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Toxicity of 50 organic chemicals to microorganisms was determined using the respirometeric approach. Using this experimental database, models for predicting toxicity (IC50 values) were developed using QSAR techniques. Toxicity measurements were also made for ten binary mixtures, and sixteen multi-component mixtures. The joint effects of organic chemicals in mixtures were analyzed by three different approaches. Using the QSAR model developed from single chemical studies, an approach was developed to analyze and predict joint effects of chemicals in mixtures. The results of this study indicate that the joint effects could be considered simply additive for the different classes of chemicals tested.

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

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Interim Progress Report
Phase I
September 1991- August 1992

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Toxicity of Mixtures of Organic Contaminants to Microorganisms
- Phase I: Study Using Polytox Cultures-

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Abstract

Toxicity of 50 organic chemicals to microorganisms was determined using the respirometeric approach. Using this experimental database, models for predicting toxicity (IC_{50} values) were developed using QSAR techniques. Toxicity measurements were also made for ten binary mixtures, and sixteen multi-component mixtures. The joint effects of organic chemicals in mixtures were analyzed by three different approaches. Using the QSAR model developed from single chemical studies, an approach was developed to analyze and predict joint effects of chemicals in mixtures. The results of this study indicate that the joint effects could be considered simply additive for the different classes of chemicals tested.

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 several 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. A study is underway 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 I study during the first year.

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Objectives of Phase I 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 the first year:

a) measure single chemical toxicity to a surrogate culture of microorganisms;
b) develop a QSAR model to predict single chemical toxicity to the surrogate organisms;
c) measure and analyze toxicity of binary mixtures to surrogate organisms and verify simple additivity;
d) measure and analyze toxicity of multi-component mixtures to surrogate organisms and verify simple additivity;
e) develop an approach to predict joint effects of mixtures of organic chemicals to the surrogate culture based purely on the molecular structures of the components of the mixtures.

Work during the second and third years will focus on the effects of mixtures on activated sludge and anaerobic cultures with a view to predict joint effects on them using molecular structures of the components and the surrogate culture results.

Several techniques and approaches in toxicity modeling reported in the literature as individual studies are integrated in this study. Some of the features of this study are use of a 20-reactor, computer integrated respirometer for toxicity determination; use of the probit transformation method to determine IC_{50}; and use of QSAR approach to predict joint effects of binary mixtures and multi-component mixtures.

This interim report covers the work done in Phase I during the first year. Results reported below include:

a) developing an experimental procedure for IC_{50} determination using the respirometeric approach;
b) evaluation of the use of Polytox (polbac Corp.) cultures as surrogate microorganisms in rapid toxicity determination;
c) development of QSAR model for single chemical toxicity;
d) testing the hypothesis that joint effects of the chosen chemicals are simply additive in binary and multi-component mixtures;
e) establishment of a methodology to analyze joint effects in binary mixtures and multi-component mixtures;
f) development and validation of a QSAR-based approach to predict the concentrations of the components in mixture that would cause 50% inhibition.

Experimental Approach

All tests were conducted using research grade chemicals as supplied by the manufacturers without any further purification. The toxicity tests were run on a 20-reactor computer interfaced N-Con Respirometer Model 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 then transformed to probit values according to Finney (1964). From these probit vs. concentration plots, the IC_{50} values were then determined [IC_{50} is the concentration of the toxicant at which a 50% inhibition is caused]. The above procedure is illustrated schematically in Fig 1.

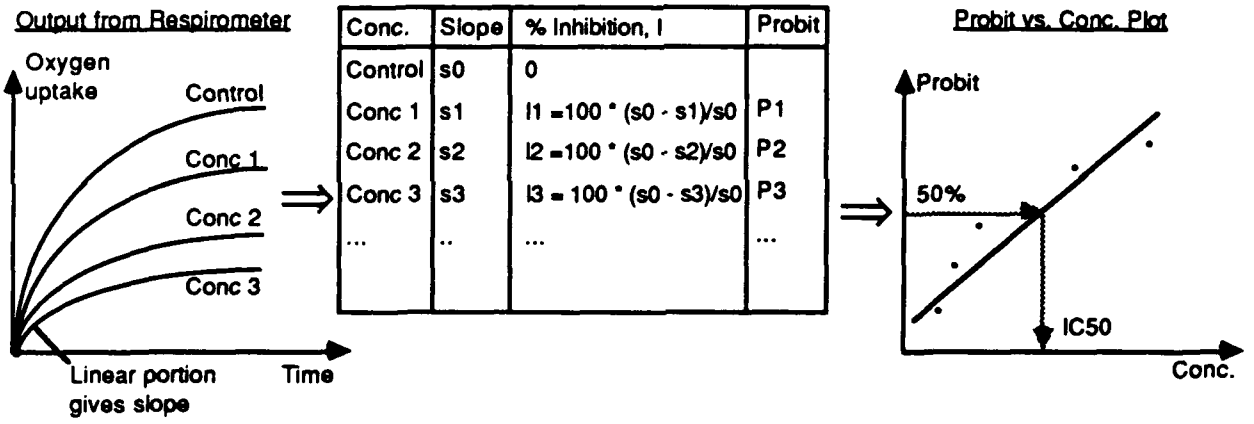

Figure 1. Schematic Illustration of Procedure to Find IC_{50}

In the single chemical studies, 50 chemicals belonging to several different congeneric classes with a diversity of molecular structures were chosen for testing. 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.

In the binary mixture tests, n-octanol was chosen as one of the toxicants and the following chemicals as the second component: chlorobenzene, 2,4 dimethyl phenol, 1,1,1 trichloroethane, cyclohexane, n-butyl amine, n-amyl acetate, ethanol, chlorodibromomethane, cyclohexanone and ethanolamine. For each combination of the two components, a matrix of four octanol concentrations by four second contaminant concentrations was assayed. The four test concentrations were chosen as 0.4, 0.6, 0.8 and 0.9 of the individual IC_{50} concentrations.

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.

Modeling Approach

The single chemical toxicity results from the 50 chemicals were used to develop a QSAR model. Molecular connectivity indexes were calculated for the 50 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 IC_{50} values as the dependent variable. The IC_{50} values calculated from the QSAR models were then compared with the experimentally measured values.

In the binary mixture studies, isobolograms were first constructed to test the hypothesis that the joint effects were according to the simple additivity model. Then, the QSAR models developed from single chemical was used to predict the joint toxicity caused by the second chemical in the presence of n-octanol. The predicted values were compared with the experimental results.

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

a) Single chemical results-

The 50 chemicals used in this study are listed in Table I. This Table also contains the respective IC_{50} values and the corresponding r^2 found from the probit vs. concentration plots from which the IC_{50} values were determined. These r^2 values range from 0.7 to 0.98, indicating a reasonably good database. The distribution of r^2 is shown in Fig 2. On preliminary evaluation, the following four chemicals were found to be "outliers": 1,4 dichlorobenzene, 1,2 dichloroethylene, triethanolamine and bromodichloromethane. At this point, no explanation could be postulated for the outlying tendency of these chemicals. Other researchers have found similar peculiarities too. These chemicals were therefore excluded from the analysis and the remaining 46 were used in the QSAR model development.

The first order molecular connectivity index, $^1\chi^v$, was found to fit the individual IC_{50} values (in mM/L) for the alkanes, aromatic compounds, alcohols, esters, ketones, amines:

Alcohols, ketones and esters:

$$\log IC_{50} = 3.690 - 0.896\ ^1\chi^v$$

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

Alkanes:

$$\log IC_{50} = 1.851 - 0.765\ ^1\chi^v$$

$n = 5$; $r = 0.999$; $r^2 = 0.999$; RMS residual = 0.018.

Amines and acids:

$$\log IC_{50} = 1.045 - 0.470\ ^1\chi^v$$

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

Aromatics:

$$\log IC_{50} = 3.258 - 1.133\ ^1\chi^v$$

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

In the case of halogenated aliphatics, the zero order molecular connectivity index, $^0\chi^v$, fitted the data set more closely:

Halogenated aliphatics:

$$\log IC_{50} = 2.670 - 0.448\ ^0\chi^v$$

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

Table I. Chemicals Assayed and Single Chemical Results

ID #	Chemical	Type	IC50 [mg/L]	r^2	Log IC50 [mM/L] Exp.	Calc.	Error	
1.	Benzene	ARO	685	0.898	0.94	0.99	-0.05	
2.	Toluene	ARO	207	0.870	0.35	0.53	-0.18	
3.	Xylene	ARO	140	0.851	0.12	0.06	0.06	
4.	Ethylbenzene	ARO	220	0.950	0.32	-0.11	0.43	**
5.	Chlorobenzene	ARO	350	0.966	0.50	0.46	0.04	
6.	1,2 Dichlorobenzene	ARO	135	0.939	-0.04	-0.10	0.06	
7.	1,3 Dichlorobenzene	ARO	40	0.903	-0.57	-0.09	-0.48	**
8.	1,4 Dichlorobenzene	ARO	6	0.908	-1.39			?
9.	1,2,4 Trichlorobenzene	ARO	23	0.810	-0.90	-0.63	-0.27	*
10.	2,4 Dimethyl phenol	ARO	240	0.953	0.29	-0.10	0.39	**
11.	Methylene chloride	HAL	1,750	0.845	1.31	1.34	-0.03	
12.	Dibromomethane	HAL	1,110	0.848	0.81	0.60	0.21	*
13.	Carbon tetrachloride	HAL	325	0.701	0.32	0.42	-0.09	
14.	1,2 Dichloroethane	HAL	685	0.979	0.84	1.02	-0.18	
15.	1,1,1 Trichloroethane	HAL	415	0.850	0.49	0.48	0.02	
16.	1,1,2,2 Tetrachloroethane	HAL	180	0.949	0.03	0.13	-0.10	
17.	1,2 Dichloropropane	HAL	500	0.954	0.65	0.63	0.01	
18.	Bromochloromethane	HAL	1,800	0.948	1.15	0.97	0.18	
19.	Bromodichloromethane	HAL	90	0.916	-0.26			?
20.	Chlorodibromomethane	HAL	425	0.959	0.31	0.15	0.17	
21.	Ethylene dibromide	HAL	520	0.939	0.45	0.37	0.08	
22.	1,2 Dichloroethylene	HAL	350	0.783	0.56			?
23.	Trichloroethylene	HAL	500	0.979	0.58	0.67	-0.09	
24.	Tetrachloroethylene	HAL	175	0.923	0.02	0.19	-0.17	
25.	Cyclohexane	ALK	74	0.964	-0.06	-0.06	0.01	
26.	Pentane	ALK	70	0.793	-0.01	0.01	-0.02	
27.	Hexane	ALK	38	0.751	-0.36	-0.38	0.02	
28.	Heptane	ALK	18	0.739	-0.75	-0.76	0.01	
29.	Octane	ALK	8	0.857	-1.15	-1.14	-0.01	
30.	Bis (2-chloroethyl) ether	AKE	1,600	0.886	1.05	0.84	0.21	*
31.	Ethanol	AKE	40,000	0.964	2.94	2.79	0.15	
32.	Propanol	AKE	7,200	0.942	2.08	2.33	-0.25	*
33.	Pentanol	AKE	2,325	0.821	1.42	1.41	0.01	
34.	Octanol	AKE	126	0.968	-0.01	0.04	-0.05	
35.	n-Butyl acetate	AKE	3,750	0.923	1.51	1.07	0.44	**

Code for type: ARO- aromatics; HAL- halogenated aliphatics; ALK- alkanes; AKE- alcohols,ketones and esters; AMI- amines.
Note: * error >1 std. dev.; ** error > 2 std. dev.; ? excluded from analysis.

Table I (contd.)

ID #	Chemical	Type	IC50 [mg/L]	r^2	Log IC50 [mM/L] Exp.	Calc.	Error	
36.	Isobutyl acetate	AKE	1,600	0.955	1.14	1.20	-0.06	
37.	n-Amyl acetate	AKE	440	0.980	0.53	0.61	-0.08	
38.	Ethyl acetate	AKE	5,400	0.916	1.79	1.98	-0.19	
39.	Acetone	AKE	48,000	0.864	2.92	2.62	0.30	*
40.	Methyl ethyl ketone	AKE	1,900	0.885	1.41	1.90	-0.49	**
41.	Methyl isobutyl ketone	AKE	2,600	0.905	1.42	1.32	0.09	
42.	Methyl n-propyl ketone	AKE	4,500	0.883	1.72	1.65	0.07	
43.	Cyclohexanone	AKE	3,750	0.964	1.58	1.51	0.07	
44.	n-Butyl amine	AMI	90	0.952	0.09	0.05	0.04	
45.	t-Butyl amine	AMI	85	0.919	0.07	0.21	-0.14	
46.	Diethylamine	AMI	104	0.769	0.15	0.05	0.11	
47.	Acetic acid	AMI	287	0.744	0.68	0.61	0.07	
48.	Cyclohexylamine	AMI	60	0.885	-0.22	-0.20	-0.02	
49.	Ethanolamine	AMI	160	0.955	0.42	0.47	-0.05	
50.	Triethanolamine	AMI	900	0.829	0.78			?
	Average			0.893				

Code for type: ARO- aromatics; HAL- halogenated aliphatics; ALK- alkanes; AKE- alcohols,ketones and esters; AMI- amines.
Note: * error >1 std. dev.; ** error > 2 std. dev.; ? excluded from analysis.

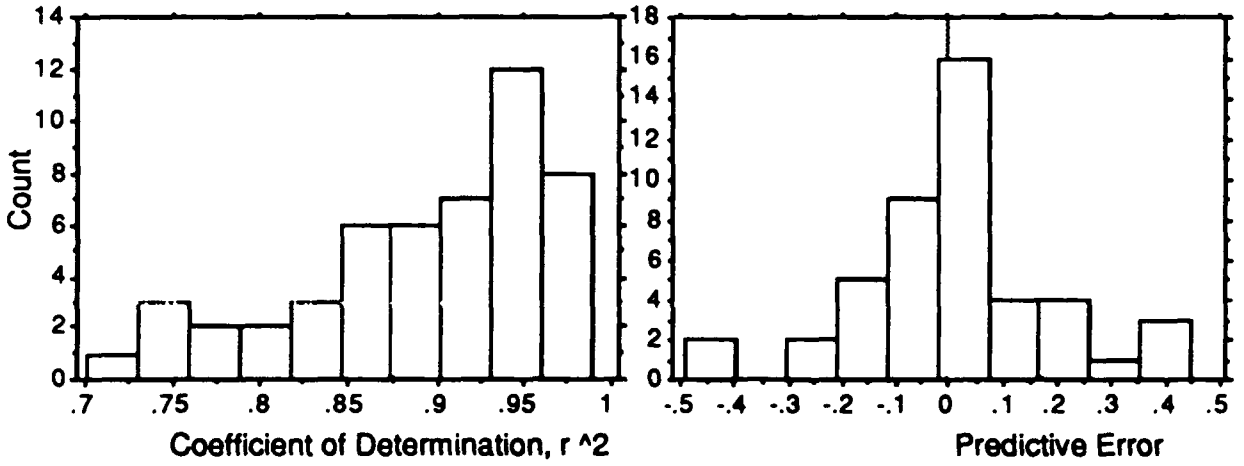

Figure 2. Frequency Distribution of
Coefficient Determination and Predictive Error in Table I

Detailed statistical parameters of these equations are included in Appendix III. These models explain about 90% of the variance in the experimental data for alcohols, ketones, esters, alkanes, amines and aromatics and over 70% in the case of the halogenated aliphatics. The average RMS residuals of 0.163 suggests that these equations can predict IC_{50} within a factor of 1.5. Except in the case of the aromatic compounds, this degree of precision is comparable to the uncertainty of the experimental IC_{50} values. The IC_{50} values calculated by these equations are tabulated in Table I and compared against the respective experimental values in Fig 3, from which the overall quality of the agreement between the two can be seen to be comparable to the experimental uncertainty in the IC_{50} values, with $r^2 = 0.954$. The distribution of the predictive error is shown in Fig 2.

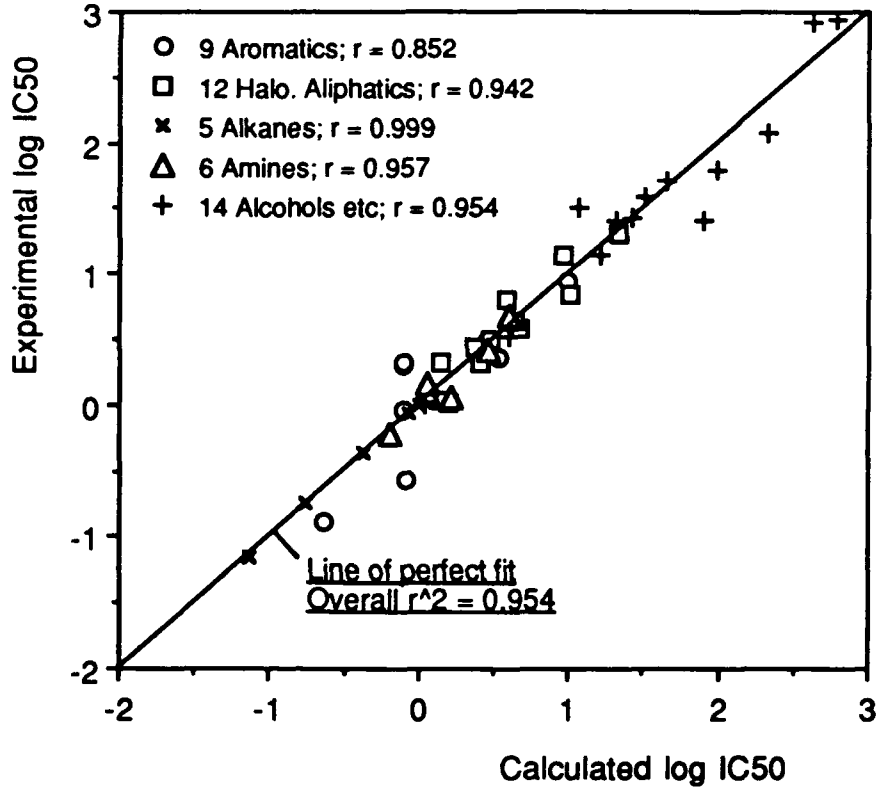

Figure 3. Comparison Between Experimental and Calculated log IC_{50} Values [mM/L].

b) Binary mixture results-

The isobologram developed from the experimental IC_{50} of the binary mixtures is shown in Fig 4. The experimental data points for the six toxicants falling on or around the diagonal confirms the hypothesis of simple additivity. Additional tests are being done now to check these results and include more chemicals.

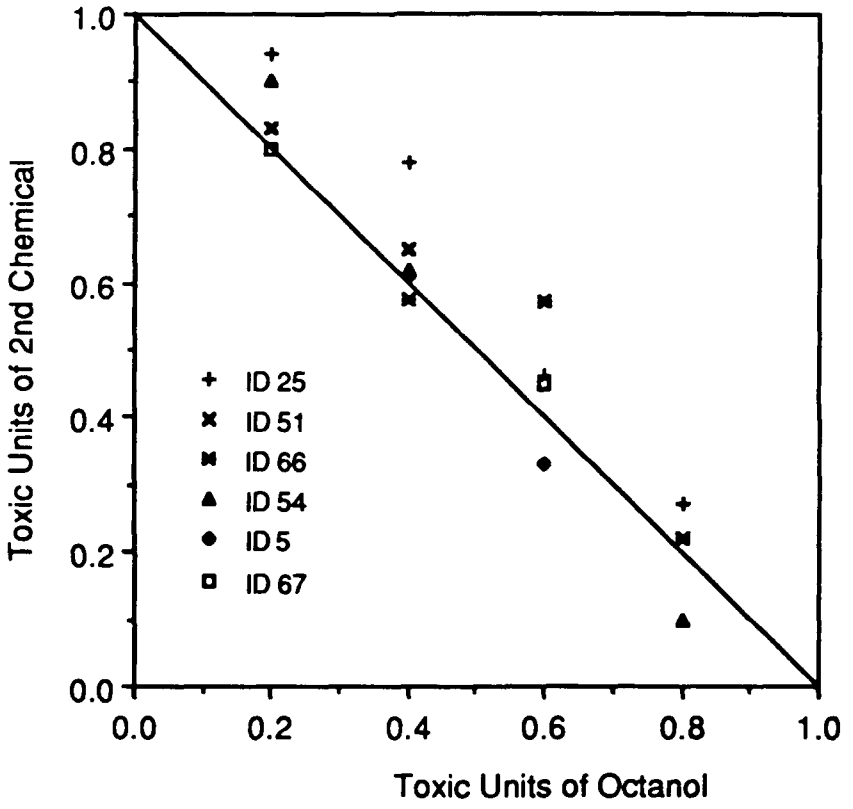

Figure 4. Isobologram- Octanol vs. Six Other Chemicals

c) Multi-component mixture results-

The results of the multi-component tests are presented in graphical form in Appendix IV-a for the 10-component mixtures and in Appendix IV-b for the 8-component mixtures. The r^2 in these plots range from 0.860 to 0.995, indicating a satisfactory database. The IC_{50} values obtained from these plots were used to analyze the joint effects by the Toxic Units, Additivity Index and Mixture Toxicity Index concepts as summarized in Table III.

Table III. Analysis of Joint Effects of Multi-component Mixtures

Mixture	Chemicals in Mixture		Joint Effects Analyzed by		
Nº	ID # of components	Total n	Toxic Units M = ∑TU	Additivity Index AI = M - 1	Toxicity Index MTI = 1 - logM/logn
10C-1	4,5,10,36,32,33,12,18,1,2	10	1.10	0.10	0.96
10C-2	4,5,10,36,32,33,12,18,22,23	10	1.00	0.00	1.00
10C-3	40,41,35,36,32,33,4,5,10,17	10	1.14	0.14	0.94
10C-4	40,41,35,36,32,33,4,5,10,2	10	1.05	0.05	0.98
10C-5	40,41,35,36,32,33,31,43,34,17	10	1.31	0.31	0.88
10C-6	40,41,35,36,31,43,12,18,1,2	10	1.10	0.10	0.96
10C-7	40,41,43,31,32,33,12,18,22,23	10	1.15	0.15	0.94
10C-8	40,41,35,36,4,5,17,43,34,17	10	0.90	-0.10	1.05
10C-9	40,41,35,36,4,5,17,18,1,2	10	0.90	-0.10	1.05
10C-10	40,41,43,4,5,17,12,18,22,23	10	0.90	-0.10	1.05
8C-1	40,41,35,36,32,33,12,30	8	1.19	0.19	0.92
8C-2	40,41,35,36,12,18,1,2	8	1.02	0.02	0.99
8C-3	40,41,35,36,32,33,22,23	8	1.07	0.07	0.97
8C-4	4,36,32,33,12,18,34,17	8	1.02	0.02	0.99
8C-5	4,10,36,32,33,18,22,23	8	0.92	-0.08	1.04
8C-6	40,35,21,15,4,5,10,2	8	0.93	-0.07	1.03
	Average		1.04	0.04	0.98

Note: ID # refer to chemicals listed in Table I;
Simple additivity implied by M = 1, AI = 0 and MTI = 1.

The results of these analyses presented in Table III support the hypothesis of simple additivity for the chemicals assayed, with average values of ∑TU = 1.04±0.12, AI = 0.04±0.12, and MTI = 0.98±0.05.

The results from the 8-component multi-mixture studies were used to test the hypothesis that the concentrations of the components of a mixture causing 50% inhibition could be predicted from single chemical results. Two different approaches were used to predict the concentrations of the components at which 50% inhibition would be caused:

- in one approach, perfect simple additivity (i.e $\Sigma TU = 1.0$) was assumed and the corresponding concentrations of the components in the mixtures were determined from *experimentally measured single chemical IC_{50} values.*
- in the second approach, perfect simple additivity (i.e $\Sigma TU = 1.0$)was assumed and the corresponding concentrations of the components in the mixtures were determined from *QSAR models for single chemical IC_{50} values.*

Results of these calculations and comparisons are presented in detail in Appendix V. The concentrations predicted by these two approaches are compared against the actual experimental concentrations in Fig 5 for the 48 sets of data points for each approach (six different mixtures, each containing 8 components). The concentrations calculated by the first approach is in near perfect agreement ($r^2 = 0.995$) with the experimentally measured concentrations. This confirms that the joint action is according to simple additivity mechanism for the components tested.

In the second approach, the agreement appears to be "poor" ($r^2= 0.799$). This deviation stems mainly from the inadequacy of the QSAR models and partly from the slight deviation from simple additivity. In addition, two of these mixtures, Mixture Nº 3 and 5, contain 1,2 dichloroethylene which was found to be an "outlier" in the single chemical studies and excluded in the QSAR model development. Thus the single chemical IC_{50} predicted by the QSAR model for this chemical results in a higher error.

Nevertheless, it must be appreciated that the second approach does not require any experimental inputs whatsoever and the predictive errors are within a factor of 3, and the predictions are practically of the same order of magnitude as the experimental values, in the span of 2 log units of IC_{50} concentrations. This degree of precision is comparable to the work reported in the literature for single chemical studies, and can be considered sufficiently adequate for predicting toxicity of multi-component mixtures.

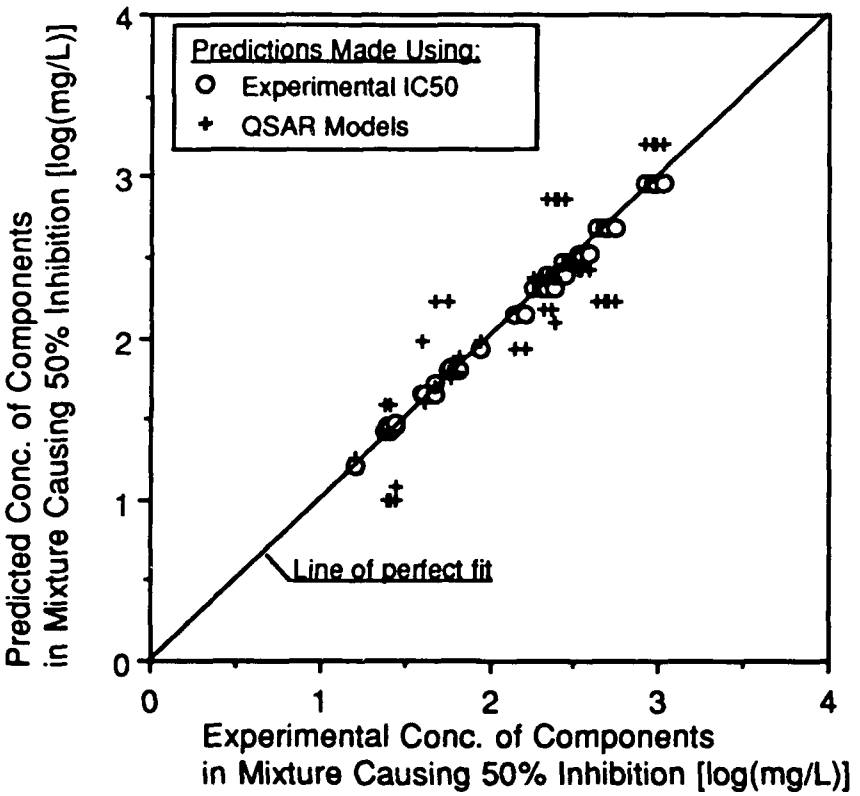

Figure 5. Comparison Between Experimental and Predicted Concentrations

Conclusions

The respirometeric technique for determining toxicity data has been demonstrated to be an efficient and reliable one. Joint effects of the range of chemicals assayed can be adequately quantified by the simple additivty concept using toxic units. The results of the above studies show clearly that QSAR approaches can be used to predict joint toxic effects of multiple chemicals on microorganisms. The quality of the predictions by the QSAR models for the range of chemicals tested in this study are comparable to the experimental uncertainty in the IC_{50} data.

Additional chemicals belonging to a wider range of molecular structures need to be assayed and used for checking the validity and utility value of these models. In addition, similar studies on activated sludge and anaerobic cultures need to be done to establish correlation between the Polytox surrogate cultures so that far reaching predictions can be made to maximize the benefits of this research.

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20. Sprague J B (1970); *Water Res*; 4, 3-32.
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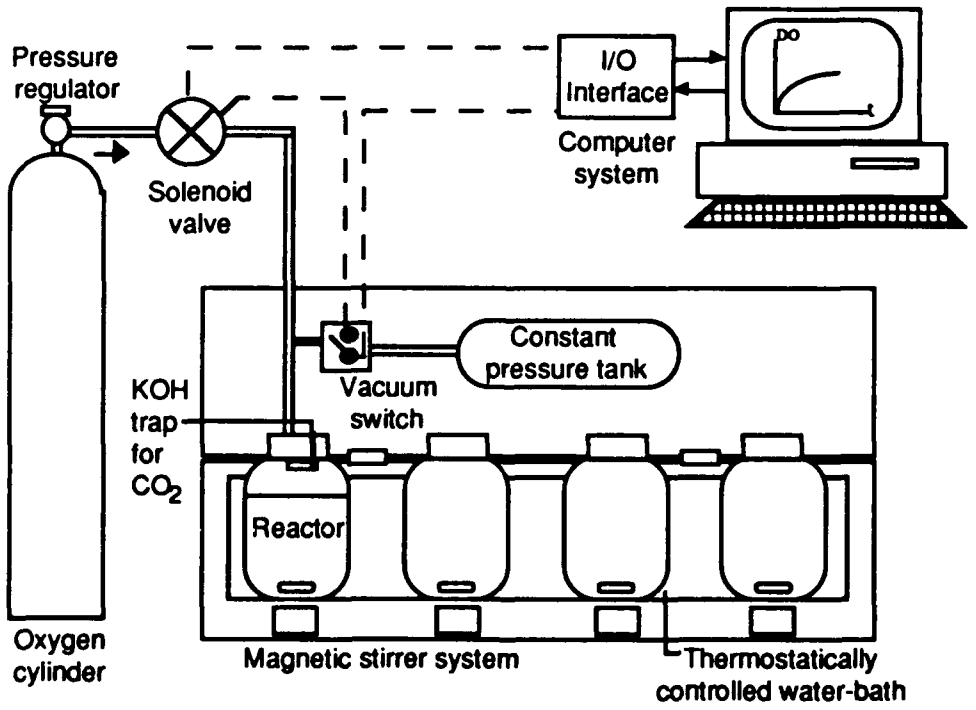
Appendix I:
Details of Respirometer System

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:
Details of the Test Procedures

In this study, a commercial culture- Polytox, was used to determine IC_{50} values of 50 organic chemicals. One vial of 8 gr of Polytox freeze dried cultures was dispersed in 250 mL of buffered dilution water, prepared according to Standard Methods. This medium was mixed for 30 minutes and then allowed to settle for 5 minutes. The supernatant was then separated for placement in eight 123 mL-reactors. Each of the eight reactors was fed with 6 mL of the supernatant and topped with buffered dilution water to bring up the final volume to 60 mL. The control reactor was topped up to 62 mL. All the reactors were then capped with potassium hydroxide pellets in the holders attached to the caps.

The capped reactors were then placed in the respirometer water bath, and oxygen was supplied to each reactor for a period of 4 hours. During this period the temperature of the water bath was maintained constant at 25ºC, and the contents of the reactors were kept well-mixed by magnetic stirrer bars. At the end of the 4-hr period, each reactor, except the control, was administered with different volumes of the toxicant being assayed. Each toxicant dose was prepared by dissolving the toxicant in 2 mL of acetone. The dosed reactors and the control reactor were then placed in the respirometer, with continued supply of oxygen. The data acquisition system was then re-initiated to monitor the oxygen uptake of each reactor for the next 6 hour period.

For each toxicant, a trial run was first made to establish the range of concentrations to bring about 50% inhibition. Once this range was determined by trial and error, a final run was made with appropriate volumes of the toxicant to cover inhibitions in the range of about 30 to 80%. The oxygen uptake as a function of time as recorded by the data acquisition system was then used to determine the IC_{50} values as described in the main text.

Appendix III

Detailed Statitics of QSAR Models

Regression Summary
log Y-RM vs. 1XV

Count	14
Num. Missing	0
R	.954
R Squared	.910
Adjusted R Squared	.903
RMS Residual	.246

Residual Statistics
log Y-RM vs. 1XV

# >= 0	7
# < 0	7
SS[e(i) - e(i-1)]	1.665
Durbin-Watson	2.288
Serial Autocorrelation	-.236

ANOVA Table
log Y-RM vs. 1XV

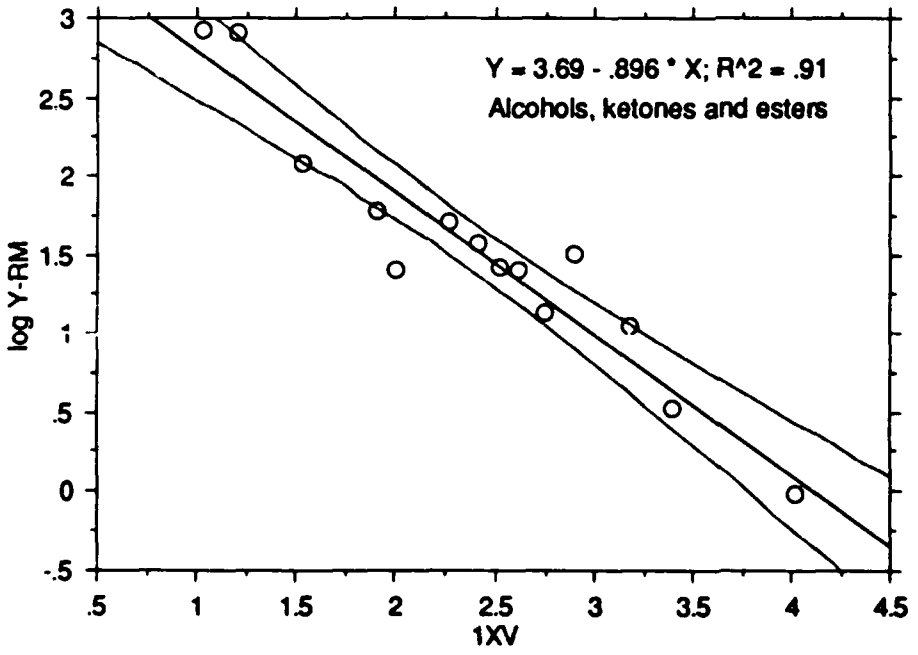
	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	7.398	7.398	122.014	<.0001
Residual	12	.728	.061		
Total	13	8.126			

Regression Coefficients
log Y-RM vs. 1XV

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	3.690	.206	3.690	17.920	<.0001
1XV	-.896	.081	-.954	-11.046	<.0001

Confidence Intervals
log Y-RM vs. 1XV

	Coefficient	95% Lower	95% Upper
Intercept	3.690	3.241	4.138
1XV	-.896	-1.072	-.719



Regression Summary
log Y-RM vs. 1XV
Split By: Type
Cell: Alkane

Count	5
Num. Missing	0
R	.999
R Squared	.999
Adjusted R Squared	.999
RMS Residual	.018

Residual Statistics
log Y-RM vs. 1XV
Split By: Type
Cell: Alkane

# >= 0	3
# < 0	2
SS[e(i) - e(i-1)]	.003
Durbin-Watson	2.578
Serial Autocorrelation	-.403

ANOVA Table
log Y-RM vs. 1XV
Split By: Type
Cell: Alkane

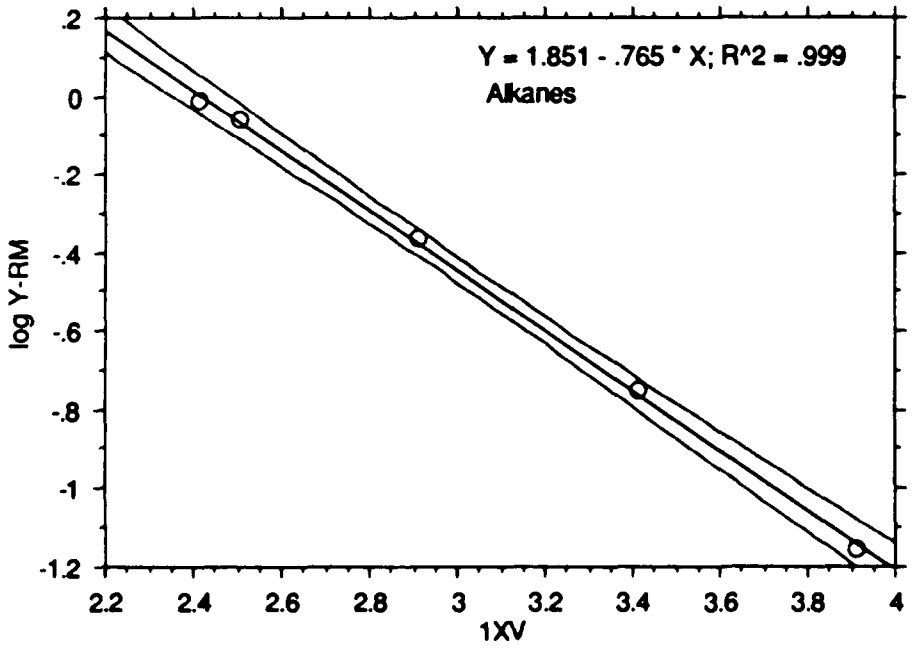
	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.936	.936	2823.077	<.0001
Residual	3	.001	3.314E-4		
Total	4	.937			

Regression Coefficients
log Y-RM vs. 1XV
Split By: Type
Cell: Alkane

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.851	.044	1.851	41.741	<.0001
1XV	-.765	.014	-.999	-53.133	<.0001

Confidence Intervals
log Y-RM vs. 1XV
Split By: Type
Cell: Alkane

	Coefficient	95% Lower	95% Upper
Intercept	1.851	1.710	1.993
1XV	-.765	-.811	-.719



Regression Summary
log Y-RM vs. 1XV
Split By: Type
Cell: Amine

Count	6
Num. Missing	0
R	.957
R Squared	.915
Adjusted R Squared	.894
RMS Residual	.101

Residual Statistics
log Y-RM vs. 1XV
Split By: Type
Cell: Amine

# >= 0	3
# < 0	3
SS[e(i) - e(i-1)]	.104
Durbin-Watson	2.549
Serial Autocorrelation	-.326

ANOVA Table
log Y-RM vs. 1XV
Split By: Type
Cell: Amine

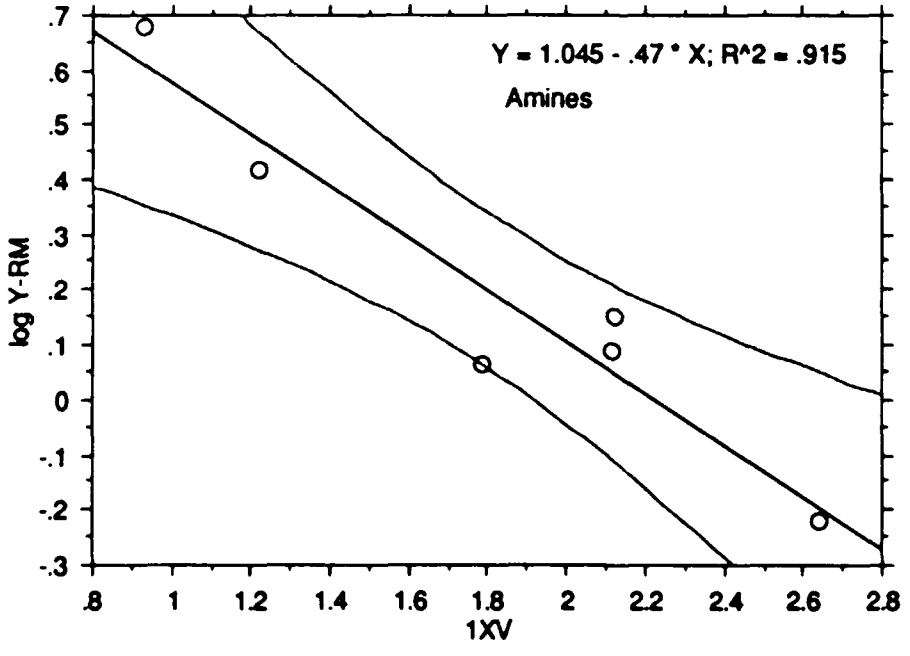
	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.443	.443	43.327	.0028
Residual	4	.041	.010		
Total	5	.484			

Regression Coefficients
log Y-RM vs. 1XV
Split By: Type
Cell: Amine

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.045	.135	1.045	7.738	.0015
1XV	-.470	.071	-.957	-6.582	.0028

Confidence Intervals
log Y-RM vs. 1XV
Split By: Type
Cell: Amine

	Coefficient	95% Lower	95% Upper
Intercept	1.045	.670	1.420
1XV	-.470	-.669	-.272



Regression Summary
log Y-RM vs. 1XV
Split By: Type
Cell: Aromatic

Count	9
Num. Missing	0
R	.852
R Squared	.726
Adjusted R Squared	.687
RMS Residual	.311

Residual Statistics
log Y-RM vs. 1XV
Split By: Type
Cell: Aromatic

# >= 0	5
# < 0	4
SS[e(i) - e(i-1)]	1.125
Durbin-Watson	1.665
Serial Autocorrelation	.053

ANOVA Table
log Y-RM vs. 1XV
Split By: Type
Cell: Aromatic

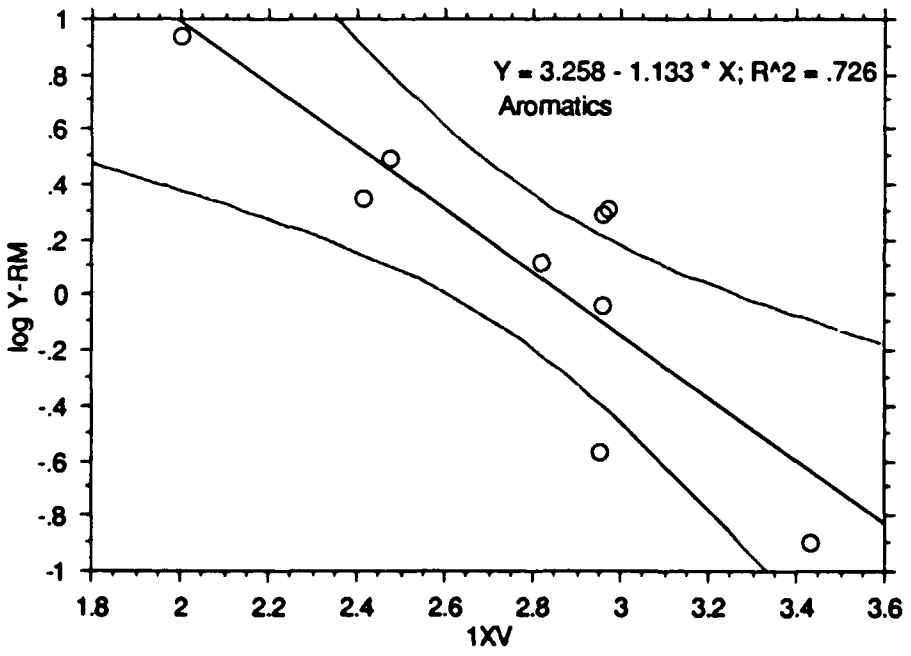
	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.792	1.792	18.566	.0035
Residual	7	.676	.097		
Total	8	2.468			

Regression Coefficients
log Y-RM vs. 1XV
Split By: Type
Cell: Aromatic

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	3.258	.737	3.258	4.420	.0031
1XV	-1.133	.263	-.852	-4.309	.0035

Confidence Intervals
log Y-RM vs. 1XV
Split By: Type
Cell: Aromatic

	Coefficient	95% Lo...	95% Upper
Interce...	3.258	1.515	5.001
1XV	-1.133	-1.755	-.511



Regression Summary
log Y-RM 2 vs. 0XV

Count	12
Num. Missing	39
R	.942
R Squared	.887
Adjusted R Squared	.876
RMS Residual	.141

Residual Statistics
log Y-RM 2 vs. 0XV

# >= 0	6
# < 0	6
SS[e(i) - e(i-1)]	.394
Durbin-Watson	1.990
Serial Autocorrelation	-.066

ANOVA Table
log Y-RM 2 vs. 0XV

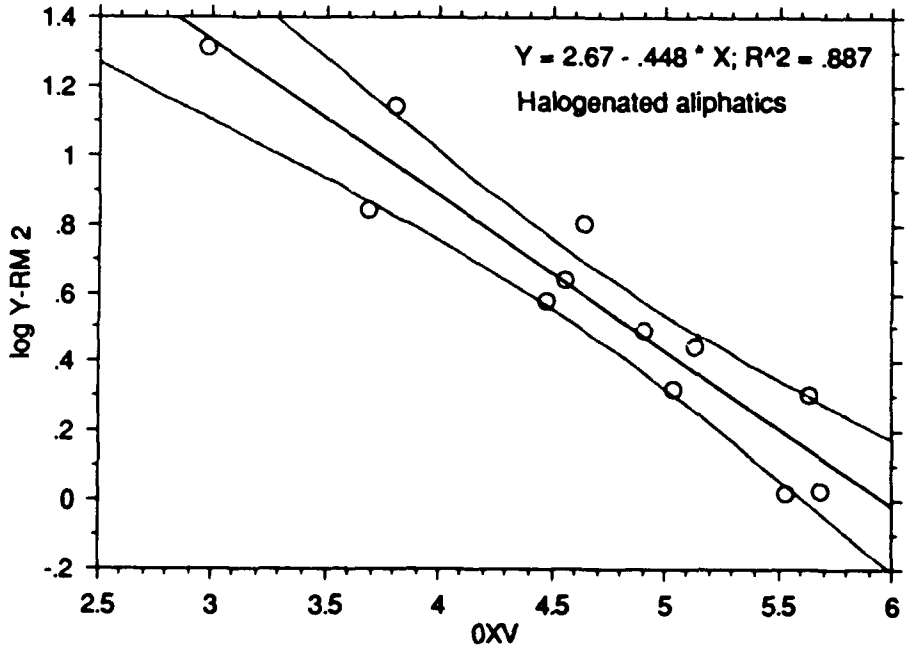
	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.558	1.558	78.803	<.0001
Residual	10	.198	.020		
Total	11	1.756			

Regression Coefficients
log Y-RM 2 vs. 0XV

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	2.670	.239	2.670	11.178	<.0001
0XV	-.448	.050	-.942	-8.877	<.0001

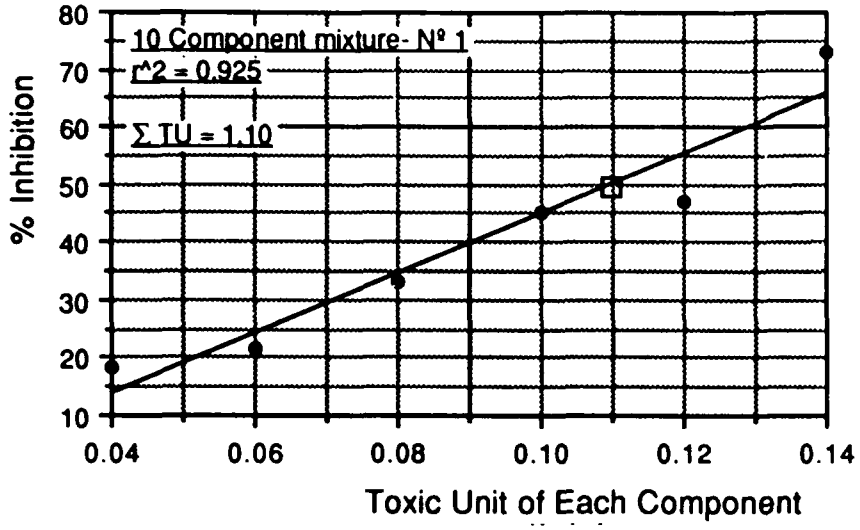
Confidence Intervals
log Y-RM 2 vs. 0XV

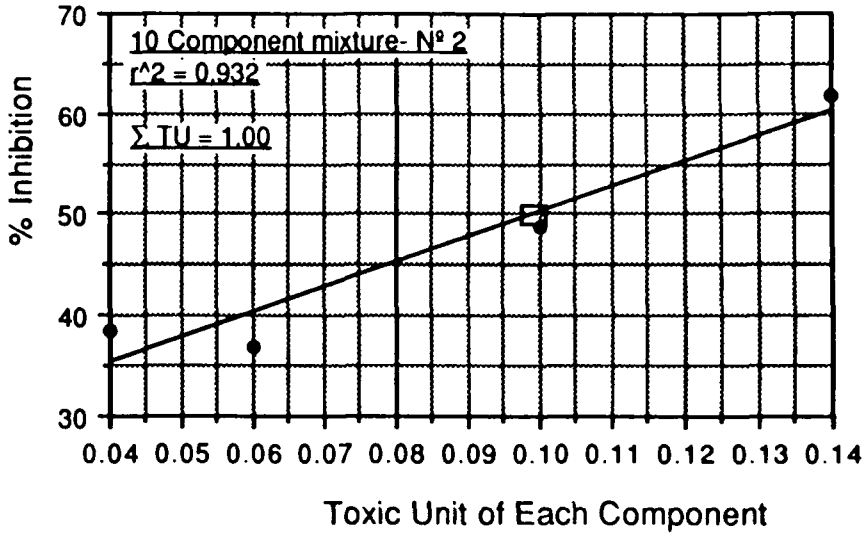
	Coefficient	95% Lower	95% Upper
Intercept	2.670	2.137	3.202
0XV	-.448	-.560	-.335

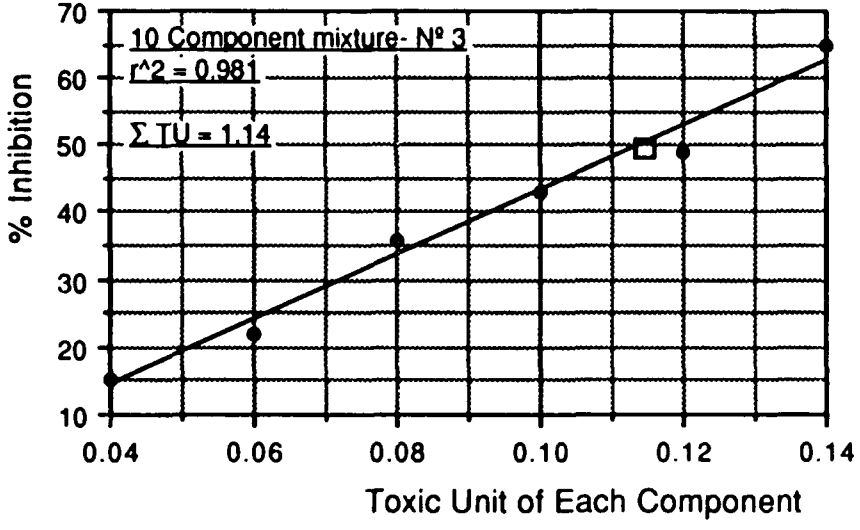


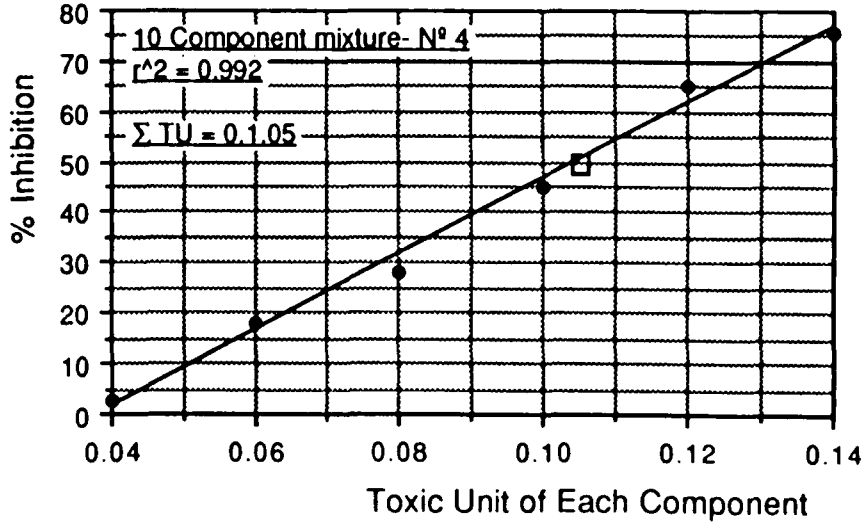
Appendix IV a

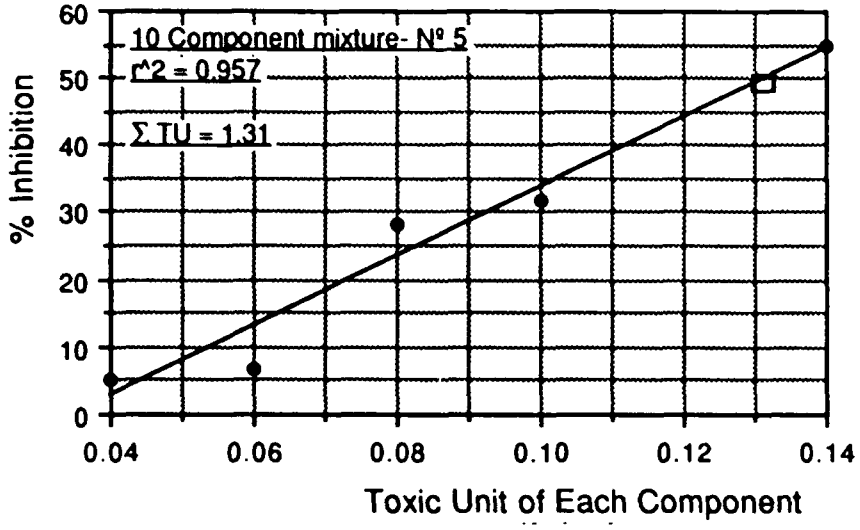
Results of 10-Component Mixtures

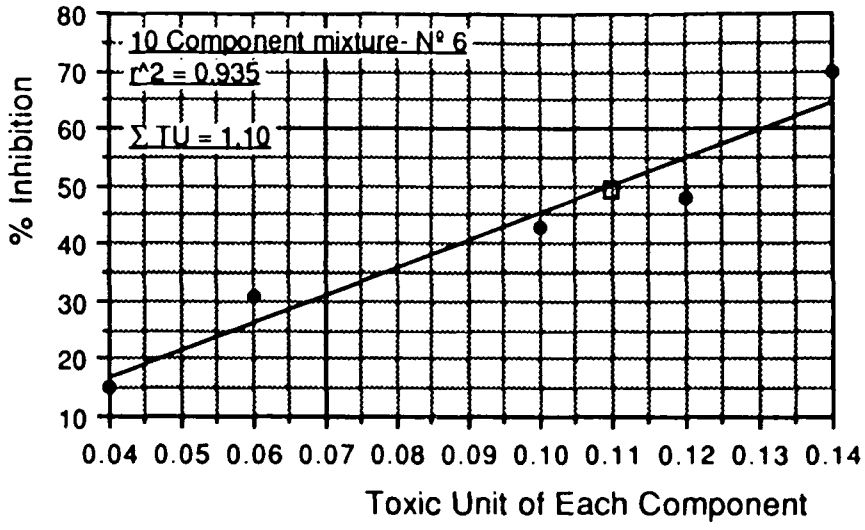

10 Component mixture- Nº 1
r^2 = 0.925
Σ TU = 1.10
% Inhibition
Toxic Unit of Each Component
80
70
60
50
40
30
20
10
0.04
0.06
0.08
0.10
0.12
0.14

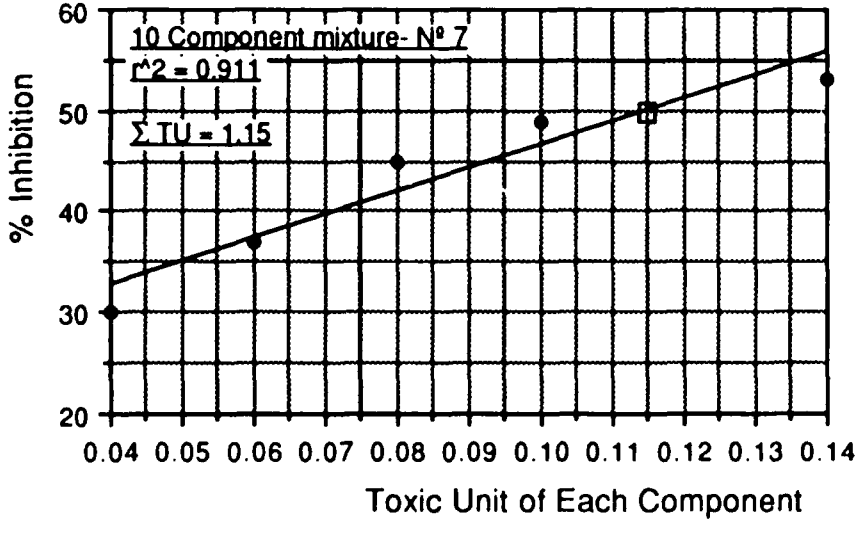

10 Component mixture- Nº 2
r^2 = 0.932
Σ TU = 1.00
% Inhibition
Toxic Unit of Each Component
70
60
50
40
30
0.04 0.05 0.06 0.07 0.08 0.09 0.10 0.11 0.12 0.13 0.14

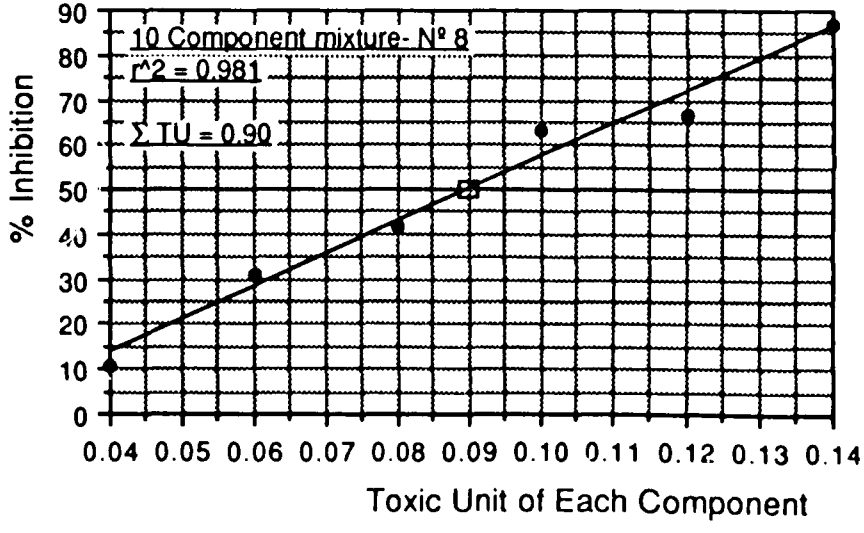

10 Component mixture- Nº 3
r^2 = 0.981
Σ TU = 1.14
% Inhibition
Toxic Unit of Each Component
70
60
50
40
30
20
10
0.04
0.06
0.08
0.10
0.12
0.14

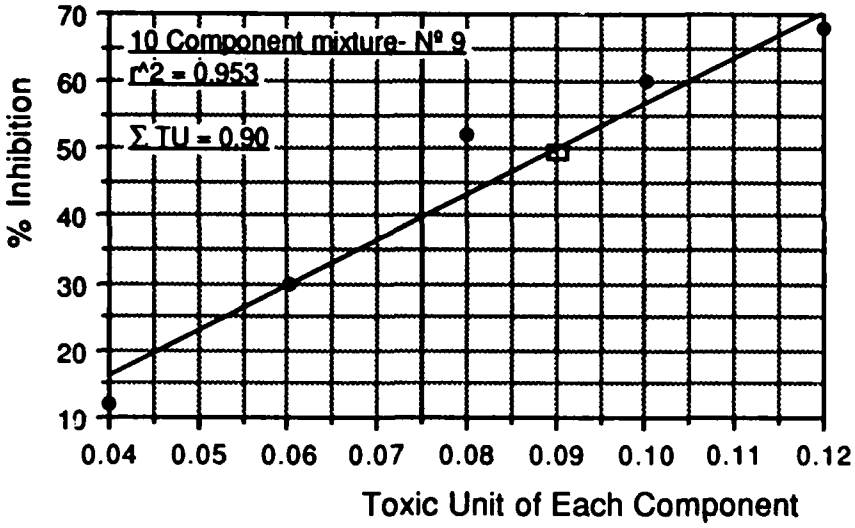

10 Component mixture- Nº 4
r^2 = 0.992
Σ TU = 0.1.05
% Inhibition
Toxic Unit of Each Component
80
70
60
50
40
30
20
10
0
0.04
0.06
0.08
0.10
0.12
0.14

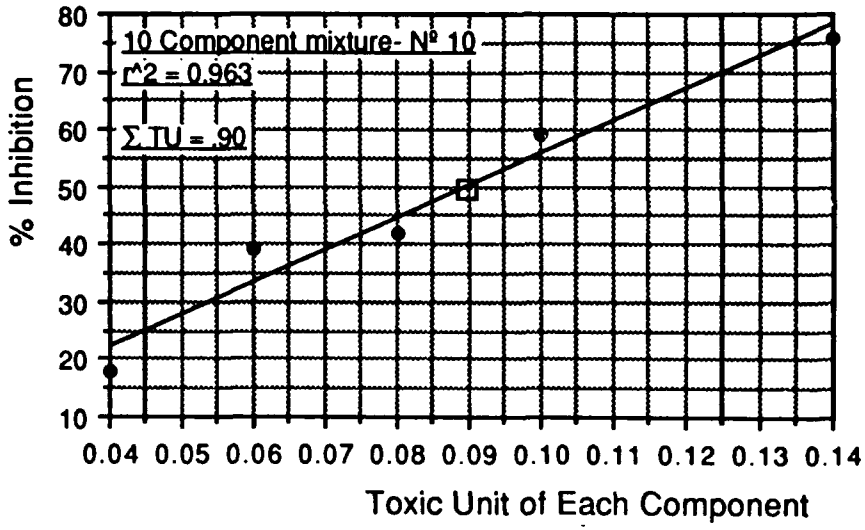

10 Component mixture- Nº 5
r^2 = 0.957
Σ TU = 1.31
% Inhibition
Toxic Unit of Each Component


10 Component mixture- Nº 6
r^2 = 0.935
Σ TU = 1.10
% Inhibition
Toxic Unit of Each Component


10 Component mixture- Nº 7
r^2 = 0.911
Σ TU = 1.15
% Inhibition
Toxic Unit of Each Component

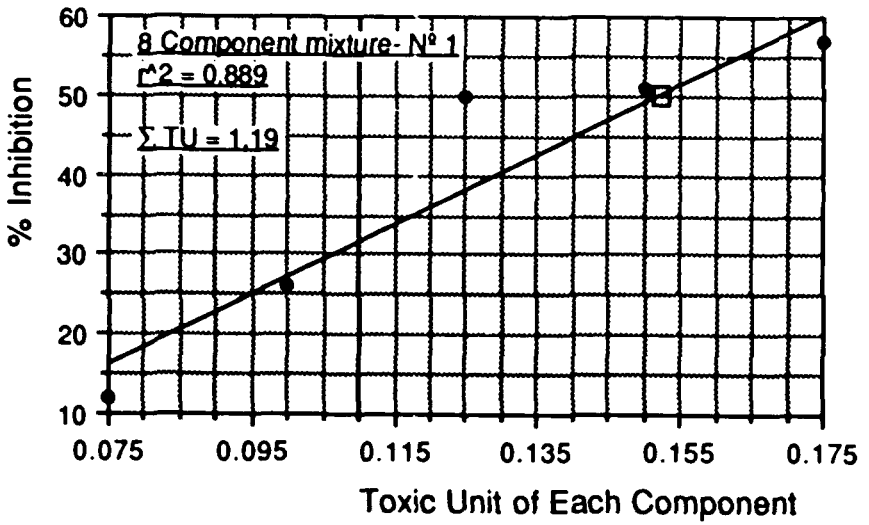

10 Component mixture- Nº 8
r^2 = 0.981
Σ TU = 0.90
% Inhibition
Toxic Unit of Each Component

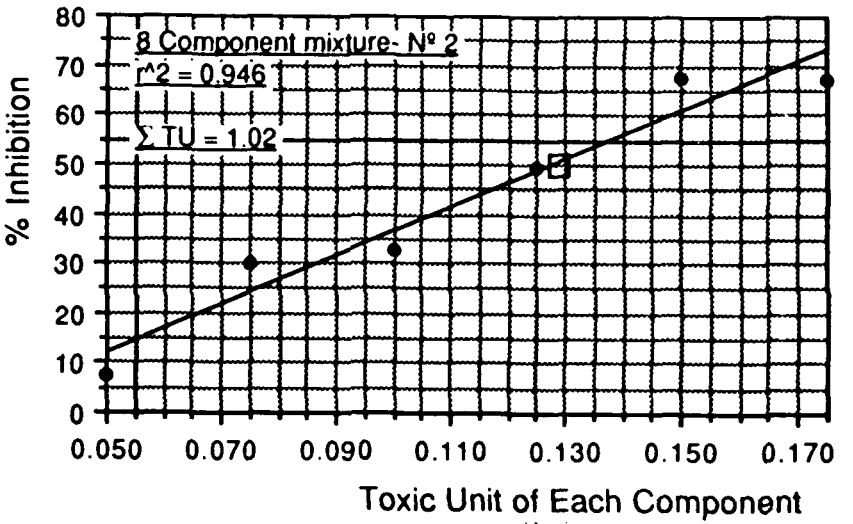

10 Component mixture- Nº 9
r^2 = 0.953
Σ TU = 0.90
% Inhibition
70
60
50
40
30
20
10
0.04 0.05 0.06 0.07 0.08 0.09 0.10 0.11 0.12
Toxic Unit of Each Component

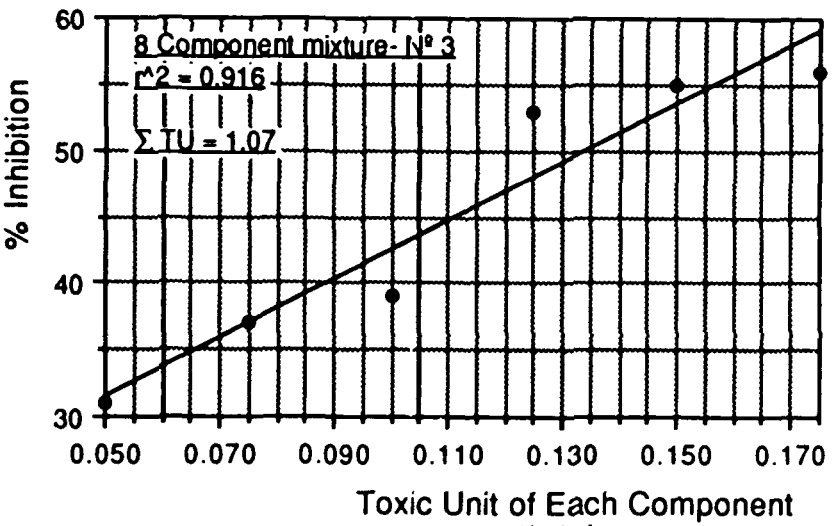

10 Component mixture- Nº 10
r^2 = 0.963
Σ TU = .90
% Inhibition
80
70
60
50
40
30
20
10
0.04 0.05 0.06 0.07 0.08 0.09 0.10 0.11 0.12 0.13 0.14
Toxic Unit of Each Component

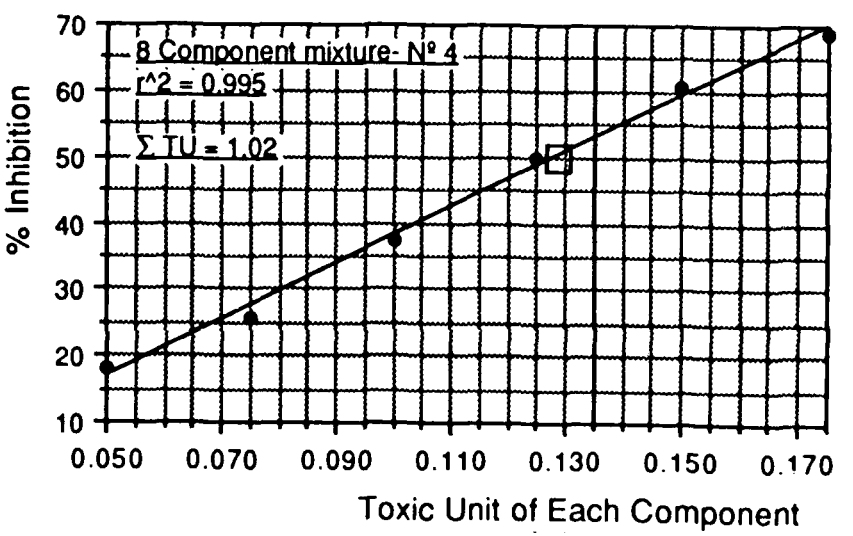
Appendix IV b

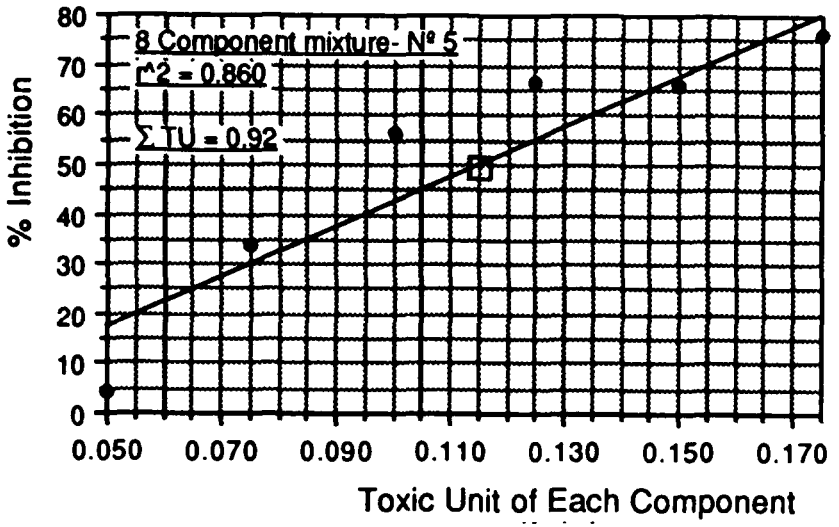
Results of 8-Component Mixtures

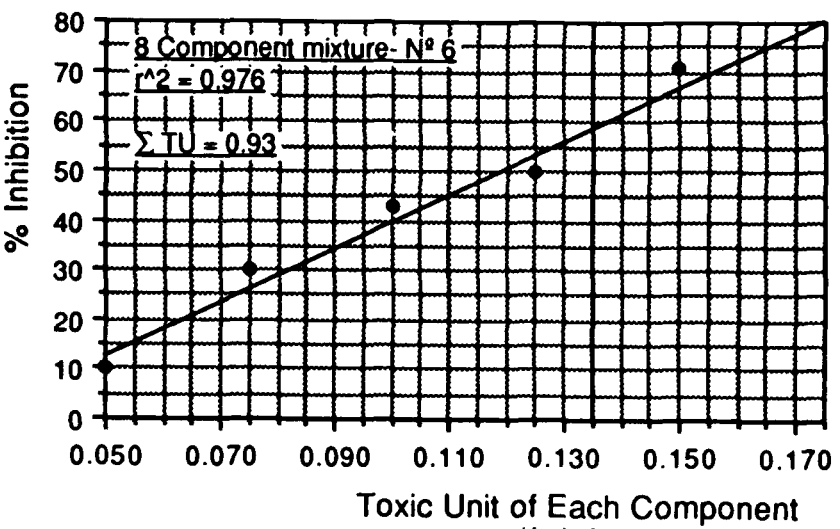

8 Component mixture- Nº 1
r^2 = 0.889
Σ TU = 1.19
% Inhibition
Toxic Unit of Each Component
60
50
40
30
20
10
0.075
0.095
0.115
0.135
0.155
0.175


8 Component mixture- Nº 2
r^2 = 0.946
Σ TU = 1.02
% Inhibition
Toxic Unit of Each Component
80
70
60
50
40
30
20
10
0
0.050
0.070
0.090
0.110
0.130
0.150
0.170


8 Component mixture- Nº 3
r^2 = 0.916
Σ TU = 1.07
% Inhibition
Toxic Unit of Each Component
60
50
40
30
0.050
0.070
0.090
0.110
0.130
0.150
0.170


8 Component mixture- Nº 4
r^2 = 0.995
Σ TU = 1.02
% Inhibition
Toxic Unit of Each Component
70
60
50
40
30
20
10
0.050
0.070
0.090
0.110
0.130
0.150
0.170


8 Component mixture- Nº 5
r^2 = 0.860
Σ TU = 0.92
% Inhibition
80
70
60
50
40
30
20
10
0
0.050
0.070
0.090
0.110
0.130
0.150
0.170
Toxic Unit of Each Component


8 Component mixture- Nº 6
r^2 = 0.976
Σ TU = 0.93
% Inhibition
80
70
60
50
40
30
20
10
0
0.050
0.070
0.090
0.110
0.130
0.150
0.170
Toxic Unit of Each Component

Appendix V

Results of 8-Component Mixtures

Comparison of Experimental Results vs. Predictions

Appendix V- Results of 8-Component Mixture Studies
Comparison of Experimental vs. Predicted Concentrations Causing 50% Inhibition

Mixture Nº Results	Components	Individual IC50 From			Conc. of Component in Mixture causing IC50		
		Experiment	QSAR Model		Measured in Experiment	Predicted Using IC50 From	
						Experiemnt	QSAR Model
		[mg/L]	[mg/L]		[mg/L]	[mg/L]	[mg/L]
1	Methyl ethyl ketone	1,900	5,865		283	238	733
	Methyl isobutyl ketone	2,600	2,099		387	325	262
r^2 = 0.889	n-Butyl acetate	3,750	1,350		559	469	169
ΣTU = 1.19	Isobutyl acetate	1,600	1,851		238	200	231
AI = 0.19	Propanol	7,200	12,798		1,073	900	1,600
MTI = 0.916	Pentanol	2,325	2,283		346	291	285
	Dibromomethane	1,110	684		165	139	86
	Bis2-chloroethyl ether	1,600	992		238	200	124
2	Methyl ethyl ketone	1,900	5,865		243	238	733
	Methyl isobutyl ketone	2,600	2,099		333	325	262
r^2 = 0.946	n-Butyl acetate	3,750	1,350		480	469	169
ΣTU = 1.02	Isobutyl acetate	1,600	1,851		205	200	231
AI = 0.02	Dibromomethane	1,110	684		142	139	86
MTI = 0.990	Bromochloromethane	1,800	1,201		230	225	150
	Benzene	685	766		88	86	96
	Toluene	207	310		26	26	39
3	Methyl ethyl ketone	1,900	5,865		255	238	733
	Methyl isobutyl ketone	2,600	2,099		348	325	262
r^2 = 0.916	n-Butyl acetate	3,750	1,350		503	469	169
ΣTU = 1.07	Isobutyl acetate	1,600	1,851		214	200	231
AI = 0.07	Propanol	7,200	12,798		965	900	1,600
MTI = 0.967	Pentanol	2,325	2,283		312	291	285
	1,2 Dichloroethylene	350	1,332		47	44	167
	Trichloroethylene	500	611		67	63	76

Appendix V- Results of 8-Component Mixture Studies (contd.)

Mixture Nº	Components	Individual IC50 From		Conc. of Component in Mixture causing IC50		
		Experiment	QSAR Model	Measured in Experiment	Predicted Using IC50 From	
					Experiemnt	QSAR Model
		[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
4	Ethyl benzene	220	83	28	28	10
	Isobutyl acetate	1,600	1,851	205	200	231
r^2 = 0.995	Propanol	7,200	12,798	922	900	1,600
ΣTU = 1.02	Pentanol	2,325	2,283	298	291	285
AI = 0.02	Dibromomethane	1,110	684	142	139	86
MTI = 0.990	Bromochloromethane	1,800	1,201	230	225	150
	Octanol	126	143	16	16	18
	1,2 Dichloropropane	500	485	64	63	61
5	Ethyl benzene	220	83	25	28	10
	2,4 Dimethyl phenol	240	98	28	30	12
r^2 = 0.860	Isobutyl acetate	1,600	1,851	184	200	231
ΣTU = 0.92	Propanol	7,200	12,798	828	900	1,600
AI = -0.08	Pentanol	2,325	2,283	267	291	285
MTI = 1.040	Bromochloromethane	1,800	1,201	207	225	150
	1,2 Dichloroethylene	350	1,332	40	44	167
	Trichloroethylene	500	611	58	63	76
6	Methyl ethyl ketone	1,900	5,865	220	238	733
	n-Butyl acetate	3,750	1,350	435	469	169
r^2 = 0.976	Ethylenedibromide	520	437	60	65	55
ΣTU = 0.93	1,1,1,Trichloroethane	415	398	48	52	50
AI = -0.07	Ethyl benzene	220	83	26	28	10
MTI = 1.035	Chlorobenzene	350	322	41	44	40
	2,4 Dimethyl phenol	240	98	28	30	12
	Toluene	207	310	24	26	39