	r^2 =	0.734	n = 8	r^2 =	0.705
	p = 1	adj. r^2 =	0.722	p = 4	adj. r^2 =	0.203	p = 1	adj. r^2 =	0.655
		SE =	0.252		SE =	0.324		SE =	0.214
ALK	n = 5	r^2 =	0.703	n = 5	r^2 =	0.674	n = 5	r^2 =	0.699
	p = 1	adj. r^2 =	0.605	p = 1	adj. r^2 =	0.566	p = 1	adj. r^2 =	0.599
		SE =	0.181		SE =	0.189		SE =	0.182
AKE	n = 9	r^2 =	0.872	n = 9	r^2 =	0.961	n = 9	r^2 =	0.802
	p = 1	adj. r^2 =	0.854	p = 4	adj. r^2 =	0.922	p = 1	adj. r^2 =	0.774
		SE =	0.243		SE =	0.178		SE =	0.304
AMI	n = 6	r^2 =	0.726	n = 7	r^2 =	0.859	n = 7	r^2 =	0.000
	p = 1	adj. r^2 =	0.657	p = 4	adj. r^2 =	0.576	p = 1	adj. r^2 =	-
		SE =	0.142		SE =	0.184		SE =	0.309

Note: * n = Nº of chemicals used in analysis
p = Nº of independent variables
† adjusted r^2 = [(n - 1) • r^2 - 1] + (n - p - 1)

APPENDIX VII

Table A-VII-1: Prediction of IC50 values for testing chemicals using A/S vs. Epx. Polytox model

[logIC50,A/S]=0.43162+0.87890x[logIC50,Polytox], R^2=0.928, [mg/L]

Slope: 0.8789

Intercept: 0.43162

Type	ID#	Chemical name	MW mg/mM	Polytox IC50 mg/L	Polytox IC50 mM/L	Polytox logIC50 mg/L	Polytox logIC50 mM/L	A/S logIC50 mg/L	A/S IC50 mg/L	A/S IC50 mM/L	A/S logIC50 mM/L
ARO	10	2,4 Dimethyl phenol	122.17	240	2.0	2.380	0.293	2.524	334	2.7	0.437
HAL	13	Carbon tetrachloride	153.82	325	2.1	2.512	0.325	2.639	436	2.8	0.452
	17	1,2 Dichloropropane	112.99	500	4.4	2.699	0.646	2.804	636	5.6	0.751
	20	Chlorodibromemethane	208.29	425	2.0	2.628	0.310	2.742	552	2.6	0.423
	23	Trichloroethylane	131.39	500	3.8	2.699	0.580	2.804	636	4.8	0.685
AKE	30	Bis (2-chloroethyl) ether	143.01	1,600	11.2	3.204	1.049	3.248	1,769	12.4	1.092
	34	Octanol	130.23	126	1.0	2.100	-0.014	2.278	190	1.5	0.163
	37	n-Amyl acetate	130.19	440	3.4	2.643	0.529	2.755	569	4.4	0.640
	41	Methyl isobutyl ketone	100.16	2,600	26.0	3.415	1.414	3.433	2,710	27.1	1.432
	43	Cyclohexanone	98.15	3,750	38.2	3.574	1.582	3.573	3,740	38.1	1.581

Table A-VII-2: Prediction of IC50 values for testing chemicals using A/S QSAR models

ARO: [logIC50]=3.364-1.191x[1xv], R^2=0.787, [mM/L]

Slope: -1.191

Intercept: 3.364

ID#	Chemical Name	1xv	MW mg/mM	Pred logIC50 mM/L	Pred IC50 mM/L	Pred IC50 mg/L	Exp IC50 mg/L
10	2,4 Dimethyl phenol	2.96	122.17	-0.1614	1.4500	84.3	224.1

HAL: [logIC50]=2.781-0.446x[0xv], R^2=0.753, [mM/L]

Slope: -0.446

Intercept: 2.781

ID#	Chemical Name	0xv	MW mg/mM	Pred logIC50 mM/L	Pred IC50 mM/L	Pred IC50 mg/L	Exp IC50 mg/L
13	Carbon tetrachloride	5.03	153.82	0.5376	0.2900	530.4	432.2
17	1,2 Dichloropropane	4.55	112.99	0.7517	0.1771	637.9	860.99
20	Chlorodibromemethane	5.64	208.29	0.2656	0.5426	383.9	206.06
23	Trichloroethylane	4.47	131.39	0.7874	0.1632	805.3	769.7

AKE: [logIC50]=3.663-0.892x[1xv], R^2=0.872, [mM/L]

Slope: -0.892

Intercept: 3.663

ID#	Chemical Name	1xv	MW mg/mM	Pred logIC50 mM/L	Pred IC50 mM/L	Pred IC50 mg/L	Exp IC50 mg/L
30	Bis (2-chloroethyl) ether	3.18	143.01	0.8264	0.1491	959.0	3024.96
34	Octanol	4.02	130.23	0.0772	0.8372	155.6	194.4
37	n-Amyl acetate	3.4	130.19	0.6302	0.2343	555.6	1030.8
41	Methyl isobutyl ketone	2.62	100.16	1.3260	0.0472	2121.6	2811.1
43	Cyclohexanone	2.41	98.15	1.5133	0.0307	3200.1	5452.4

APPENDIX VIII

Tab A-VIII-1: Comparison of IC50 values between QSAR predictions and Exp. results of different A/S sources

ID#	Chemical name	Type	0xv	1xv	MW mg/mM	QSAR model predictions IC50 mg/L	Exp. results of diff. sources This study IC50 mg/L	Blum's IC50 mg/L	V & G's IC50 mg/L
1	Benzene	ARO		2.00	78.11	749	993	520	
2	Toluene	ARO		2.41	92.14	287	292	110	
3	Xylene	ARO		2.82	106.17	107	166		
4	Ethylbenzene	ARO		2.97	106.17	71	222	130	
5	Chlorobenzene	ARO		2.47	112.56	298	155	310	140
6	1,2 Dichlorobenzene	ARO		2.96	147.01	101	49		
7	1,3 Dichlorobenzene	ARO		2.95	147.01	104	63		
8	1,4 Dichlorobenzene	ARO		2.95	147.01	104	14		
9	1,2,4 Trichlorobenzene	ARO		3.43	181.45	34	35		
10	2,4 Dimethyl phenol	ARO		2.96	122.17	84	224		190
11	Methylene chloride	HAL	2.97		84.93	2,429	1,994		
12	Dibromomethane	HAL	4.63		173.85	904	1,572		
13	Carbon tetrachloride	HAL	5.03		153.82	530	432	130	240
14	1,2 Dichloroethane	HAL	3.68		98.96	1,365	1,385	470	
15	1,1,1 Trichloroethane	HAL	4.90		133.41	526	659	450	360
16	1,1,2,2 Tetrachloroethane	HAL	5.68		167.85	297	197	130	
17	1,2 Dichloropropane	HAL	4.55		112.99	638	861		520
18	Bromochloromethane	HAL	3.80		129.39	1,578	2,672		
19	Bromodichloromethane	HAL	4.80		163.83	715	249		
20	Chlorodibromomethane	HAL	5.64		208.29	384	206		
21	Ethylene dibromide	HAL	5.13		187.87	585	1,271		
22	1,2 Dichloroethylene	HAL	3.42		96.94	1,747	1,249		
23	Trichloroethylene	HAL	4.47		131.39	805	770	130	260
24	Tetrachloroethylene	HAL	5.53		165.83	342	299		170
25	Cyclohexane	ALK		2.50	84.16	119	133	29	
26	Pentane	ALK		2.41	72.15	110	150		
27	Hexane	ALK		2.91	86.18	85	47		
28	Heptane	ALK		3.41	100.21	64	58		
29	Octane	ALK		3.91	114.23	47	60		
30	Bis (2-chloroethyl) ether	AKE		3.18	143.01	959	3,025		
31	Ethanol	AKE		1.02	46.07	26,096	26,311	24,000	
32	Propanol	AKE		1.52	60.1	12,191	10,875	9,600	
33	Pentanol	AKE		2.52	88.15	2,293	3,528		
34	Octanol	AKE		4.02	130.23	156	194	200	
35	n-Butyl acetate	AKE		2.90	116.16	1,384	1,649		
36	Isobutyl acetate	AKE		2.75	116.16	1,884	2,156		
37	n-Amyl acetate	AKE		3.40	130.19	556	1,031		
38	Ethyl acetate	AKE		1.90	88.11	8,189	5,427		
39	Acetone	AKE		1.20	58.08	22,731	48,619	16,000	
40	Methyl ethyl ketone	AKE		1.99	72.11	5,571	1,873		
41	Methyl isobutyl ketone	AKE		2.62	100.16	2,122	2,811		
42	Methyl n-propyl ketone	AKE		2.26	86.13	3,822	4,267		

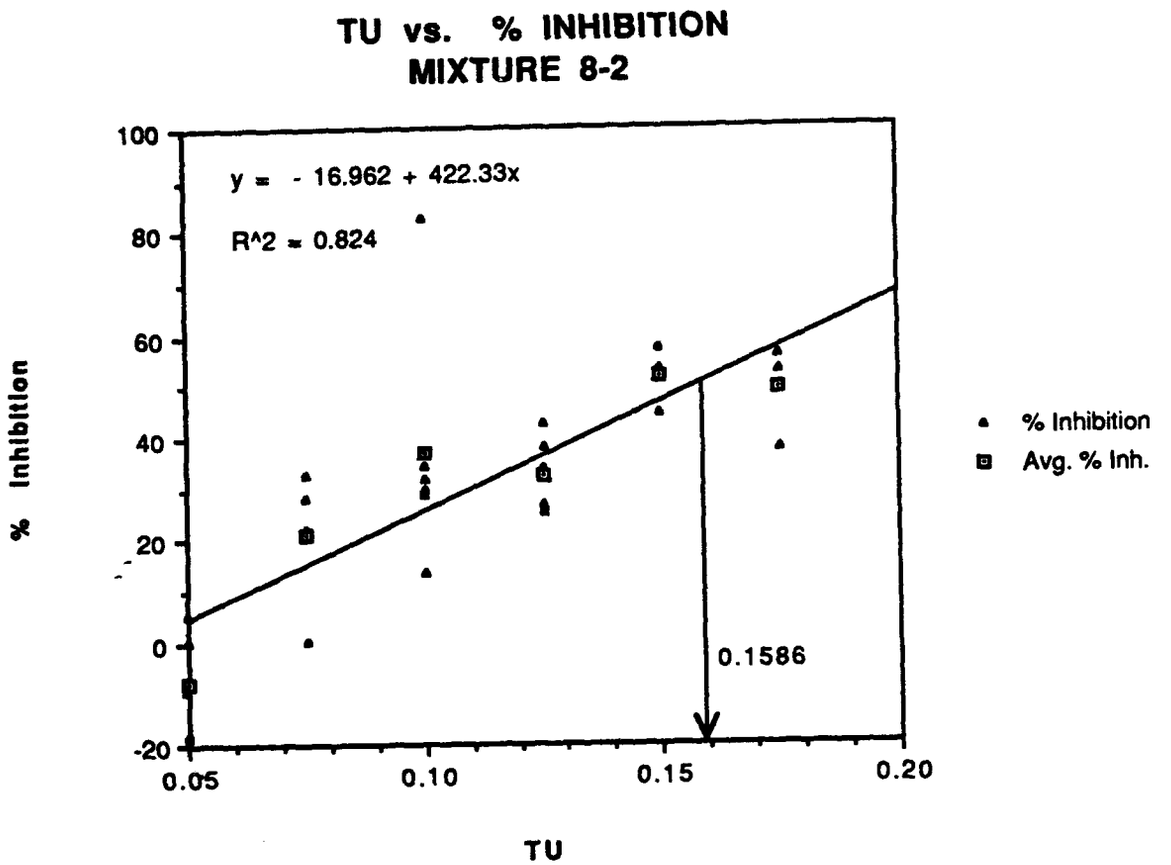
Table A-VIII-1 (cont'd)

ID#	Chemical name	Type	0xv	1xv	MW mg/mM	QSAR model predictions IC50 mg/L	Exp. results of diff. sources This study IC50 mg/L	Blum's IC50 mg/L	V & G's IC50 mg/L
43	Cyclohexanone	AKE		2.41	98.15	3,200	5,452		
44	n-Butylamine	AMI		2.11	73.14	98	111		
45	t-Butylamine	AMI		1.78	73.14	126	90		
46	Diethylamine	AMI		2.12	73.14	98	100		
47	Acetic acid	AMI		0.93	60.65	198	299		
48	Cyclohexylamine	AMI		2.64	99.18	90	103		
49	Ethanolamine	AMI		1.22	61.08	161	115		
50	Triethanolamine	AMI		3.39	149.19	77	741		
New chemicals:									
51	Pentachlorophenol	ARO		4.73	266.34	1			3
52	Phenol	ARO		2.13	94.11	625		1,100	520
53	Benzyl alcohol	ARO		2.73	108.13	140		2,100	
54	m-Cresol	ARO		2.54	108.14	236		440	
55	p-Cresol	ARO		2.54	108.14	236		260	
56	2 Chlorophenol	ARO		2.61	128.56	232		360	
57	3 Chlorophenol	ARO		2.61	128.56	232		160	
58	4 Chlorophenol	ARO		2.61	128.56	232		98	
59	2,3 Dichlorophenol	ARO		3.10	163	77		210	
60	2,5 Dichlorophenol	ARO		3.10	163	77		180	
61	2,6 Dichlorophenol	ARO		3.10	163	77		410	
62	2,3,4 Trichlorophenol	ARO		3.58	197.45	25		8	
63	2,3,6 Trichlorophenol	ARO		3.58	197.45	25		14	
64	2,4,5 Trichlorophenol	ARO		3.58	197.45	25		23	
65	4 Bromoethane	ARO		3.02	173.01	101		120	
66	1,1,2 Trichloroethane	HAL	4.90		133.41	526		240	440
67	Chloroform	HAL	3.97		119.39	1,223		640	500
68	1,1 Dichloroethane	HAL	3.84		98.96	1,158		620	
69	1,1,1,2 Tetrachloroethane	HAL	5.74		167.85	279		230	
70	Pentachloroethane	HAL	6.74		202.3	120		150	
71	1 Chloropropane	HAL	3.54		78.54	1,251		700	
72	2 Chloropropane	HAL	3.71		78.54	1,051		440	
73	1,3 Dichloropropane	HAL	4.38		112.99	760		210	
74	1,2,3 Trichloropropane	HAL	5.39		147.43	351		290	
75	1 Chlorobutane	HAL	4.25		92.57	711		230	
76	1 Chloropentane	HAL	5.03		106.6	368		68	
77	1 Chlorohexane	HAL	5.66		120.62	218		83	
78	1,3 Dichloropropene	HAL	4.12		112.99	992		120	
79	5 Chloro 1 pentyne	HAL	4.33		102	722		86	
80	Methanol	AKE		0.45	32.04	58,518		20,000	
81	1 Butanol	AKE		2.02	74.12	5,384		3,900	
82	Ethyl ether	AKE		1.99	74.12	5,726		17,000	
83	2 Butanone	AKE		1.77	72.11	8,753		11,000	

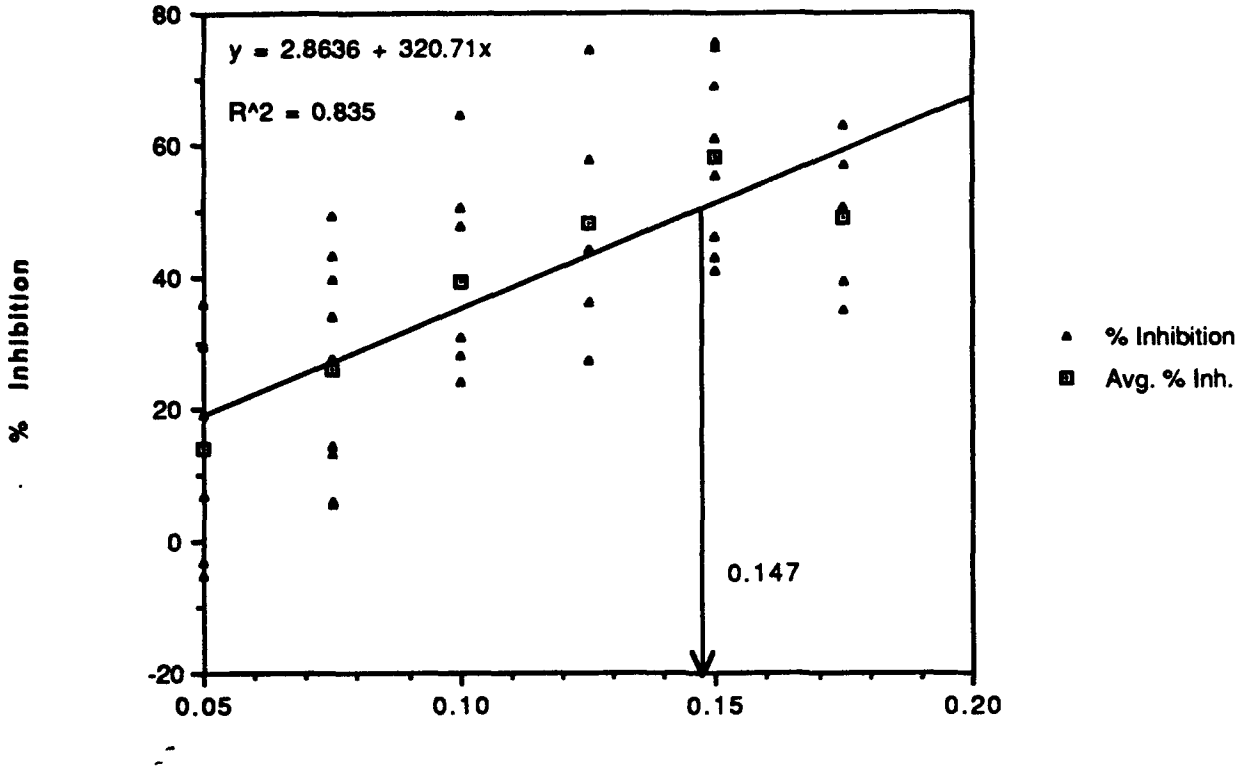
APPENDIX IX

8 CHEMICAL MIXTRUE LIST COMBINATIONS

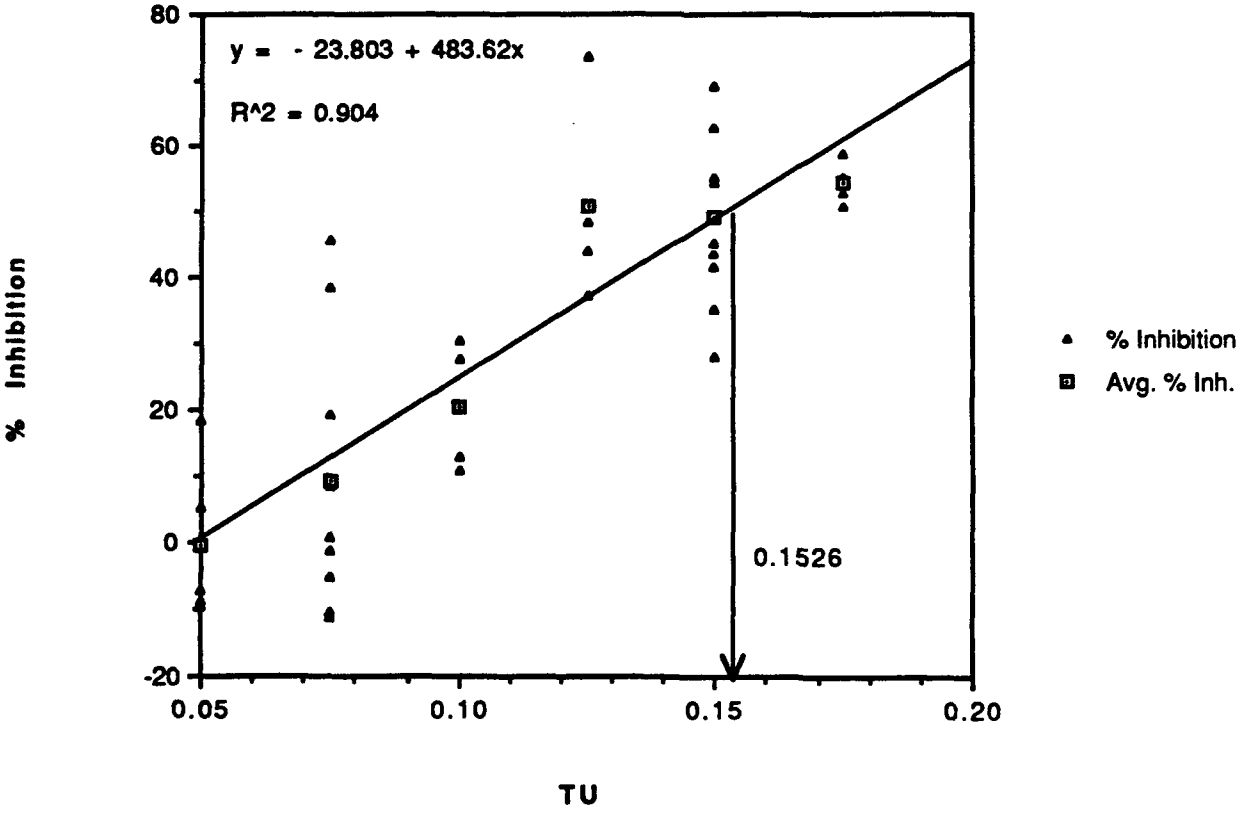
Mixture Nº	Chemical Nº	Chemical Name	Statistics	
8-1	12	Dibromomethane		
	30	Bis (2-chloroethyl) ether		
	32	Propanol	r^2 =	0.115
	33	Pentanol		
	35	n-Butyl acetate	TU =	0.517
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
8-2	1	Benzene		
	2	Toluene		
	12	Dibromomethane	r^2 =	0.824
	18	Bromochloromethane		
	35	n-Butyl acetate	TU =	0.1586
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
8-3	22	1,2 Dichloroethylene		
	23	Trichloroethylene		
	32	Propanol	r^2 =	0.835
	33	Pentanol		
	35	n-Butyl acetate	TU =	0.147
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
8-4	4	Ethylbenzene		
	12	Dibromomethane		
	17	1,2 Dichloropropane	r^2 =	0.904
	18	Bromochloromethane		
	32	Propanol	TU =	0.1526
	33	Pentanol		
	34	Octanol		
	36	Isobutyl acetate		
8-5	4	Ethylbenzene		
	10	2,4 Dimethyl phenol		
	18	Bromochloromethane	r^2 =	0.92
	22	1,2 Dichloroethylene		
	23	Trichloroethylene	TU =	0.1373
	32	Propanol		
	33	Pentanol		
	36	Isobutyl acetate		
8-6	2	Toluene		
	4	Ethylbenzene		
	5	Chlorobenzene	r^2 =	0.953
	10	2,4 Dimethyl phenol		
	15	1,1,1 Trichloroethane	TU =	0.1483
	21	Ethylene dibromide		
	35	n-Butyl acetate		
	40	Methyl ethyl ketone		


TU vs. % INHIBITION
MIXTURE 8-2
y = - 16.962 + 422.33x
R^2 = 0.824
100
80
60
40
20
0
-20
0.05
0.10
0.15
0.20
0.1586
% Inhibition
TU
% Inhibition
Avg. % Inh.

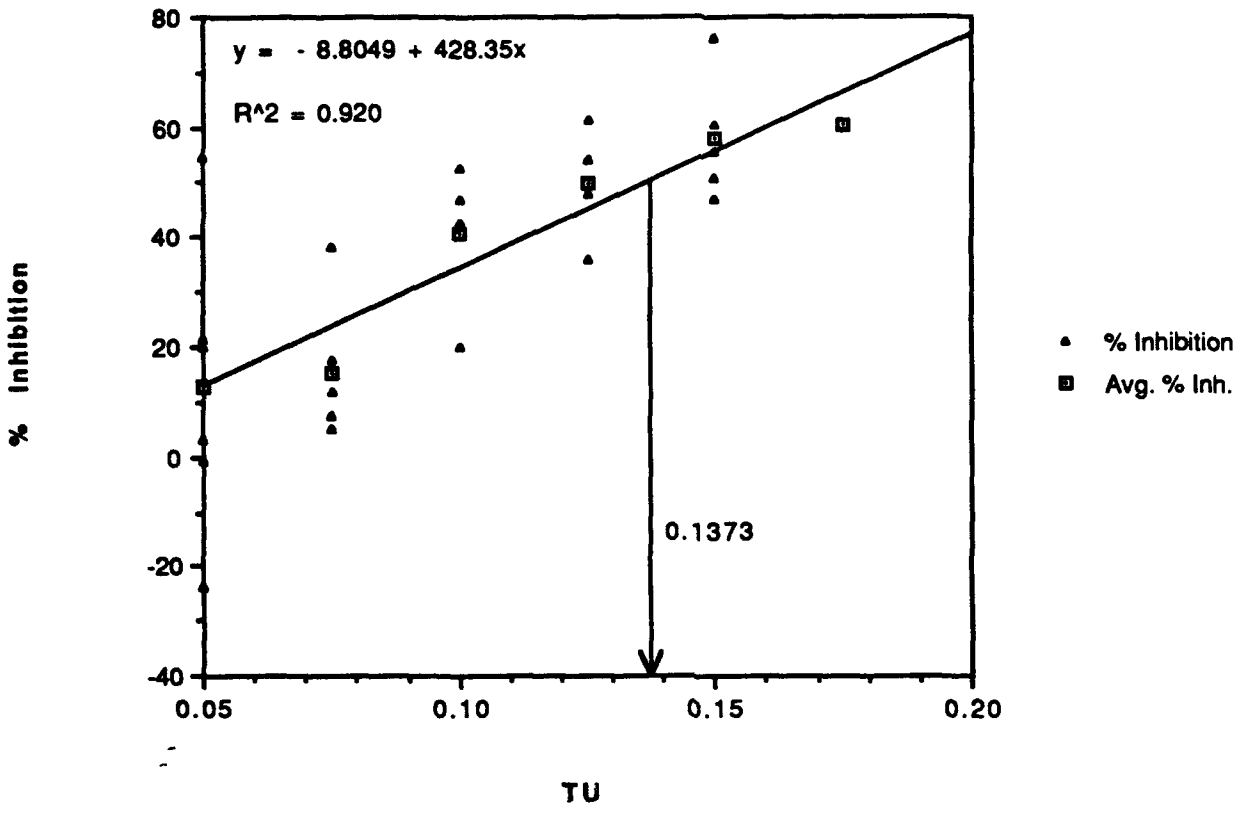
TU vs. % INHIBITION
MIXTURE 8-3



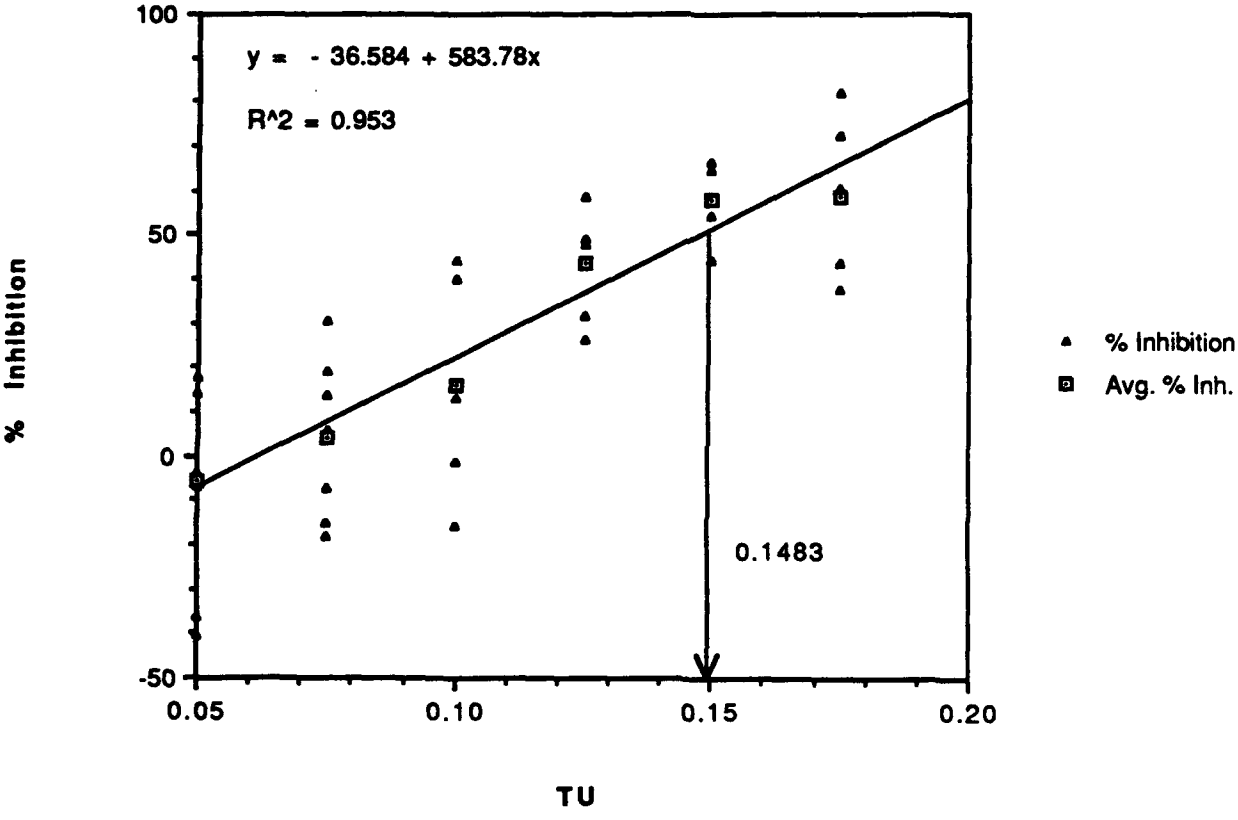
TU vs. % INHIBITION
MIXTURE 8-4



TU vs. % INHIBITION
MIXTURE 8-5



TU vs. % INHIBITION
MIXTURE 8-6



APPENDIX X

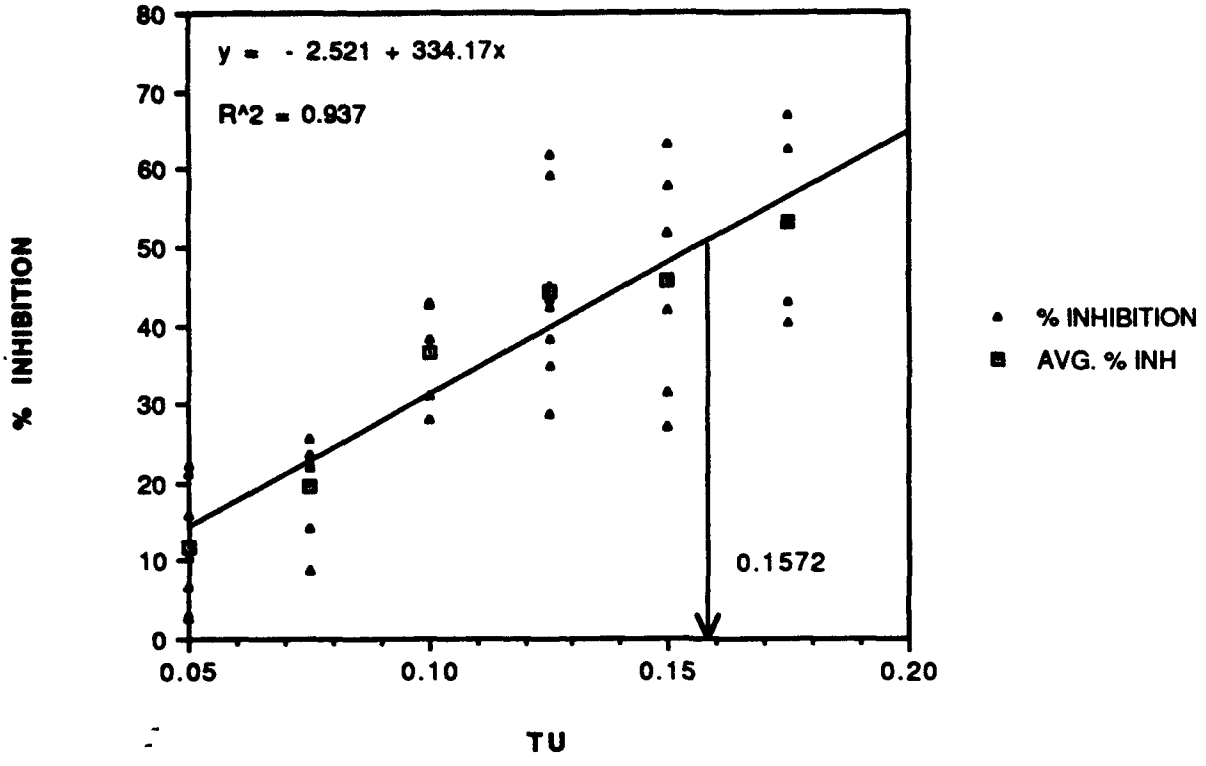
10 CHEMICAL MIXTRUE LIST COMBINATIONS

Mixture Nº	Chemical Nº	Chemical Name	Statistics	
10-1	1	Benzene		
	2	Toluene		
	4	Ethylbenzene	r^2 =	0.937
	5	Chlorobenzene		
	10	2,4 Dimethyl phenol	TU =	0.1572
	12	Dibromomethane		
	18	Bromochloromethane		
	32	Propanol		
	33	Pentanol		
	36	Isobutyl acetate		
10-2	4	Ethylbenzene		
	5	Chlorobenzene		
	10	2,4 Dimethyl phenol	r^2 =	0.863
	12	Dibromomethane		
	18	Bromochloromethane	TU =	0.1604
	22	1,2 Dichloroethylene		
	23	Trichloroethylene		
	32	Propanol		
	33	Pentanol		
	36	Isobutyl acetate		
10-3	4	Ethylbenzene		
	5	Chlorobenzene		
	10	2,4 Dimethyl phenol	r^2 =	0.947
	17	1,2 Dichloropropane		
	32	Bromochloromethane	TU =	0.1703
	33	1,2 Dichloroethylene		
	35	n-Butyl acetate		
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
10-4	2	Toluene		
	4	Ethylbenzene		
	5	Chlorobenzene	r^2 =	0.963
	10	2,4 Dimethyl phenol		
	32	Bromochloromethane	TU =	0.1496
	33	1,2 Dichloroethylene		
	35	n-Butyl acetate		
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
10-5	17	1,2 Dichloropropane		
	31	Ethanol		
	32	Propanol	r^2 =	0.987
	33	Pentanol		
	34	Octanol	TU =	0.1843
	35	n-Butyl acetate		
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
	43	Cyclohexanone		

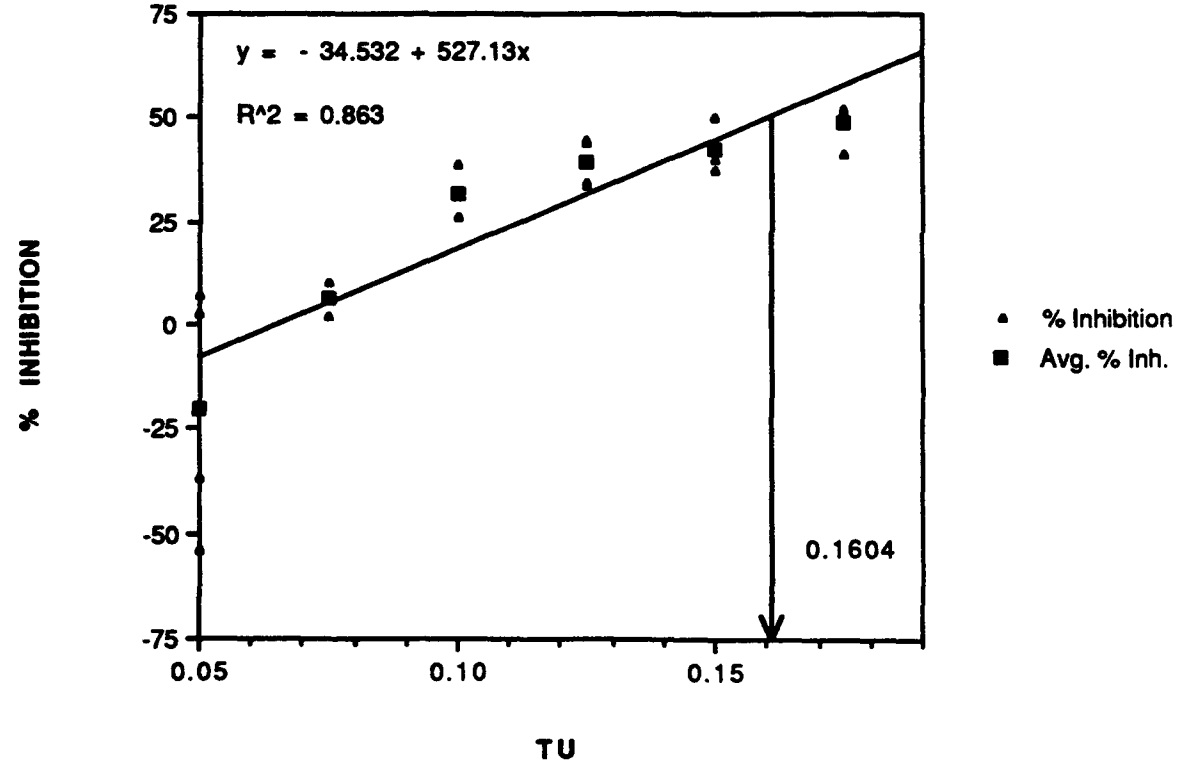
10 CHEMICAL MIXTRUE LIST COMBINATIONS (Continued)

Mixture Nº	Chemical Nº	Chemical Name	Statistics	
10-6	1	Benzene		
	2	Toluene		
	12	Dibromomethane	r^2 =	0.931
	18	Bromochloromethane		
	31	Ethanol	TU =	0.1535
	35	n-Butyl acetate		
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
	43	Cyclohexanone		
10-7	12	Dibromomethane		
	18	Bromochloromethane		
	22	1,2 Dichloroethylene	r^2 =	0.906
	23	Trichloroethylene		
	31	Ethanol	TU =	0.1913
	32	Propanol		
	33	Pentanol		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
	43	Cyclohexanone		
10-8	4	Ethylbenzene		
	5	Chlorobenzene		
	17	1,2 Dichloropropane	r^2 =	0.890
	32	Propanol		
	34	Octanol	TU =	0.1834
	35	n-Butyl acetate		
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
	43	Cyclohexanone		
10-9	1	Benzene		
	2	Toluene		
	4	Ethylbenzene	r^2 =	0.965
	5	Chlorobenzene		
	17	1,2 Dichloropropane	TU =	0.157
	18	Bromochloromethane		
	35	n-Butyl acetate		
	36	Isobutyl acetate		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
10-10	4	Ethylbenzene		
	5	Chlorobenzene		
	12	Dibromomethane	r^2 =	0.946
	17	1,2 Dichloropropane		
	18	Bromochloromethane	TU =	0.1309
	22	1,2 Dichloroethylene		
	23	Trichloroethylene		
	40	Methyl ethyl ketone		
	41	Methyl isobutyl ketone		
	43	Cyclohexanone		

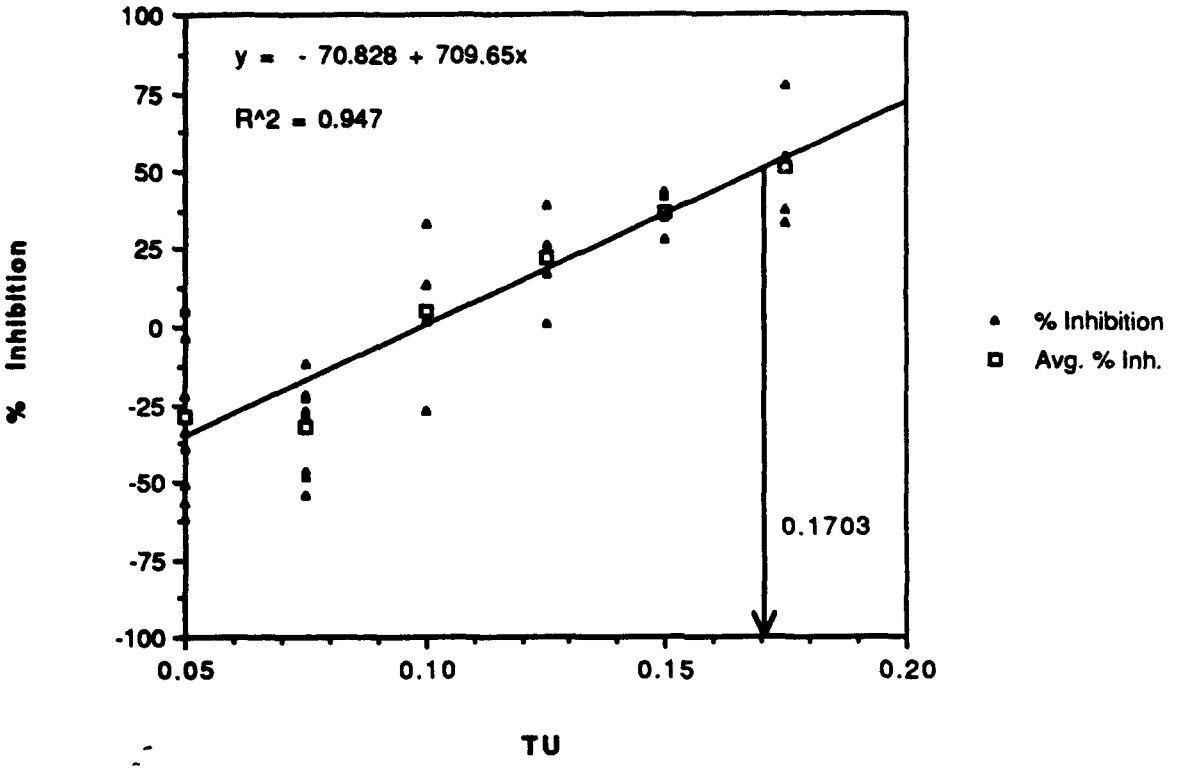
TU vs. % INHIBITION
MIXTURE 10-1



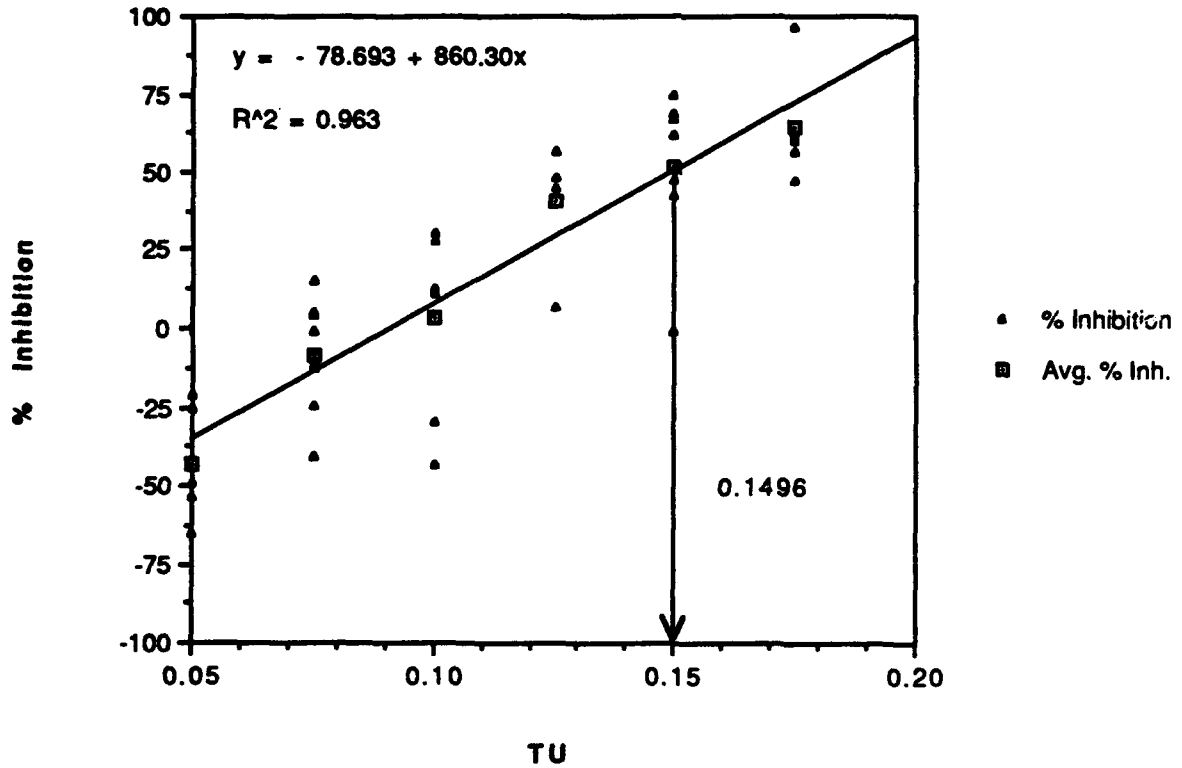
TU vs. % INHIBITION
MIXTURE 10-2



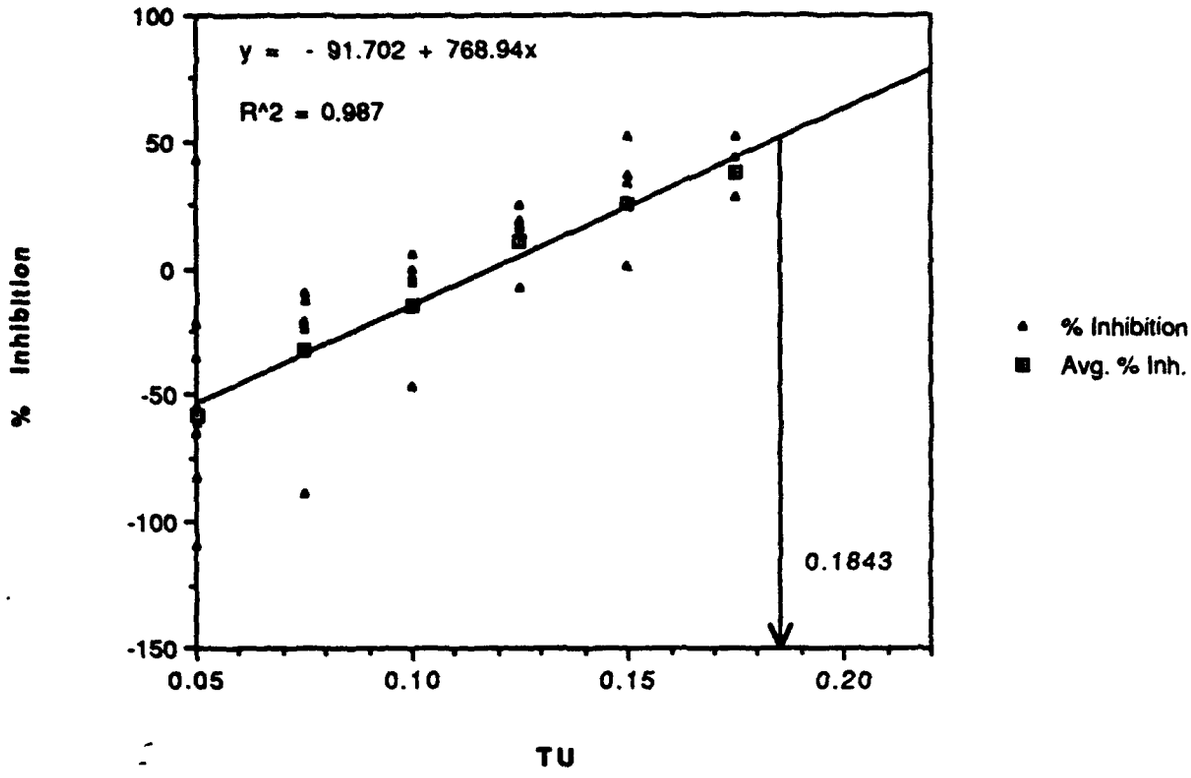
TU vs. % INHIBITION
MIXTURE 10-3



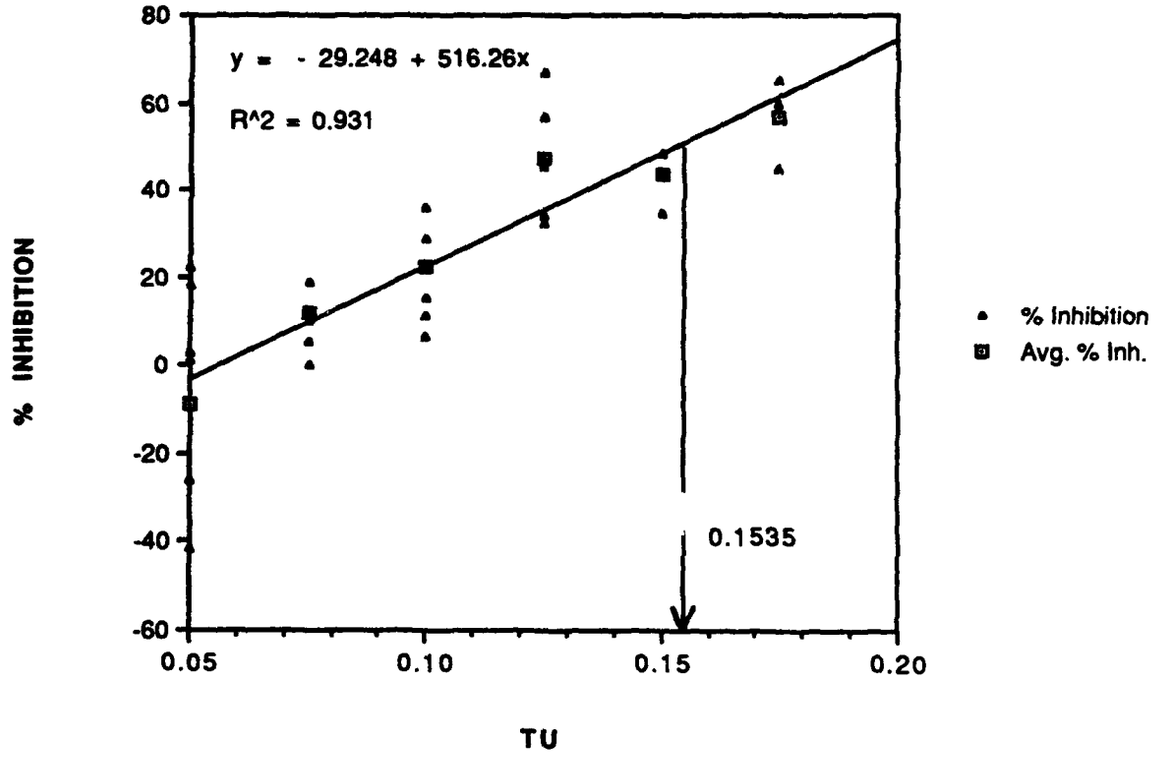
TU vs. % INHIBITION
MIXTURE 10-4



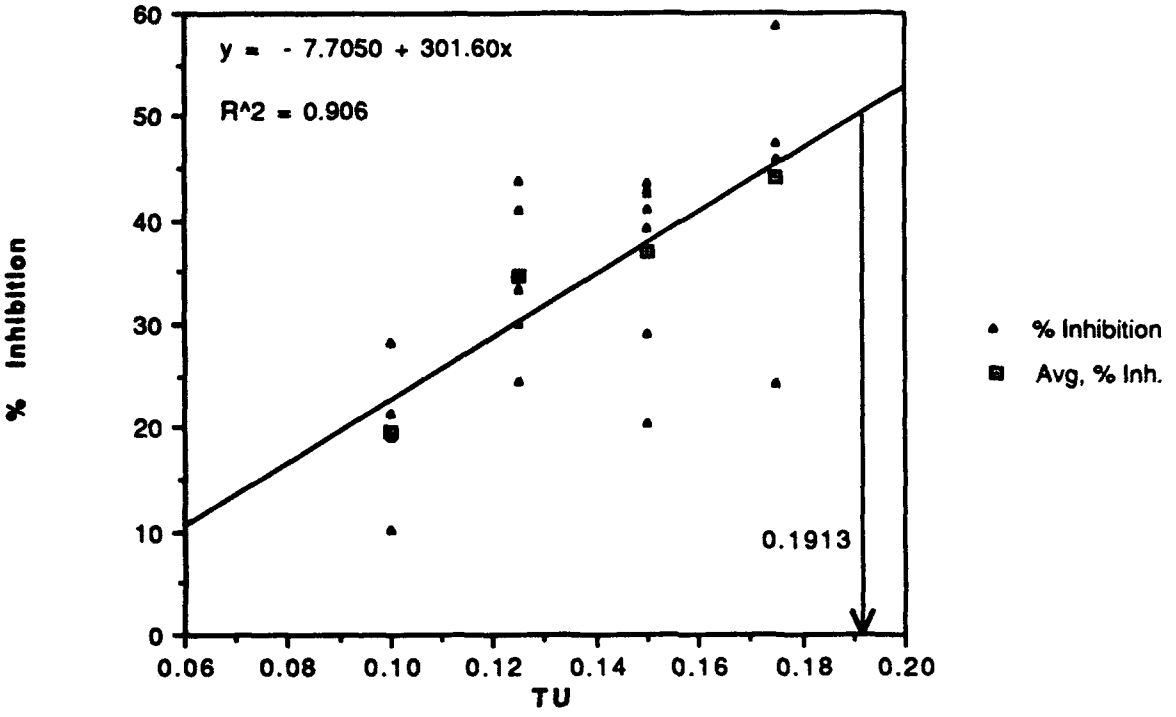
TU vs. % INHIBITION
MIXTURE 10-5



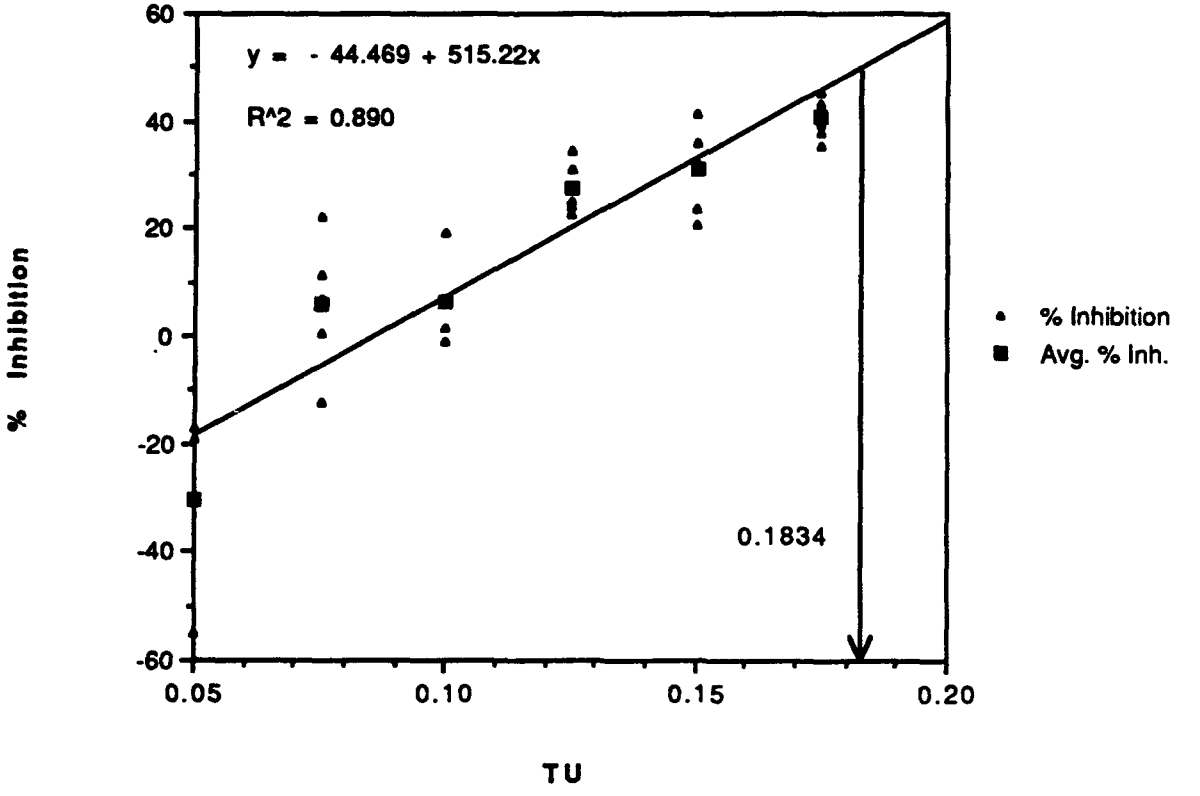
TU vs. % INHIBITION
MIXTURE 10-6



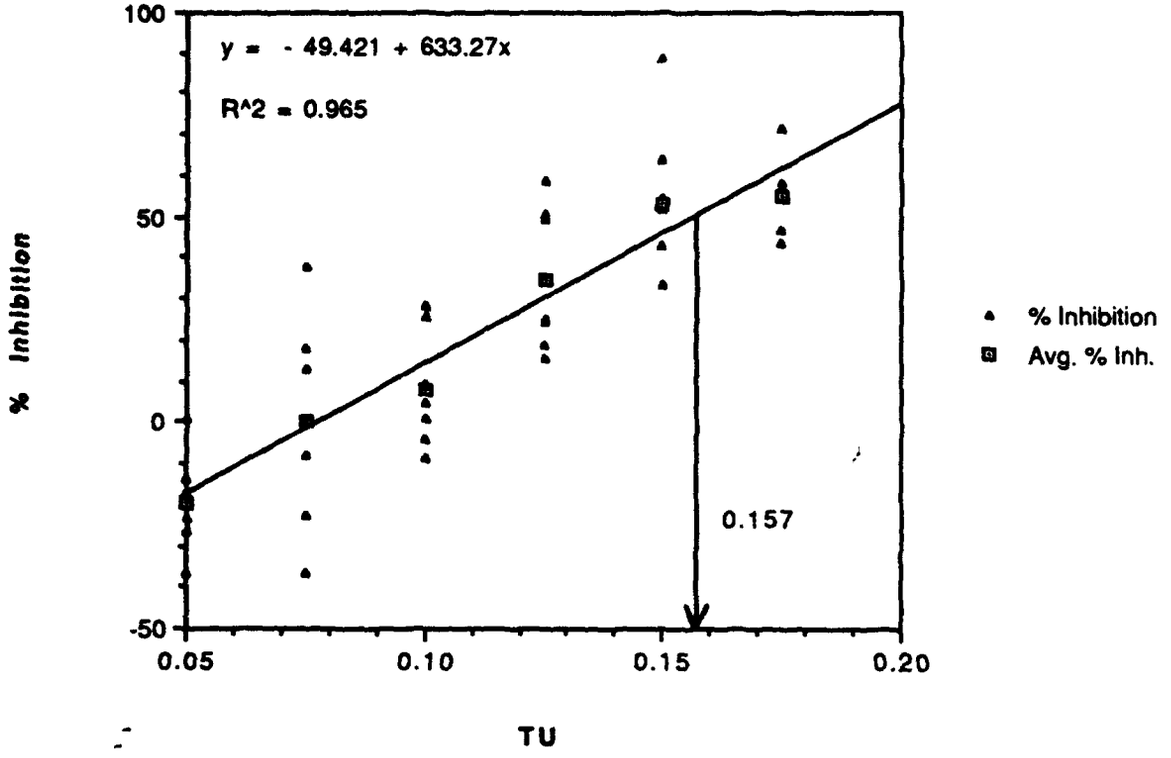
TU vs. % INHIBITION
Mixture 10-7



TU vs. % INHIBITION
MIXTURE 10-8



TU vs. % INHIBITION
MIXTURE 10-9



TU vs. % INHIBITION
MIXTURE 10-10