REPORT DOCUMENTATION PAGE Dist: A

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
		FINAL 23 Oct 91 TO 22 Oct 94

4. TITLE AND SUBTITLE
MODELING JOINT EFFECTS OF MIXTURES OF CHEMICALS ON MICROORGANISMS USING QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

5. FUNDING NUMBERS
AFOSR-91-0394
61102F
2312/AS

6. AUTHOR(S)
Dr Nirmalak Kandan

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
dept of civil Engineering
New Mexico State University
Espina Street, Box 30001
Las Cruces NM 88003-0001

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 95 0015

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
110 Duncan Ave Suite B115
Bolling AFB DC 20332-0001
Dr Kozumbo

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited.

12b. DISTRIBUTION CODE
A

13. ABSTRACT *(Maximum 200 words)*

A laboratory procedure was developed to measure the toxicity of 35 organic chemicals in the soil medium using the respirometeric technique. These toxicity assays were carried out using a commercially available surrogate test culture of microorganisms. Reproducibility test were done on 12 of the chemicals yielding an average standard deviation of 0.034 and a coefficient of variation of 0.08. These tests were also repeated at different moisture holding capacities of 33%, 50%, 80% and 100% for six chemicals yielding an average standard deviation of 0.20 and coefficient of variation of 0.27. Using a part of the experimental IC50 results as a training set, Quantitative structure Activity Relationship (QSAR) models were developed to predict the toxicity of 12 chemicals in the testing set. Joint toxiciti of 35 different combinations of mixtures in the soil were also measured at equitoxic ratios. The joint effects in there mixtures were analyzed for simple additivity. Result indicate that the test chemcials exhibited simple additivity when acting jointly in a uniform mixture. A QSAR approach is proposed to predict mixture toxicity based on single chemical QSAR models.

14. SUBJECT TERMS	15. NUMBER OF PAGES
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(U)

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

Grant Nº AFOSR - 91 -0394

Interim Progress Report

Phase III

August 1993 - October 1994

By

N. Nirmalakhandan,
V. R. J. Arulgnanendran,
J. Prakash, B. Sun, J. Peace,
R. Maynes, R. Little, R. Walsh

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

Grant Nº AFOSR - 91 -0394

Interim Progress Report

Phase III

August 1993 - October 1994

By

N. Nirmalakhandan,
V. R. J. Arulgnanendran,
J. Prakash, B. Sun, J. Peace,
R. Maynes, R. Little, R. Walsh

Table of Contents

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

- Phase III: Microbial Toxicity in Soils -

ABSTRACT

A laboratory procedure was developed to measure the toxicity of 35 organic chemicals in the soil medium using the respirometeric technique. These toxicity assays were carried out using a commercially available surrogate test culture of microorganisms. Reproducibility tests were done on 12 of the chemicals yielding an average standard deviation of 0.034 and a coefficient of variation of 0.08. These tests were also repeated at different moisture holding capacities of 33%, 50%, 80% and 100% for six chemicals yielding an average standard deviation of 0.20 and coefficient of variation of 0.27. Using a part of the experimental IC_{50} results as a training set, Quantitative Structure Activity Relationship (QSAR) models were developed to predict the toxicity of 12 chemicals in the testing set. Joint toxicities of 35 different combinations of mixtures in the soil were also measured at equitoxic ratios. The joint effects in these mixtures were analyzed for simple additivity. Results of this study indicate that the test chemicals exhibited simple additivity when acting jointly in a uniform mixture. A QSAR approach is proposed to predict mixture toxicity based on single chemical QSAR models.

INTRODUCTION

The widespread use of organic chemicals and their release into the ecosphere cause concern due to their toxic effects even at very low concentrations. Chemical contaminants may enter the soil compartment of the ecosphere from leaking underground storage tanks, municipal or industrial wastes, accidental spills, and from different agricultural practices. Moreover leachate from landfill sites, by - products of oil refineries, and gaseous pollutants in the atmosphere also contribute to this problem. In addition to the anthropogenic contribution of organic contaminants, certain organic chemicals enter the soil system from the

metabolic processes of the soil inhabitants. The impact of these chemicals on the soil compartment of the ecosphere has been recognized.

The current emergence of different bioremediation technologies to cleanup contaminated sites has aroused attention in determining the effects of these contaminants on the organisms. The objective of any remediation process is to reduce the concentration of the contaminant so as to substantially eliminate the toxic effects on the environment. While preliminary data on potential toxicity may be obtained from the available literature, it is imperative that direct toxicity testing be done to assess the problem at hand prior to and subsequent to remediation. The determination of toxicity is one of the essential features in the evaluation of possible remedial action. This, together with other site characteristics will determine the type and level of treatment required.

Many bioassays have been developed to assess toxicity of organic chemicals in the aqueous medium for various test organisms. The different approaches are to evaluate the effects of the contaminants on: the number of organisms by direct count or viable count, the diversity or composition of organisms, biomass, and, microbial activity (Bartha 1982). However these test procedures may not be used directly to assess the toxicity of a chemical in the soil medium as these procedures are designed to measure the toxic effect of the chemical in the aqueous medium on the test organism. Under these circumstances a direct approach designed to test the toxicity of these chemicals in the soil would be more acceptable as a test procedure. Since the toxicants are released into the soil medium from time to time, a predictive model to evaluate the microbial toxicity in soils would be a useful tool. These models can be used to flag new chemicals introduced by the various industries for their toxicity, as well as for existing chemicals, without extensive laboratory testing.

Predicting Chemical Toxicity : Application of QSAR Techniques

Quantitative Structure Activity Relationship (QSAR) techniques have been used by the pharmaceutical and pesticide industries in the development of new chemicals. In recent years they have been applied for the prediction of toxicity. The Office of Toxic Substances (OTS) of the US Environmental Protection

Agency has utilized QSAR techniques for hazard assessment since 1981. Charged with the responsibility of ecological hazard assessment of new chemicals, the Environmental Effects Branch of the Health and Environmental Review Division of OTS has developed more than 50 QSARs. These are being used regularly in the assessment of toxicity to aquatic organisms (EPA - 560/6 - 88 - 001, July 1988).

QSAR is based on the premise that a definite relationship exists between the chemical/biological activity and molecular properties of the organic chemicals. Different molecular descriptors have been used by many researchers to derive suitable QSAR models. These molecular descriptors provide quantitative information as to how the modification of a chemical structure results in changes in chemical or biological activity.

By using a set of experimental data as a "training set", QSAR models can be developed correlating the toxicity and the molecular descriptors. Using these QSAR models and the molecular descriptors, toxicity of new chemicals in a "testing set" can be predicted to validate the QSAR model. By employing suitable descriptors of the molecule, and experimentally measured toxicity values, QSAR techniques have been used to predict the toxicity of chemicals in the aqueous medium. In this manner QSAR techniques can supplement and expand the applicability of experimental results.

OBJECTIVE OF STUDY - PHASE III

The objective of this study was to develop and demonstrate a laboratory procedure to determine the microbial toxicity of organic chemicals in the soil medium; and, to develop QSAR models to predict toxicity of chemicals acting singularly or jointly in a mixture. A set of new chemicals, whose toxicity has been determined experimentally, is used to test these models. Joint toxicities of 8 component and 10 component mixtures are determined experimentally at equitoxic ratios of these chemicals. Using the concepts of Toxicity Units, Additivity Index and Mixture Toxicity Index, these mixtures are tested for simply additive, synergistic or antagonistic effects of the components. These concepts are further validated on different combinations of 8 component mixtures tested

in the laboratory. The ultimate purpose of the research is to develop and demonstrate a protocol to predict joint microbial toxicity of different mixtures of organic chemicals with varying molecular features acting by the same mode of toxicity.

EXPERIMENTAL METHODOLOGY

A total of 35 organic chemicals selected from the list of chemicals of concern to the US Air Force were assayed. Toxicity of these chemicals in the soil medium to a surrogate test culture, Polytox, was measured using the respirometric technique developed in this research. Details of the materials and methods are given in Appendix II.

MODELING OF THE EXPERIMENTAL SYSTEM

The chemical dose is administered to the soil medium in the form of liquid. From the bulk liquid, the chemical partitions between the soil, the water, the microbial cells and the head space in the reactor.

In this research it is modeled that the toxic effect on the microorganisms is caused by the concentration of the chemical available as the dissolved form in the soil moisture. This concentration is determined by mechanistic modeling of the experimental system as shown in Figure 1.

Developing a mathematical relationship based on the above model, by mass balance for the chemical within each reactor of the respirometer,

$$M_{Total} = M_{Water} + M_{Soil} + M_{Cells} + M_{Headspace} \quad (1)$$

$$= C_w{}^*V_{water} + C_{soil}{}^*m_{soil} + C_{cell}{}^*m_{cells} + C_{air}{}^*V_{air} \quad (2)$$

where

C_w = equilibrium concentration of the chemical in soil moisture in mg/l,

C_{soil} = equilibrium concentration of the chemical in soil in mg/g,

C_{cell} = equilibrium concentration of the chemical in cells in mg/mg, and

C_{air} = equilibrium concentration of the chemical in head space of reactor in mg/l,

V_{water} = Volume of liquid added to each reactor in ml
m_{soil} = mass of soil in each reactor in grams
m_{cells} = mass of microbial cells in each reactor in mg
V_{air} = volume of headspace in the reactor in liters

The adsorption of soil is given by the linear model

$$C_{soil} = K_d * C_w \quad (3)$$

where Kd [in l/g] is the adsorption coefficient of the soil.

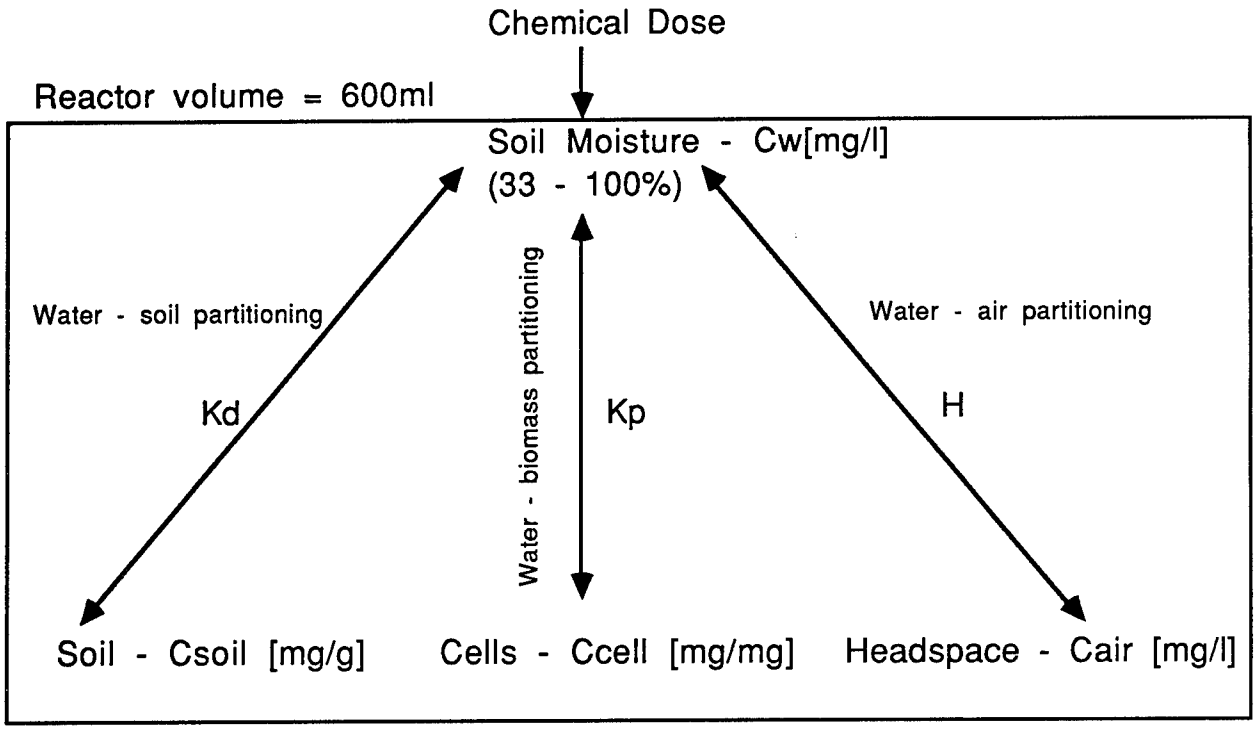


Figure 1. Mechanistic modeling of the experimental system

The biosorption of the chemical into the cell is obtained from the linear model

$$C_{cell} = K_p * C_w \quad (4)$$

where Kp is the partition coefficient [in l/mg] between the aqueous medium and the cell.

For the partition of the chemical into the head space of the reactor bottle,

$$C_{air} = H^* C_w \quad (5)$$

where H is the Henry's constant [dimensionless].

Using equations 3-5 in equation 2, we have

$$M_{Total} = C_w V_{water} + K_d * C_w * m_{soil} + K_p * C_w * m_{cells} + H * C_w * V_{air}$$

$$= C_w [V_{water} + K_d * m_{soil} + K_p * m_{cells} + H * V_{air}] \quad (6)$$

Hence

C_w= equilibrium concentration of the chemical in soil moisture in mg/l,

$$= M_{Total} / [V_{water} + K_d * m_{soil} + K_p * m_{cells} + H * V_{air}] \quad (7)$$

The experimental procedures used in the determination of the values of K_d and K_p are detailed in Appendix II.

RESULTS AND DISCUSSION

Single chemical experimental results

Typical data output from the computer interfaced respirometer system and the determination of the 50 % inhibition concentration are shown in Figures 2 & 3 respectively. Test results from single runs for 35 chemicals by the above experimental technique are given in Table 1. The high r^2 values listed in Table 1 for the dose - response plots explain the clear linear variation between chemical concentration and the percentage inhibition of the rate of oxygen uptake for the ranges of values tested.

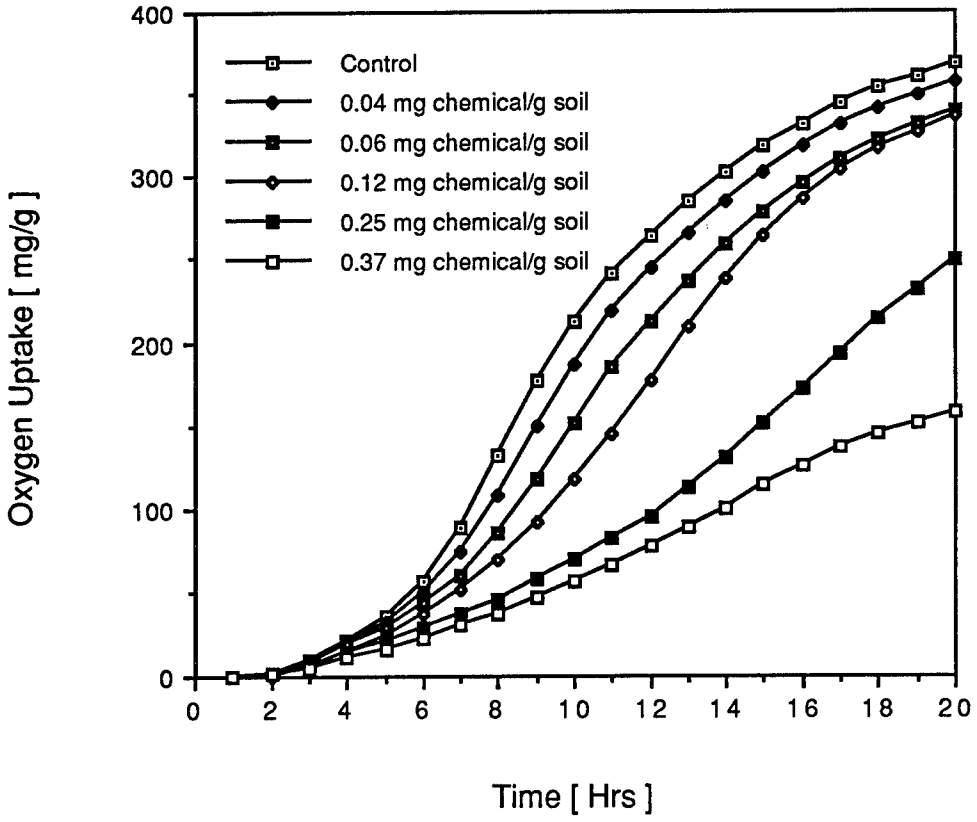


Fig 2. Typical respirometer data output: Chlorodibromomethane [ID # 19]

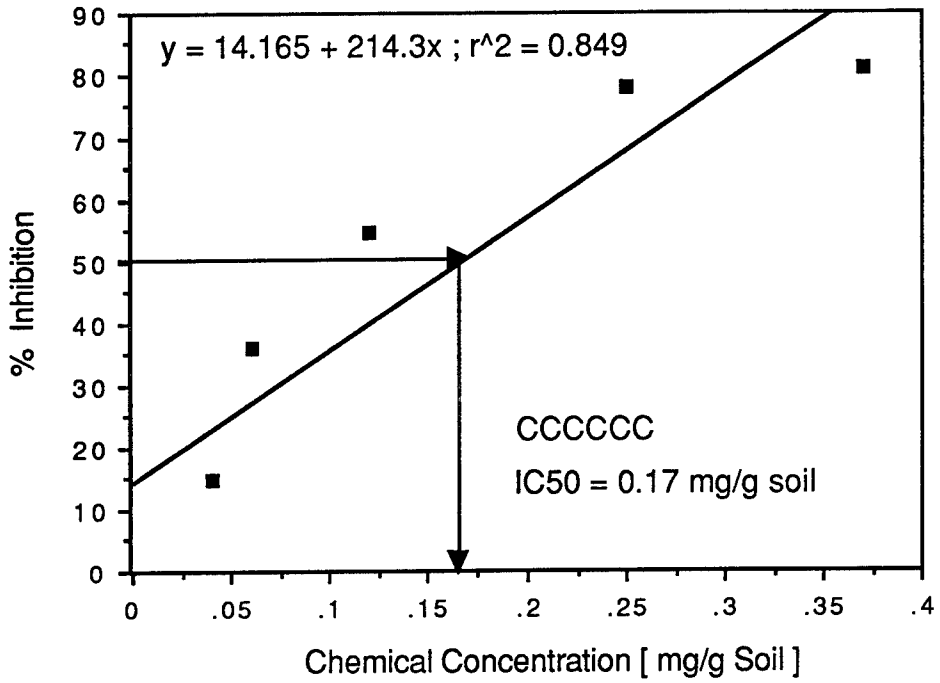


Fig 3. Percentage inhibition of oxygen uptake rate Vs chemical concentration [ID # 19]

TABLE 1. EXPERIMENTAL IC_{50} VALUES

ID #	Chemical Name	Type**	IC_{50} [mg/g]	r^2
1	Benzene	ARO	0.51	0.916
2	Toluene	ARO	0.37	0.905
3	O-Xylene	ARO	0.22	0.808
4	Ethylbenzene	ARO	0.21	0.921
5	Chlorobenzene	ARO	0.33	0.909
6	1,2 Dichlorobenzene	ARO	0.12	0.819
7	1,3 Dichlorobenzene	ARO	0.14	0.917
8	1,2,4 Trichlorobenzene	ARO	0.24	0.983
9	2,4 Dimethyl phenol	ARO	0.13	0.956
10	Dichloromethane	HAL	0.94	0.722
11	Dibromomethane	HAL	0.68	0.919
12	Carbontetrachloride	HAL	0.45	0.979
13	1,2 Dichloroethane	HAL	0.51	0.909
14	1,1,1 Trichloroethane	HAL	0.59	0.981
15	1,1,2,2 Tetrachloroethane	HAL	0.12	0.856
16	1,2 Dichloropropane	HAL	0.32	0.987
17	Bromochloromethane	HAL	0.91	0.953
18	Bromodichloromethane	HAL	0.21	0.984
19	Chlorodibromomethane	HAL	0.17	0.849
20	Ethylene dibromide	HAL	0.35	0.962
21	*cis* - 1,2 Dichloroethylene	HAL	0.45	0.915
22	Trichloroethylene	HAL	0.56	0.955
23	Tetrachloroethylene	HAL	0.34	0.913
24	Ethanol	AKE	2.59	0.729
25	Propanol	AKE	1.13	0.960
26	Pentanol	AKE	0.45	0.886
27	Octanol	AKE	0.12	0.960
28	N- Butyl acetate	AKE	0.45	0.635
29	Isobutyl acetate	AKE	0.57	0.972
30	N- Amyl acetate	AKE	0.34	0.945
31	Ethyl acetate	AKE	0.97	0.934
32	Acetone	AKE	4.48	0.975
33	Methyl isobutyl ketone	AKE	0.56	0.828
34	Methyl N- propyl ketone	AKE	0.39	0.787
35	Cyclohexanone	AKE	0.95	0.970

**ARO - Aromatic; HAL - Halogenated aliphatic; AKE - Alcohols, esters and ketones.

Reproducibility Studies

To demonstrate the reproducibility of the proposed test procedure, duplicate tests were run on 12 of the 35 chemicals. Results of this reproducibility runs are given in Table 2. The mean and standard deviation of the replicability of IC_{50} values for these twelve chemicals from two runs are shown in Figure 4. The reproducibility tests yielded an average standard deviation of 0.034 and coefficient of variation of 0.08 for the twelve chemicals. These variations are comparable to toxicity tests in aqueous medium with activated sludge, Microtox and Polytox found in this research as well as those reported in the literature.

TABLE 2. REPRODUCIBILITY OF IC_{50} VALUES FROM TWO RUNS

ID #	Chemical	Type	IC_{50} [mg/g] Run 1	r^2	IC_{50} [mg/g] Run 2	r^2
1	Benzene	ARO	0.51	0.916	0.48	0.985
2	Toluene	ARO	0.37	0.905	0.31	0.986
4	Ethylbenzene	ARO	0.21	0.921	0.18	0.902
11	Dibromomethane	HAL	0.68	0.919	0.70	0.901
12	Carbontetrachloride	HAL	0.45	0.979	0.58	0.852
13	1,2 Dichloroethane	HAL	0.51	0.909	0.50	0.915
15	1,1,2,2 Tetrachloroethane	HAL	0.12	0.856	0.11	0.979
17	Bromochloromethane	HAL	0.91	0.953	0.84	0.968
22	Trichloroethylene	HAL	0.56	0.955	0.45	0.930
26	Pentanol	AKE	0.45	0.886	0.52	0.915
27	Octanol	AKE	0.12	0.960	0.12	0.952
30	N-Amyl acetate	AKE	0.34	0.945	0.36	0.948

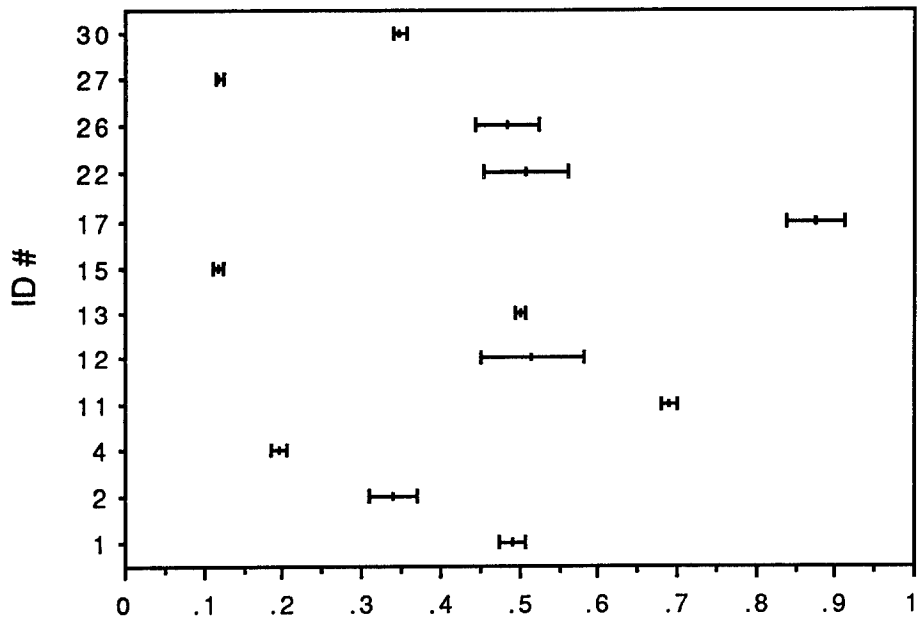


Fig 4. Results of reproducibility tests on 12 chemicals

Tests at Different Moisture holding Capacities

A series of tests was done to evaluate the effect of soil moisture content. Results from the tests done at different moisture holding capacities of the soil are shown in Table 3. The mean, standard deviation and coefficient of variation of the IC_{50} values are illustrated in Figure 5. These variations are comparable to the ones shown in Figure 4, where the tests were repeated at identical conditions. From these values it can be concluded that the test procedure is valid at different moisture holding capacities. Though the actual values of the toxicity of the chemical may be slightly different, they are within statistically acceptable levels.

TABLE 3. IC_{50} VALUES AT DIFFERENT MOISTURE HOLDING CAPACITIES

ID #	Chemical	Type		Moisture holding capacity 33%	50% Run1	50% Run2	80%	100%	Mean	SD	CV
1	Benzene	ARO	$IC_{50[mg/g]}$	0.51	0.51	0.48	0.75	0.71	0.59	0.127	0.215
			r^2	0.990	0.916	0.985	0.844	0.965			
2	Toluene	ARO	$IC_{50[mg/g]}$	0.31	0.37	0.31	0.38	0.43	0.36	0.051	0.142
			r^2	0.971	0.905	0.986	0.977	0.971			
11	Dibromomethane	HAL	$IC_{50[mg/g]}$	0.52	0.68	0.70	1.10	1.25	0.85	0.309	0.364
			r^2	0.995	0.919	0.901	0.997	0.712			
13	1,2 Dichloroethane	HAL	$IC_{50[mg/g]}$	0.45	0.51	0.50	0.65	1.23	0.67	0.323	0.482
			r^2	0.931	0.909	0.915	0.905	0.920			
30	N - Amyl acetate	AKE	$IC_{50[mg/g]}$	0.35	0.34	0.36	0.51	0.51	0.41	0.088	0.215
			r^2	0.985	0.945	0.948	0.858	0.950			
31	Ethyl acetate	AKE	$IC_{50[mg/g]}$	1.10	0.97	1.18	1.26	1.71	1.24	0.282	0.227
			r^2	0.952	0.934	0.948	0.812	0.863			
									Mean	0.20	0.27

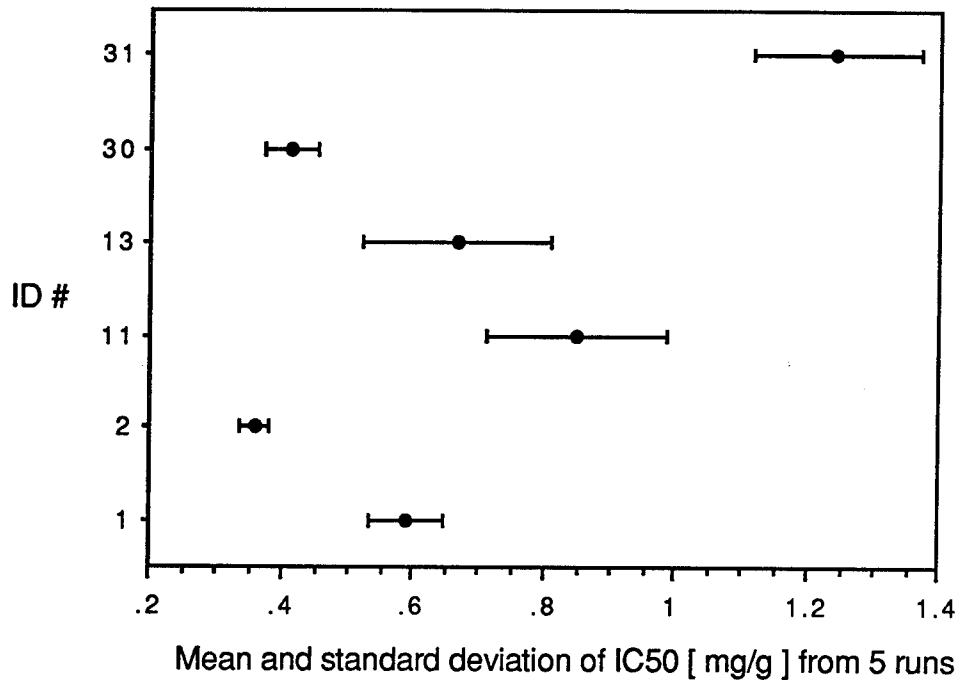


Fig. 5. Reproducibility results with different moisture holding capacities

Isotherm studies

Table 4 shows the C_w values determined from the experimental values using the mechanistic modeling approach as given in Equation (7). Details of the isotherm studies done on soils and microbial cells are given in Appendix VII and VIII. The experimentally determined values of K_d and K_p are given in Tables IX -I and IX - II in Appendix IX.. Table IX - III and Table IX - IV in Appendix IX give the Henry's constants and the aqueous solubilities of the chemicals. These were used in the determination of K_d and K_p.

TABLE 4. VALUES OF C_w [mM/L] FROM EXPERIMENTATION AND MECHANISTIC MODELING

ID #	Chemical Name	C_w [mM/l]
1	Benzene	0.026
2	Toluene	0.010
3	O-Xylene	0.003
4	Ethylbenzene	0.002
5	Chlorobenzene	0.007
6	1,2 Dichlorobenzene	0.001
7	1,3 Dichlorobenzene	0.001
8	1,2,4 Trichlorobenzene	0.001
9	2,4 Dimethyl phenol	0.001
10	Dichloromethane	0.092
11	Dibromomethane	0.033
12	Carbontetrachloride	0.012
13	1,2 Dichloroethane	0.022
14	1,1,1 Trichloroethane	0.018
15	1,1,2,2 Tetrachloroethane	0.003
16	1,2 Dichloropropane	0.008
17	Bromochloromethane	0.059
18	Bromodichloromethane	0.007
19	Chlorodibromomethane	0.004
20	Ethylene dibromide	0.017
21	*cis* - 1,2 Dichloroethylene	0.036
22	Trichloroethylene	0.020
23	Tetrachloroethylene	0.006
24	Ethanol	0.475
25	Propanol	0.084
26	Pentanol	0.006
27	Octanol	1.66E-4
28	N- Butyl acetate	0.003
29	Isobutyl acetate	0.004
30	N- Amyl acetate	0.001
31	Ethyl acetate	0.026
32	Acetone	0.703
33	Methyl isobutyl ketone	0.008
34	Methyl N- propyl ketone	0.011
35	Cyclohexanone	0.019

Single chemical QSAR models

Experimental IC_{50} results of 23 test chemicals were used as training set to develop QSAR models. Three approaches, namely; Molecular Connectivity Index (MCI), Linear Solvation Energy Relationship (LSER) and Octanol water partition coefficient (log P) were evaluated in the QSAR model development.

MCI approach

Three models are developed for the three congeneric groups of chemicals.

Aromatics:

$$\log IC_{50}\ (Dissolved) = 0.559 - 1.089\ {}^{1}\chi \qquad (8)$$

$n = 6$; $r = 0.994$; $r^2 = 0.989$; $SE = 0.058$.

Halogenated aliphatics:

$$\log IC_{50}\ (Dissolved) = 0.243 - 1.046\ {}^{1}\chi \qquad (9)$$

$n = 9$; $r = 0.938$; $r^2 = 0.881$; $SE = 0.143$

Alcohols, esters and ketones:

$$\log IC_{50}\ (Dissolved) = 0.659 - 1.110\ {}^{1}\chi^{V} \qquad (10)$$

$n = 8$; $r = 0.997$; $r^2 = 0.994$; $SE = 0.093$,

where IC_{50} *(Dissolved)* is the concentration, (mM/l) of the chemical (i.e. C_w in mg/l in Equation 7) in the dissolved form which causes 50% inhibition. Details of the regression analysis are given in Table III, in Appendix III for the above three models. The comparison between the experimental and calculated values of the inhibition concentrations is shown in Figure 6.

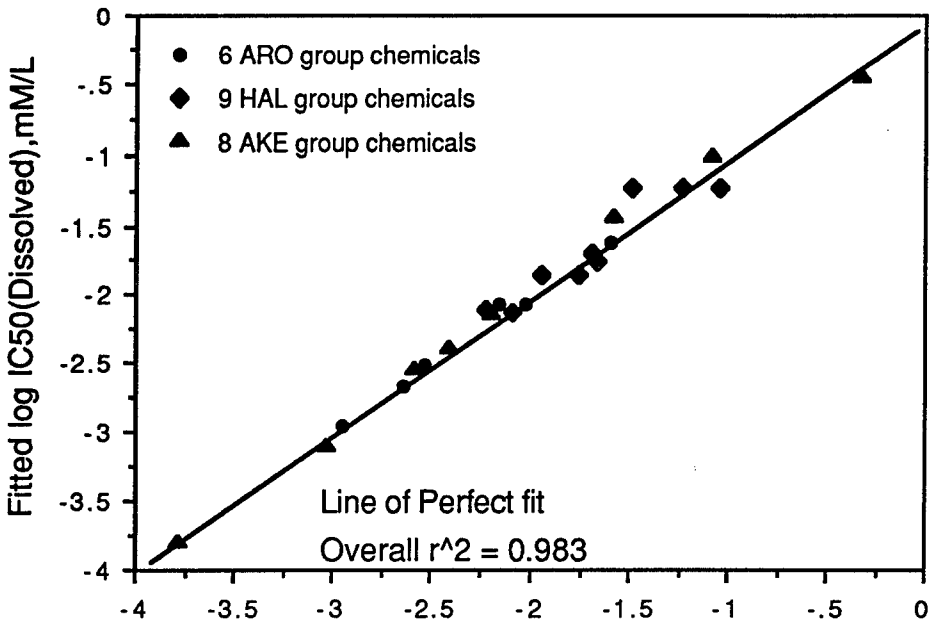


Fig. 6 Comparison of experimental & QSAR fitted IC_{50} values

LSER approach

Equation (11) and Table IV in Appendix IV give the statistical details of the model developed by multiple regression using the LSER approach for the entire set of 23 chemicals in the training set.

$$\log IC_{50}\ (\text{Dissolved}) = 1.002 - 5.339\ V_i/100 - 0.139\pi^* - 0.351\alpha + 0.474\beta \quad (11)$$

$n = 23$; $r = 0.985$; $r^2 = 0.971$; $SE = 0.142$.

Log P approach

The approach using the log P yielded the model given in equation (12). Details of the statistical analysis are given in Table V, Appendix V.

$$\log IC_{50}\ (\text{Dissolved}) = -0.980 - 0.491\ \log P \quad (12)$$

$n = 23$; $r = 0.571$; $r^2 = 0.326$; $SE = 0.635$.

Comparison of the three approaches

A summary the above three approaches is given in Table 5. Considering the adjusted r^2 values of the three approaches, both the MCI and LSER approaches give high values for the three groups of chemicals analyzed. However the calculation of MCI values is more direct and is error free in comparison to the LSER values. Hence the MCI approach was used in the development of predictive models.

Table 5: Comparison of three QSAR models among MCI, LSER and log P

Three QSAR Models

Type	MCI		LSER		log P	
ARO	n=6	r^2 = 0.989	n=6	r^2 = 0.996	n=6	r^2 = 0.979
	p=1	adj. r^2= 0.986	p=3	adj. r^2= 0.990	p=1	adj. r^2= 0.974
		SE = 0.058		SE = 0.050		SE = 0.080
HAL	n=9	r^2 = 0.881	n=9	r^2 = 0.955	n=9	r^2 = 0.280
	p=1	adj. r^2= 0.863	p=4	adj. r^2= 0.911	p=1	adj. r^2= 0.177
		SE = 0.143		SE = 0.116		SE = 0.351
AKE	n=8	r^2 = 0.994	n=8	r^2 = 0.997	n=8	r^2 = 0.985
	p=1	adj. r^2= 0.993	p=4	adj. r^2= 0.993	p=1	adj. r^2= 0.983
		SE = 0.093		SE = 0.090		SE = 0.144

n = Nº of chemicals used in the "Training set"

p = Nº of independent variables in the model

Prediction of IC50 values for the testing set

Twelve chemicals representing three congeneric groups and assayed for toxicity were used as testing set to validate the QSAR models developed on the twenty three chemicals from the training set. Using the model equations by the MCI approach (Equations 8-10) the IC_{50} of these 12 chemicals were predicted. The comparison of these predicted values and experimental values are shown in Figure 7.

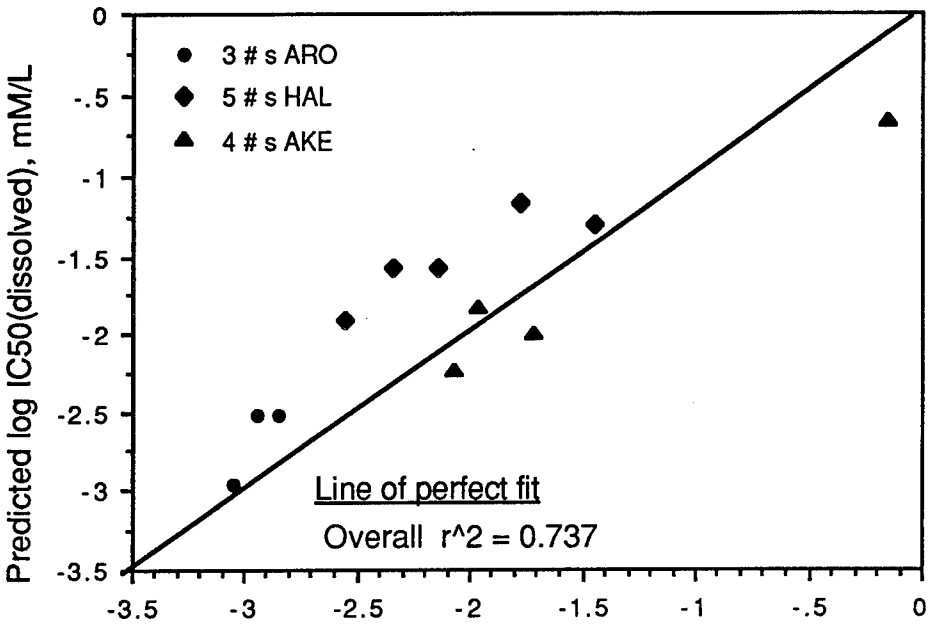


Fig. 7 Comparison of experimental & QSAR predicted IC_{50} values for 12 chemicals in testing set

Joint Toxicity of Chemical Mixtures

Results of the mixture toxicity tests are shown in Table 6. Appendix VI gives the details of the individual chemicals used in the mixture combinations. Details of the concepts of Toxicity Units (TU), Additivity Index (AI), and Mixture Toxicity Index (MTI) are given in published literature from Phase I of this project. For simple additivity the values of Toxicity Units, Additivity Index and Mixture Toxicity Index should be equal to 1, 0, and 1 respectively whereas the results in Table 6 gives average values of $\Sigma TU = 0.97 \pm 0.10$, $AI = 0.04 \pm 0.11$, $MTI = 1.02 \pm 0.05$. Based on these results it can be concluded that the chemicals exhibit simple additivity when acting jointly in a mixture.

Mixture Predictions

Based on the conclusion that the mixtures exert the joint effects by perfect simple additivity, the concentrations of any one chemical in the mixture

combination is predicted. In an N component mixture, as equitoxic ratios of the chemicals were used in the assays, each chemical will exert a toxic effect of 1/N under simple additivity. Based on this, the prediction of the N^{th} chemical in a mixture can be made using the MCI model equations (Equations 8-10). The results of these predictions are shown in Table 7. The comparison of the experimental test results and the predictions based on perfect simple additivity of joint effects of mixture is shown in Figure 8.

TABLE 6. EIGHT AND TEN COMPONENT MIXTURE TOXICITY RESULTS

Mixture Nº	Chemicals in Mixture ID# of chemical	r^2	ΣTU	AI	MTI
	10 component mixtures				
10C-1	1,14,9,18,20,17,22,35,33,27	0.924	0.82	0.22	1.09
10C-2	1,10,9,18,20,16,22,35,33,27	0.820	0.85	0.18	1.07
10C-3	5,15,12,18,20,13,22,30,29,27	0.936	0.86	0.16	1.07
10C-4	6,2,11,28,20,13,22,30,29,27	0.991	0.95	0.05	1.02
10C-5	6,2,11,18,29,13,22,35,33,27	0.954	0.99	0.01	1.00
10C-6	7,2,11,18,30,13,22,35,33,27	0.956	1.05	-0.05	0.98
10C-7	1,14,9,18,2,17,22,35,33,27	0.890	1.00	0.00	1.00
10C-8	1,10,9,18,2,16,22,35,33,27	0.887	0.98	0.02	1.01
10C-9	5,3,11,18,2,13,22,34,33,1	0.900	0.93	0.08	1.03
10C-10	5,1,12,2,20,13,22,30,29,27	0.895	0.82	0.22	1.09
10C-11	6,2,11,28,1,13,22,30,29,27	0.879	1.00	0.00	1.00
10C-12	6,2,11,1,29,13,22,35,33,27	0.969	0.98	0.02	1.01
10C-13	7,2,11,1,30,13,22,35,33,27	0.945	0.95	0.05	1.02
10C-14	7,2,11,1,20,31,22,35,33,27	0.955	0.98	0.02	1.01
10C-15	8,2,11,1,22,13,35,5,33,29	0.971	0.90	0.11	1.05
10C-16	8,2,11,22,1,13,21,35,33,29	0.957	1.06	-0.06	0.98
	8 component mixtures				
8C-1	9,18,20,17,22,35,33,27	0.986	0.81	0.24	1.10
8C-2	9,18,20,16,22,35,33,27	0.974	1.26	-0.26	0.89
8C-3	5,18,20,13,22,30,29,27	0.923	1.02	-0.02	0.99
8C-4	6,2,11,28,22,30,29,27	0.992	0.83	0.21	1.09
8C-5	6,2,11,18,13,22,33,27	0.943	0.83	0.21	1.09
8C-6	7,2,11,18,30,13,22,35	0.922	0.99	0.01	1.00
8C-7	7,2,11,18,20,22,33,27	0.927	0.93	0.08	1.04
8C-8	8,2,11,18,19,13,21,4	0.977	1.00	0.00	1.00
8C-9	8,2,11,23,21,35,33,26	0.884	1.06	-0.06	0.97
8C-10	1,14,18,2,17,35,33,27	0.921	1.00	0.00	1.00
8C-11	1,10,9,2,16,35,33,27	0.958	1.11	-0.11	0.95
8C-12	5,11,2,13,22,34,33,1	0.942	0.91	0.10	1.05
8C-13	5,1,12,2,13,22,30,29	0.865	0.95	0.05	1.03
8C-14	6,2,11,1,13,22,29,27	1.000	0.96	0.04	1.02
8C-15	2,11,1,29,22,35,33,27	0.992	1.17	-0.17	0.92
8C-16	2,11,1,30,13,22,33,27	0.956	1.06	-0.06	0.97
8C-17	7,2,11,1,31,22,35,27	0.963	1.13	-0.13	0.94
8C-18	8,2,11,1,22,13,35,5	0.996	0.96	0.04	1.02
8C-19	8,2,11,22,1,13,21,35	0.978	0.94	0.06	1.03
		Mean	0.97	0.04	1.02
		SD	0.10	0.11	0.05
		CV	0.11	2.75	0.05

TABLE 7. PREDICTION OF MIXTURE TOXICITY

Mixture Nº	Chemicals in Mixture ID# of chemical	N^{th} chemical ID #	Observed Σ TU	Obs. IC_{50} of N^{th} Chemical mg/L	Observed concn. of N^{th} chemical mg/L	Predicted concn. of N^{th} chemical mg/L
	10 component mixtures					
10C-1	1,14,18,20,17,22,35,33,27	9	0.82	0.11	0.01	0.01
10C-2	1,10,9,20,16,22,35,33,27	18	0.85	1.19	0.10	0.44
10C-3	5,15,18,20,13,22,30,29,27	12	0.86	1.79	0.15	0.22
10C-4	6,2,11,28,13,22,30,29,27	20	0.95	3.13	0.30	1.27
10C-5	6,2,11,18,13,22,35,33,27	29	0.99	0.45	0.04	0.03
10C-6	2,11,18,30,13,22,35,33,27	7	1.05	0.21	0.02	0.05
10C-7	1,14,9,18,2,22,35,33,27	17	1.00	7.69	0.77	0.76
10C-8	1,10,9,18,2,22,35,33,27	16	0.98	0.91	0.09	0.08
10C-9	5,11,18,2,13,22,34,33,1	3	0.93	0.31	0.03	0.03
10C-10	5,1,12,20,13,22,30,29,27	2	0.82	0.88	0.07	0.08
10C-11	6,2,28,1,13,22,30,29,27	11	1.00	5.63	0.57	1.02
10C-12	6,2,11,1,29,22,35,33,27	13	0.98	2.17	0.21	0.17
10C-13	7,2,11,1,13,22,35,33,27	30	0.95	0.12	0.01	0.01
10C-14	7,2,11,1,20,31,22,35,33,	27	0.98	0.02	0.002	0.002
10C-15	8,2,11,1,22,13,35,5,29	33	0.90	0.85	0.08	0.06
10C-16	8,2,11,22,1,13,21,33,29	35	1.06	1.87	0.20	0.09
	8 component mixtures					
8C-1	9,18,20,17,22,35,33	27	0.81	0.02	0.002	0.003
8C-2	9,18,20,16,22,35,27	33	1.26	0.85	0.13	0.07
8C-3	5,18,20,13,22,29,27	30	1.02	0.12	0.01	0.01
8C-4	6,2,11,28,22,30,27	29	0.83	0.45	0.05	0.06
8C-5	6,2,11,18,13,33,27	22	0.83	2.68	0.28	0.33
8C-6	2,11,18,30,13,22,35	7	0.99	0.21	0.03	0.06
8C-7	7,11,18,20,22,33,27	2	0.93	0.88	0.10	0.10
8C-8	8,2,18,19,13,21,4	11	1.00	5.63	0.70	1.27
8C-9	8,2,11,21,35,33,26	23	1.06	0.98	0.13	0.16
8C-10	1,14,2,17,35,33,27	18	1.00	1.19	0.15	0.56
8C-11	1,9,2,16,35,33,27	10	1.11	7.75	1.07	0.62
8C-12	5,11,2,13,22,33,1	34	0.91	0.92	0.10	0.15
8C-13	5,1,12,2,22,30,29	13	0.95	2.17	0.26	0.22
8C-14	2,11,1,13,22,29,27	6	0.96	0.16	0.02	0.06
8C-15	2,11,29,22,35,33,27	1	1.17	2.05	0.30	0.23
8C-16	11,1,30,13,22,33,27	2	1.06	0.88	0.12	0.10
8C-17	7,2,11,1,31,22,27	35	1.13	1.87	0.26	0.12
8C-18	8,2,11,1,22,13,35,	5	0.96	0.78	0.09	0.12
8C-19	8,2,11,22,1,13,35	21	0.94	3.44	0.40	0.60

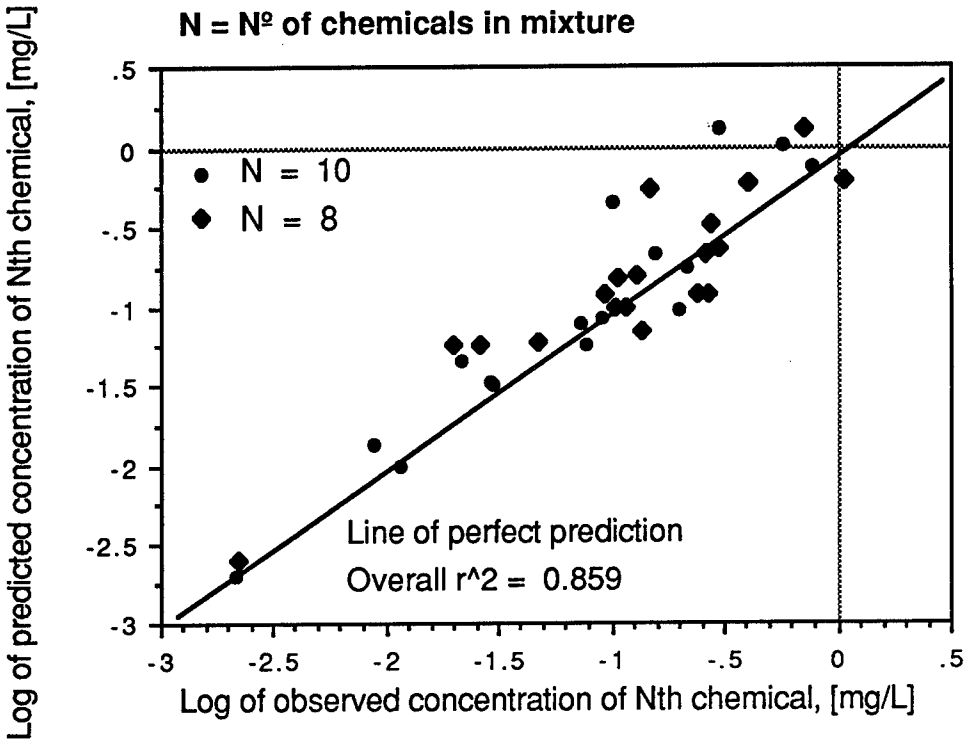


Fig. 8. Comparison of observed and predicted N^{th} chemical concentrations in 8 and 10 component mixtures

CONCLUSIONS

Experimental Protocol

The Polytox surrogate organisms used in this study are convenient to use and microbial toxicity in soil medium can be measured within 8-10 hours. Almost in all chemicals, the variation of the inhibition percentage with the contaminant concentration is explained by the high r^2 values as shown in Table 1. It has been demonstrated that these test results can be reproduced within statistically acceptable levels with an average standard deviation of 0.034 and coefficient of variation of 0.08 for the 12 chemicals. It has also been demonstrated that these tests can be carried out at different moisture holding capacities of the soil. This is particularly useful when different soil moisture levels are encountered in practice.

Single Chemical QSAR Modeling

The three QSAR approaches for the different classes of chemicals yield high adjusted r^2 values except for the halogenated aliphatics with the log P model. The correlation between the QSAR calculated values using the MCI model equations and experimental results has an overall r^2 of 0.983 for the 23 chemicals in the testing set indicating the applicability of the models proposed in this study.

Toxicity of Multicomponent Mixtures

These results indicate a simple additivity mechanism for the 35 different mixtures assayed. The prediction made by utilizing the MCI models for a chemical selected at random from these mixture combinations had an overall r2 of 0.859.

REFERENCES

1. **Bartha R.**, Pesticide Effects on Non Target Microorganisms in Agricultural Soils, Impact of Xenobiotic Chemicals in Microbial Ecosystems, Technical Paper 107, US Fish and Wildlife Service, Washington, DC., 6 -10

2. **Blum D. J. W., and Speece R. E.,** Determining Chemical Toxicity to Aquatic Species, *Environmental Science & Technology,* Critical Review, 24, 284, 1990.

3. **Blum D. J. W. and Speece R. E.,** Quantitative Structure-Activity Relationships for Chemical Toxicity to Environmental Bacteria, *Ecotoxicology and Environmental Safety,* 22, 198, 1991.

4. **Clements R. G., Nabholz J. V., Johnson D. W., and Zeeman M.,** The Use and Application of QSARs in the Office of Toxic Substances for Ecological Hazard Assessment of New Chemicals, *Environmental Toxicology and Risk Assessment, ASTM STP 1179,* Landis W. G., Hughes J . S., and Lewis M. A. Eds., American Society for Testing and Materials, Philadelphia, 56,1993.

5. **Clements R. G., Johnson D. W., Lipnick R. L., Nabholz J. V., and Newsome L. D.,** Estimating Toxicity of Industrial Chemicals to Aquatic Organisms Using Structure Activity Relationships, Vol. 1 EPA-560-6-88-001, US Environmental Protection Agency, Washington, DC, 1988.

6. **Elanbaraway M. T., Robideau R. R., and Beach S. A.** *Toxicity Assessment,* John Wiley & Sons Inc., 1988, 361.

7. **Nirmalakhandan N, Speece R. E.,** QSAR Model for Predicting Henry's Constant, Environmental Science and Technology, 1988, 1349 - 1357.

8. **Nirmalakhandan N, Speece R. E.,** Prediction of Aqueous Solubility of Organic Chemicals Based on Molecular Structure, Environmental Science and Technology, 1988, 22, 328 - 338.

9. **Nirmalakhandan N., Arulgnanendran V. R. J., Mohshin M., Bangxin S., and Cadena F.,** Toxicity of Mixtures of Chemicals to Microorganisms, *Water Research,* 28, 543, 1994.

10. **Nirmalakhandan N., Sun B., Arulgnanendran V. R. J., Mohshin M., Wang X. H., Prakash J., and Hall N.** Submitted to *Water Science & Technology* , 1993.

11. **Nirmalakhandan et al**, Interim Progress Report, Phase II, AFOSR - 91 - 0394, October 1993.

12. **Tang N. H., Blum D. J. W., Nirmalakhandan N., and Speece R. E.,** QSAR Parameters for Toxicity of Organic Chemicals to Nitrobactor, *ASCE, Journal of Environmental Engineering,* 118, 17, 1992.

13. **Tu C. M.,** Effects of Insecticides on Populations of Microflora, Nitrification and Respiration in Soil, *Commn. in Soil Science and Plant Analysis,* 9(7), 629, 1978.

APPENDIX I

Appendix I

Details of Respirometer system

Same as Phase II

APPENDIX II

Experimental Methods and Materials

Appendix II

EXPERIMENTAL METHODOLOGY

Soil

Sandy loam soil was collected from a depth of 15 cm at an agricultural field in Mesilla, New Mexico. The soil was sieved using a 2 mm sieve to remove leaves and other organic material. The measured organic content of the soil was 0.7%. The soil was autoclaved for seven hours daily for four days and oven dried for 3 hours at 105^{o}C to sterilize the soil.

Test Chemicals

Thirty-five organic chemicals from three congeneric groups with a range of molecular structures were selected for the testing of toxicity in the soil medium. These chemicals represented common solvents, petroleum constituents and halogenated compounds.

Polytox Surrogate Microbial Cultures

A commercially available surrogate culture of microorganisms, Polytox ™, was evaluated in the test procedure. An 8 gram vial of Polytox in the freeze dried state was dissolved in 280 ml of buffered solution and nutrients prepared according to Standard Methods. This mixture was supplied with oxygen for four hours while being stirred continuously. At the end of four hours 20 ml of the supernatant from the microbial culture was mixed with 200 grams of the autoclaved soil in each of the 600 ml respirometer reactor bottles.

In order to maintain 50% moisture holding capacity , the required amount of water was added to the soil. Different concentrations of the toxicant, dissolved in 0.5 ml of acetone were added to each of the reactor bottles, except for the control reactor that received only 0.5 ml of acetone. After mixing the chemicals with the soil, potassium hydroxide pellets were placed in the holder provided in the caps of the reactors. A 12 reactor computer interfaced respirometer (N -

CON Corporation, NY) was used for the assays. These reactors were placed in the respirometer bath maintained at 25°C. The oxygen uptake of each reactor was monitored by the data acquisition system in the respirometer for the next 8 - 10 hours. The concentration of the toxicant causing inhibition of the organisms' respiration by 50%, i.e., IC_{50}, was calculated by comparing the oxygen uptake of each reactor with that of the control that was free of the contaminant. The inhibition percentage at different concentrations of the toxicants was calculated based on the reduction in oxygen uptake rate in each of the reactors with the toxicant in comparison to the toxicant free control. The tests were repeated for 12 chemicals selected at random with identical conditions. Tests on 6 chemicals at moisture holding capacities 33%, 80% and 100% were done while other conditions remained the same.

Joint Toxicity of Mixtures of Chemicals

Equitoxic ratios of the different single chemicals assayed were used to experimentally determine the joint toxicity of 8 component and 10 component mixtures. These mixture combinations were selected at random from the single chemical list of 35 chemicals. These combinations of chemicals at differing concentrations were dissolved in 0.5 ml of acetone and added to the respirometer reactors. The rate of oxygen uptake from these reactors were compared against a control reactor which received 0.5 ml of acetone.

Isotherms Studies on Soils and Microbial Cells

Preparation of Saturated Solutions for Chemicals

Based on the aqueous solubility of individual chemicals saturated solutions were prepared for the test chemicals by dissolving the chemicals in water and mixing them on a mechanical shaker for 96 hours. These solutions were prepared in 13 ml test tubes with a Teflon screw cap septum. Three glass beads were included in each of the tubes in order to enhance proper mixing. Five different concentrations of the saturated solution were withdrawn from the middle section of the tubes by micro syringes and injected into tubes containing

nanopure water. These were mixed continuously for 24 hours at the end of which they were injected into the gas chromatrograph. Each concentration of this samples used for determining the calibration equation were repeated thrice.

The above procedure was repeated with the same concentrations used in the calibration equation and 2 grams of autoclaved, oven dried soil as used in the toxicity assays. These isotherm tests were also done by the same procedure with the test chemicals and 200 micro liters of supernatant from the Polytox microbial culture to maintain the same ratio of soil to microbial cells as in the respirometer reactors.

Assuming a Freundlich isotherm with $x/m = KC_0^{1/n}$
where x = mass of solute adsorbed;
m = mass of adsorbent;
C_0 = equilibrium concentration of solute, mass/volume;
K, n = experimental constants.

The results were tested for either linear or log linear relationships for the isotherms to determine the adsorption of the chemical to the soil and the bio-sorption on to the microbial cells. The confidence intervals on the values of n for a linear relationship are given in Tables 7 and 8. Details of the isotherm results are given in Appendix VII and VIII.

APPENDIX III

Table A - III -1: Correlation between log IC_{50} (Dissolved) and $^1\chi$ for ARO group

Regression Summary
logIC50(Dissolved),mM/L vs. 1X

Count	6
Num. Missing	0
R	.994
R Squared	.989
Adjusted R Squared	.986
RMS Residual	.058

ANOVA Table
logIC50(Dissolved),mM/L vs. 1X

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.191	1.191	353.491	<.0001
Residual	4	.013	.003		
Total	5	1.204			

Regression Coefficients
logIC50(Dissolved),mM/L vs. 1X

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	.559	.155	.559	3.614	.0225
1X	-1.089	.058	-.994	-18.801	<.0001

Confidence Intervals
logIC50(Dissolved),mM/L vs. 1X

	Coefficient	95% Lower	95% Upper
Intercept	.559	.130	.988
1X	-1.089	-1.249	-.928

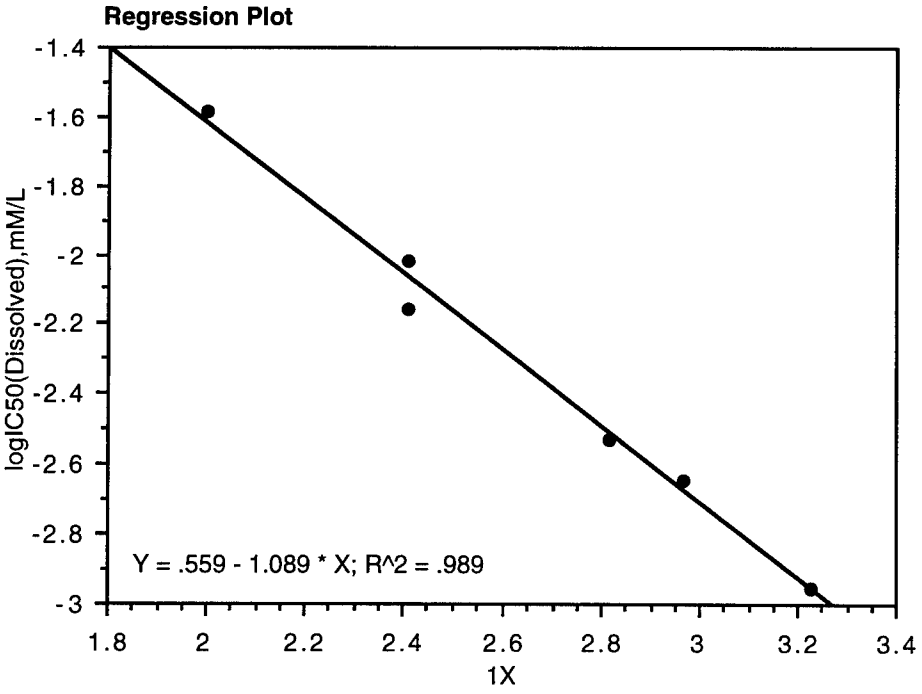


Fig. A- III-1: Correlation between log IC_{50} (Dissolved) and $^1\chi$ for ARO group

Table A - III-2: Correlation between log IC_{50} (Dissolved) and $^1\chi$ for HAL group

Regression Summary
logIC50(Dissolved),mM/L vs. 1X

Count	9
Num. Missing	0
R	.938
R Squared	.881
Adjusted R Squared	.863
RMS Residual	.143

ANOVA Table
logIC50(Dissolved),mM/L vs. 1X

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.056	1.056	51.587	.0002
Residual	7	.143	.020		
Total	8	1.199			

Regression Coefficients
logIC50(Dissolved),mM/L vs. 1X

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	.243	.272	.243	.894	.4012
1X	-1.046	.146	-.938	-7.182	.0002

Confidence Intervals
logIC50(Dissolved),mM/L vs. 1X

	Coefficient	95% Lower	95% Upper
Intercept	.243	-.400	.885
1X	-1.046	-1.391	-.702

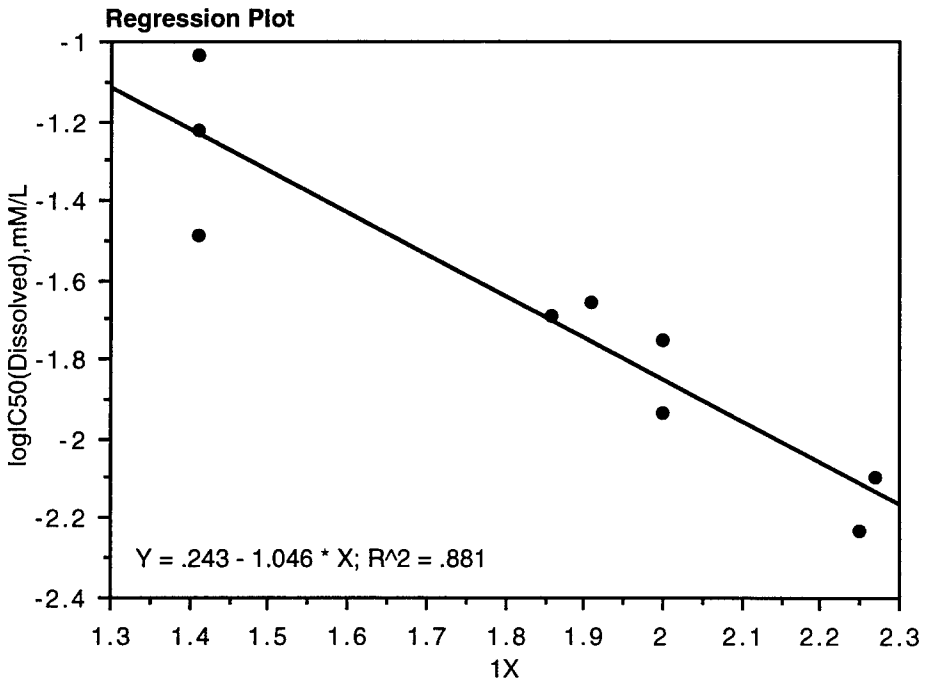


Fig. A- III - 2: Correlation between log IC_{50} (Dissolved) and $^1\chi$ for HAL group

Table A - III -3: Correlation between log IC_{50} (Dissolved) and $^{1}\chi^{v}$ for AKE group

Regression Summary
logIC50(Dissolved),mM/L vs. 1XV

Count	8
Num. Missing	0
R	.997
R Squared	.994
Adjusted R Squared	.993
RMS Residual	.093

ANOVA Table
logIC50(Dissolved),mM/L vs. 1XV

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	8.448	8.448	967.749	<.0001
Residual	6	.052	.009		
Total	7	8.500			

Regression Coefficients
logIC50(Dissolved),mM/L vs. 1XV

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	.659	.095	.659	6.917	.0005
1XV	-1.110	.036	-.997	-31.109	<.0001

Confidence Intervals
logIC50(Dissolved),mM/L vs. 1XV

	Coefficient	95% Lower	95% Upper
Intercept	.659	.426	.892
1XV	-1.110	-1.198	-1.023

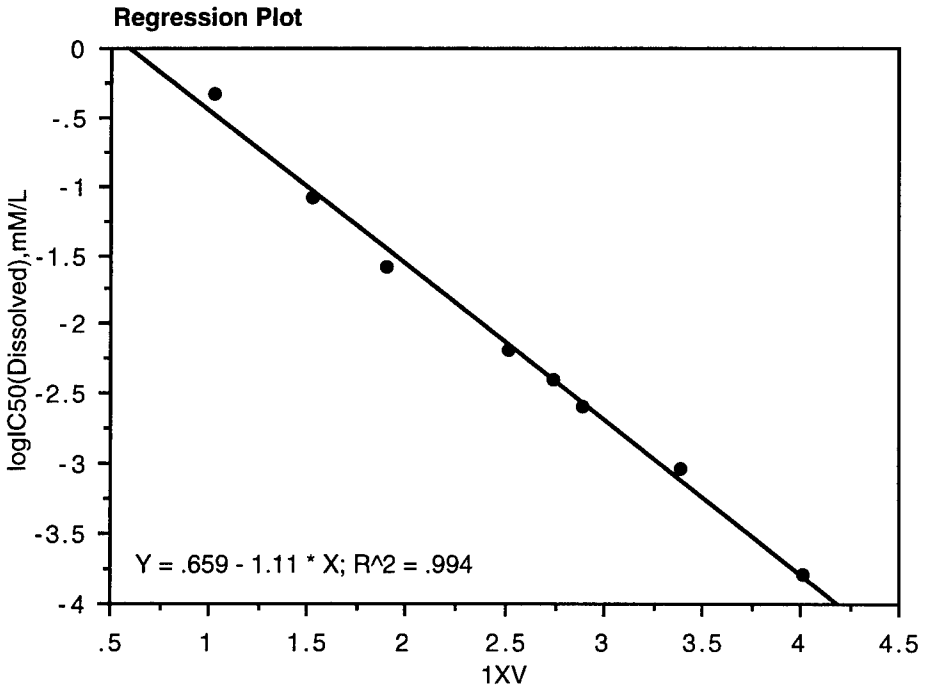


Fig. A - III -3: Correlation between log IC_{50} (Dissolved) and $^{1}\chi^{v}$ for AKE group

APPENDIX IV

Table A - IV -1: Correlation between log IC_{50} (Dissolved) and LSER for all chemicals

Regression Summary
logIC50(Dissolved),mM/L vs. 4 Independents

Count	23
Num. Missing	0
R	.985
R Squared	.971
Adjusted R Squared	.965
RMS Residual	.142

ANOVA Table
logIC50(Dissolved),mM/L vs. 4 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	4	12.186	3.046	151.799	<.0001
Residual	18	.361	.020		
Total	22	12.547			

Regression Coefficients
logIC50(Dissolved),mM/L vs. 4 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.002	.197	1.002	5.096	<.0001
Vi/100	-5.339	.221	-1.003	-24.185	<.0001
Phi*	-.139	.201	-.031	-.693	.4973
Alpha	-.351	.224	-.071	-1.568	.1343
Beta	.474	.207	.112	2.286	.0346

Confidence Intervals
logIC50(Dissolved),mM/L vs. 4 Independents

	Coefficient	95% Lower	95% Upper
Intercept	1.002	.589	1.416
Vi/100	-5.339	-5.803	-4.875
Phi*	-.139	-.562	.283
Alpha	-.351	-.822	.119
Beta	.474	.038	.909

Fig. - A - IV -1: Correlation between log IC_{50} (Dissolved) and LSER for all chemicals

Table A - IV -2: Correlation between log IC_{50} (Dissolved) and LSER for ARO group

Regression Summary
logIC50(Dissolved),mM/L vs. 3 Independents

Count	6
Num. Missing	0
R	.998
R Squared	.996
Adjusted R Squared	.990
RMS Residual	.050

ANOVA Table
logIC50(Dissolved),mM/L vs. 3 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	3	1.199	.400	160.278	.0062
Residual	2	.005	.002		
Total	5	1.204			

Regression Coefficients
logIC50(Dissolved),mM/L vs. 3 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	1.338	.230	1.338	5.810	.0284
Vi/100	-5.255	.242	-1.016	-21.715	.0021
Phi*	-.429	.222	-.110	-1.927	.1938
Beta	-1.310	.516	-.149	-2.541	.1262

Confidence Intervals
logIC50(Dissolved),mM/L vs. 3 Independents

	Coefficient	95% Lower	95% Upper
Intercept	1.338	.347	2.328
Vi/100	-5.255	-6.297	-4.214
Phi*	-.429	-1.386	.529
Beta	-1.310	-3.529	.909

Fig. - A - IV -2: Correlation between log IC_{50} (Dissolved) and LSER for ARO group

Table A - IV -3: Correlation between log IC_{50} (Dissolved) and LSER for HAL group

Regression Summary
logIC50(Dissolved),mM/L vs. 4 Independents

Count	9
Num. Missing	0
R	.977
R Squared	.955
Adjusted R Squared	.911
RMS Residual	.116

ANOVA Table
logIC50(Dissolved),mM/L vs. 4 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	4	1.145	.286	21.360	.0058
Residual	4	.054	.013		
Total	8	1.199			

Regression Coefficients
logIC50(Dissolved),mM/L vs. 4 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	.897	.542	.897	1.655	.1732
Vi/100	-5.104	.877	-1.006	-5.818	.0043
Phi*	-.150	.267	-.088	-.562	.6043
Alpha	.146	.655	.041	.223	.8347
Beta	-1.051	1.766	-.068	-.595	.5837

Confidence Intervals
logIC50(Dissolved),mM/L vs. 4 Independents

	Coefficient	95% Lower	95% Upper
Intercept	.897	-.607	2.401
Vi/100	-5.104	-7.540	-2.668
Phi*	-.150	-.891	.591
Alpha	.146	-1.672	1.963
Beta	-1.051	-5.954	3.852

Fig. - A - IV -3: Correlation between log IC_{50} (Dissolved) and LSER for HAL group

Table A - IV -4: Correlation between log IC_{50} (Dissolved) and LSER for AKE group

Regression Summary
logIC50(Dissolved),mM/L vs. 4 Independents

Count	8
Num. Missing	0
R	.999
R Squared	.997
Adjusted R Squared	.993
RMS Residual	.090

ANOVA Table
logIC50(Dissolved),mM/L vs. 4 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	4	8.476	2.119	259.304	.0004
Residual	3	.025	.008		
Total	7	8.500			

Regression Coefficients
logIC50(Dissolved),mM/L vs. 4 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	3.888	1.144	3.888	3.398	.0425
Vi/100	-5.897	.196	-.995	-30.073	<.0001
Phi*	-2.971	1.356	-.205	-2.192	.1161
Alpha	-1.775	.633	-.276	-2.803	.0677
Beta	-1.656	.892	-.175	-1.855	.1606

Confidence Intervals
logIC50(Dissolved),mM/L vs. 4 Independents

	Coefficient	95% Lower	95% Upper
Intercept	3.888	.247	7.530
Vi/100	-5.897	-6.521	-5.273
Phi*	-2.971	-7.285	1.343
Alpha	-1.775	-3.790	.240
Beta	-1.656	-4.495	1.184

Fig. - A - IV -4: Correlation between log IC_{50} (Dissolved) and LSER for AKE group

APPENDIX V

Table A - V -1: Correlation between log IC_{50} (Dissolved) and log P for all chemicals

Regression Summary
logIC50(Dissolved),mM/L vs. Log P

Count	23
Num. Missing	0
R	.571
R Squared	.326
Adjusted R Squared	.294
RMS Residual	.635

ANOVA Table
logIC50(Dissolved),mM/L vs. Log P

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	4.091	4.091	10.160	.0044
Residual	21	8.456	.403		
Total	22	12.547			

Regression Coefficients
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	-.980	.346	-.980	-2.834	.0099
Log P	-.491	.154	-.571	-3.187	.0044

Confidence Intervals
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	95% Lower	95% Upper
Intercept	-.980	-1.699	-.261
Log P	-.491	-.811	-.171

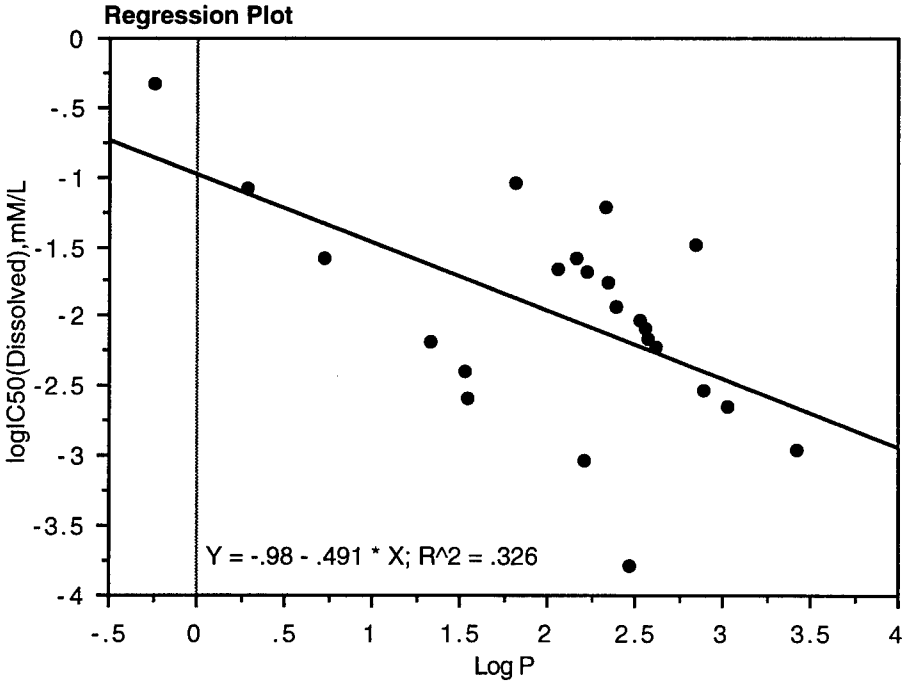


Fig. - A - V -1: Correlation between log IC_{50} (Dissolved) and log P for all chemicals

Table A - V -2: Correlation between log IC_{50} (Dissolved) and log P for ARO group

Regression Summary
logIC50(Dissolved),mM/L vs. Log P

Count	6
Num. Missing	0
R	.989
R Squared	.979
Adjusted R Squared	.974
RMS Residual	.080

ANOVA Table
logIC50(Dissolved),mM/L vs. Log P

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.179	1.179	185.261	.0002
Residual	4	.025	.006		
Total	5	1.204			

Regression Coefficients
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	.739	.227	.739	3.258	.0311
Log P	-1.102	.081	-.989	-13.611	.0002

Confidence Intervals
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	95% Lower	95% Upper
Intercept	.739	.109	1.368
Log P	-1.102	-1.326	-.877

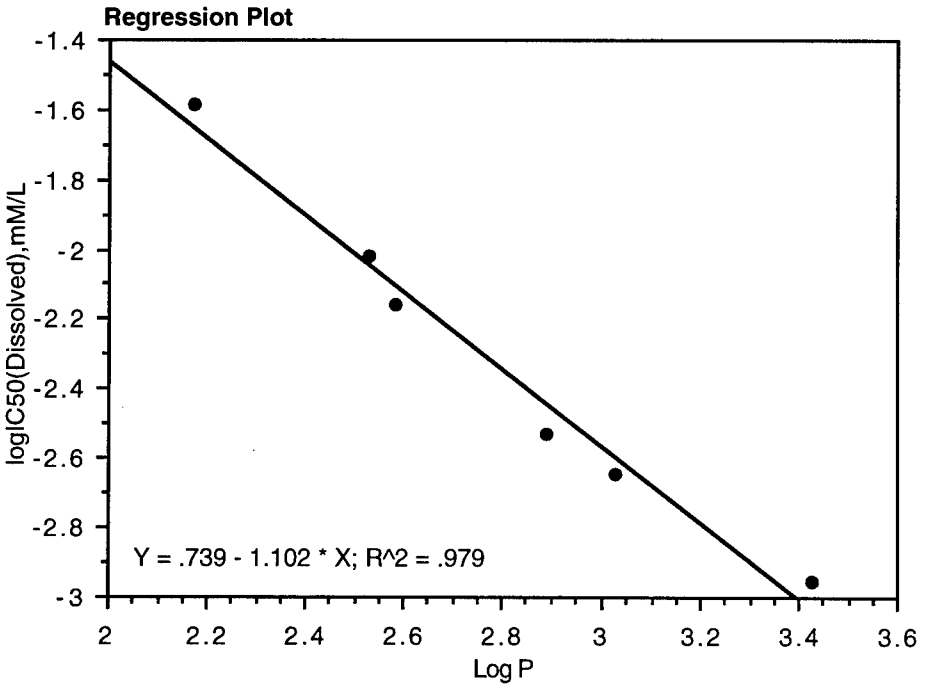


Fig. A - V -2: Correlation between log IC_{50} (Dissolved) and log P for ARO group

Table A - V -3: Correlation between log IC_{50} (Dissolved) and log P for HAL group

Regression Summary
logIC50(Dissolved),mM/L vs. Log P

Count	9
Num. Missing	0
R	.529
R Squared	.280
Adjusted R Squared	.177
RMS Residual	.351

ANOVA Table
logIC50(Dissolved),mM/L vs. Log P

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.335	.335	2.717	.1433
Residual	7	.864	.123		
Total	8	1.199			

Regression Coefficients
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	-.099	.965	-.099	-.103	.9209
Log P	-.669	.406	-.529	-1.648	.1433

Confidence Intervals
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	95% Lower	95% Upper
Intercept	-.099	-2.381	2.183
Log P	-.669	-1.629	.291

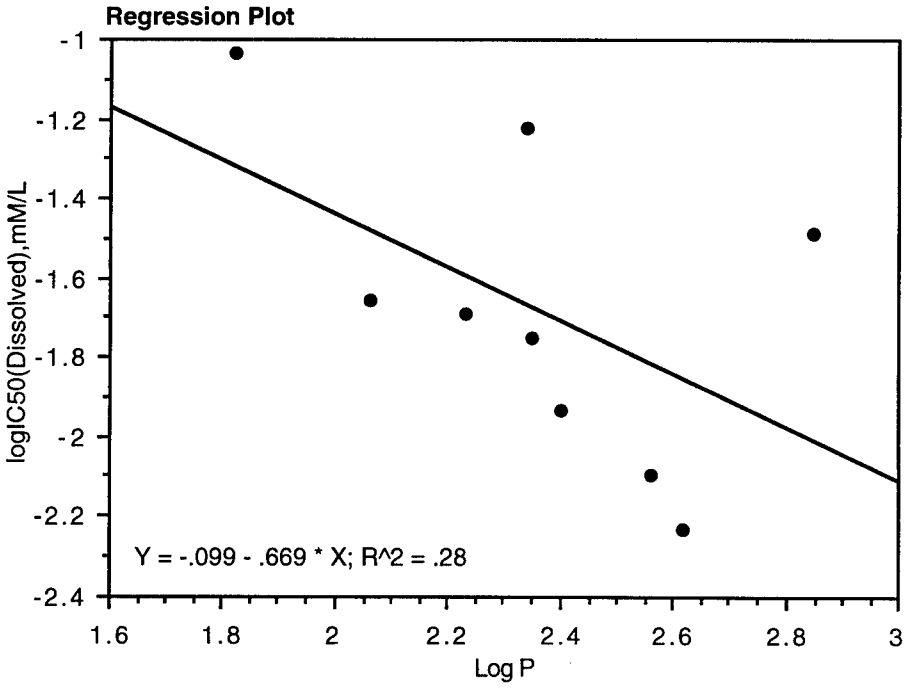


Fig. A - V -3: Correlation between log IC_{50} (Dissolved) and log P for HAL group

Table A - V -4: Correlation between log IC_{50} (Dissolved) and log P for AKE group

Regression Summary
logIC50(Dissolved),mM/L vs. Log P

Count	8
Num. Missing	0
R	.993
R Squared	.985
Adjusted R Squared	.983
RMS Residual	.144

ANOVA Table
logIC50(Dissolved),mM/L vs. Log P

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	8.376	8.376	403.253	<.0001
Residual	6	.125	.021		
Total	7	8.500			

Regression Coefficients
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	-.661	.089	-.661	-7.449	.0003
Log P	-1.182	.059	-.993	-20.081	<.0001

Confidence Intervals
logIC50(Dissolved),mM/L vs. Log P

	Coefficient	95% Lower	95% Upper
Intercept	-.661	-.878	-.444
Log P	-1.182	-1.326	-1.038

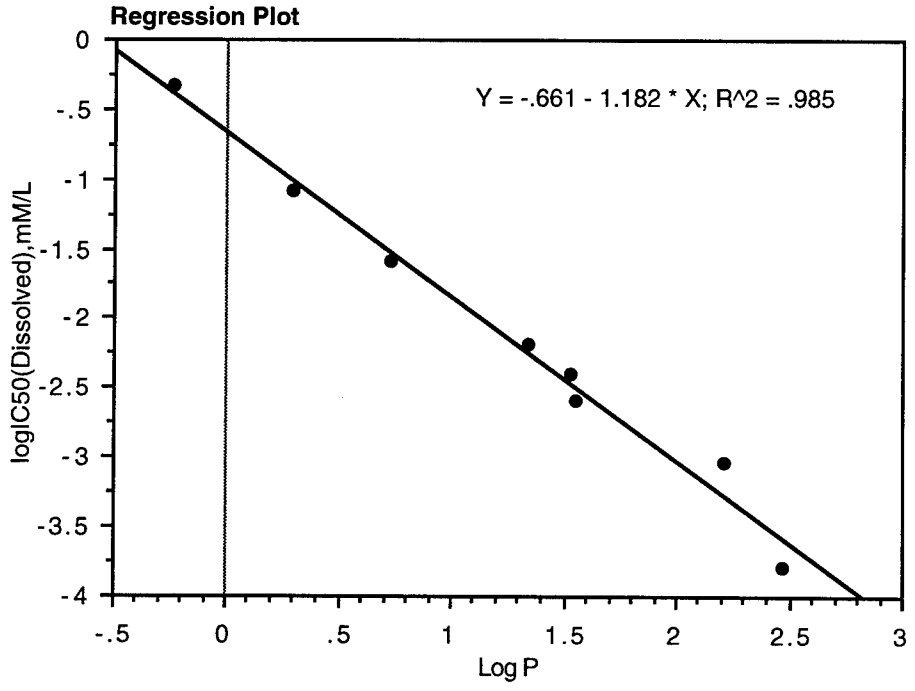


Fig. A - V -4: Correlation between log IC_{50} (Dissolved) and log P for AKE group

APPENDIX VI

APPENDIX VI

8 CHEMICAL MIXTURE COMBINATIONS

Mixture Nº	Chemical Nº	Chemical Name	Results
8 - 1	9	2,4 Dimethyl phenol	r^2 = 0.986; TU = 0.10
	18	Bromodichloromethane	AI = 0.24; MTI = 1.10
	20	Ethylene dibromide	
	17	Bromochloromethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
8 - 2	9	2,4 Dimethyl phenol	r^2 = 0.974; TU = 0.16
	18	Bromodichloromethane	AI = -0.26; MTI = .89
	20	Ethylene dibromide	
	16	1,2 Dichloropropane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
8 - 3	5	Chlorobenzene	r^2 = 0.923; TU = 0.13
	18	Bromodichloromethane	AI = -0.02; MTI = 0.99
	20	Ethylene dibromide	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	30	N - Amyl acetate	
	29	Isobutyl acetate	
	27	Octanol	
8 - 4	6	1,2 Dichlorobenzene	r^2 = 0.992; TU = 0.10
	2	Toluene	AI = 0.21; MTI = 1.09
	11	Dibromomethane	
	28	N-Butyl acetate	
	22	Trichloroethylene	
	30	N - Amyl acetate	
	29	Isobutyl acetate	
	27	Octanol	

Mixture Nº	Chemical Nº	Chemical Name	Results
8 - 5	6	1,2 Dichlorobenzene	r^2 = 0.943; TU = 0.10
	2	Toluene	AI = 0.21; MTI = 1.09
	11	Dibromomethane	
	18	Bromodichloromethane	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	33	Methyl isobutyl ketone	
	27	Octanol	
8 - 6	7	1,3 Dichlorobenzene	r^2 = 0.922; TU = 0.12
	2	Toluene	AI = 0.01; MTI = 1.00
	11	Dibromomethane	
	18	Bromodichloromethane	
	30	N - Amyl acetate	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
8 - 7	7	1,3 Dichlorobenzene	r^2 = 0.927; TU = 0.12
	2	Toluene	AI = 0.08; MTI = 1.04
	11	Dibromomethane	
	18	Bromodichloromethane	
	20	Ethylene dibromide	
	22	Trichloroethylene	
	33	Methyl isobutyl ketone	
	27	Octanol	
8 - 8	8	1,2,4 Trichlorobenzene	r^2 = 0.977; TU = 0.125
	2	Toluene	AI = 0.00; MTI = 1.00
	11	Dibromomethane	
	18	Bromodichloromethane	
	19	Chlorodibromomethane	
	13	1,2 Dichloroethane	
	21	*cis* - 1,2 Dichloroethylene	
	4	Ethylbenzene	
8 - 9	8	1,2,4 Trichlorobenzene	r^2 = 0.884; TU = 0.13
	2	Toluene	AI = -0.06; MTI = 0.97
	11	Dibromomethane	
	23	Tetrachloroethylene	
	21	*cis* - 1,2 Dichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	26	Pentanol	

Mixture Nº	Chemical Nº	Chemical Name	Results
8-10	1	Benzene	r^2 = 0.921; TU = 0.125
	14	1,1,1 Trichloroethane	AI = 0.00; MTI = 1.00
	18	Bromodichloromethane	
	2	Toluene	
	17	Bromochloromethane	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
8-11	1	Benzene	r^2 = 0.958; TU = 0.14
	10	Dichloromethane	AI = -0.11; MTI = 0.95
	9	2,4 Dimethyl phenol	
	2	Toluene	
	16	1,2 Dichloropropane	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
8-12	5	Chlorobenzene	r^2 = 0.942; TU = 0.11
	11	Dibromomethane	AI = 0.10; MTI = 1.05
	2	Toluene	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	34	Methyl N - propyl ketone	
	33	Methyl isobutyl ketone	
	1	Benzene	
8-13	5	Chlorobenzene	r^2 = 0.865; TU = 0.12
	1	Benzene	AI = 0.05; MTI = 1.03
	12	Carbontetrachloride	
	2	Toluene	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	30	N - Amyl acetate	
	29	Isobutyl acetate	
8-14	6	1,2 Dichlorobenzene	r^2 = 1.000; TU = 0.12
	2	Toluene	AI = 0.04; MTI = 1.02
	11	Dibromomethane	
	1	Benzene	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	29	Isobutyl acetate	
	27	Octanol	

Mixture Nº	Chemical Nº	Chemical Name	Results
8-15	2	Toluene	r^2 = 0.992; TU = 0.15
	11	Dibromomethane	AI = -0.17; MTI = 0.92
	1	Benzene	
	29	Isobutyl acetate	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
8-16	2	Toluene	r^2 = 0.956; TU = 0.13
	11	Dibromomethane	AI = -0.06; MTI = 0.97
	1	Benzene	
	30	N - Amyl acetate	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	33	Methyl isobutyl ketone	
	27	Octanol	
8-17	7	1,3 Dichlorobenzene	r^2 = 0.963; TU = 0.14
	2	Toluene	AI = -0.13; MTI = 0.94
	11	Dibromomethane	
	1	Benzene	
	31	Ethyl acetate	
	22	Trichloroethylene	
	35	Cyclohexanone	
	27	Octanol	
8-18	8	1,2,4 Trichlorobenzene	r^2 = 0.996; TU = 0.12
	2	Toluene	AI = 0.04; MTI = 1.02
	11	Dibromomethane	
	1	Benzene	
	22	Trichloroethylene	
	13	1,2 Dichloroethane	
	35	Cyclohexanone	
	5	Chlorobenzene	
8-19	8	1,2,4 Trichlorobenzene	r^2 = 0.978; TU = 0.12
	2	Toluene	AI = 0.06; MTI = 1.03
	11	Dibromomethane	
	22	Trichloroethylene	
	1	Benzene	
	13	1,2 Dichloroethane	
	21	*cis* - 1,2 Dichloroethylene	
	35	Cyclohexanone	

10 CHEMICAL MIXTURE COMBINATIONS

Mixture Nº	Chemical Nº	Chemical Name	Results
10-1	1	Benzene	r^2 = 0.924; TU = 0.08
	14	1,1,1 Trichloroethane	AI = 0.22; MTI = 1.09
	9	2,4 Dimethyl phenol	
	18	Bromodichloromethane	
	20	Ethylene dibromide	
	17	Bromochloromethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
10-2	1	Benzene	r^2 = 0.820; TU = 0.09
	10	Dichloromethane	AI =0.18; MTI = 1.07
	9	2,4 Dimethyl phenol	
	18	Bromodichloromethane	
	20	Ethylene dibromide	
	16	1,2 Dichloropropane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
10-3	5	Chlorobenzene	r^2 = 0.936; TU = 0.09
	15	1,1,2,2 Tetrachloroethane	AI = 0.16; MTI = 1.07
	12	Carbontetrachloride	
	18	Bromodichloromethane	
	20	Ethylene dibromide	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	30	N - Amyl acetate	
	29	Isobutyl acetate	
	27	Octanol	
10-4	6	1,2 Dichlorobenzene	r^2 = 0.991; TU = 0.10
	2	Toluene	AI = 0.05; MTI = 1.02
	11	Dibromomethane	
	28	N-Butyl acetate	
	20	Ethylene dibromide	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	30	N - Amyl acetate	
	29	Isobutyl acetate	
	27	Octanol	

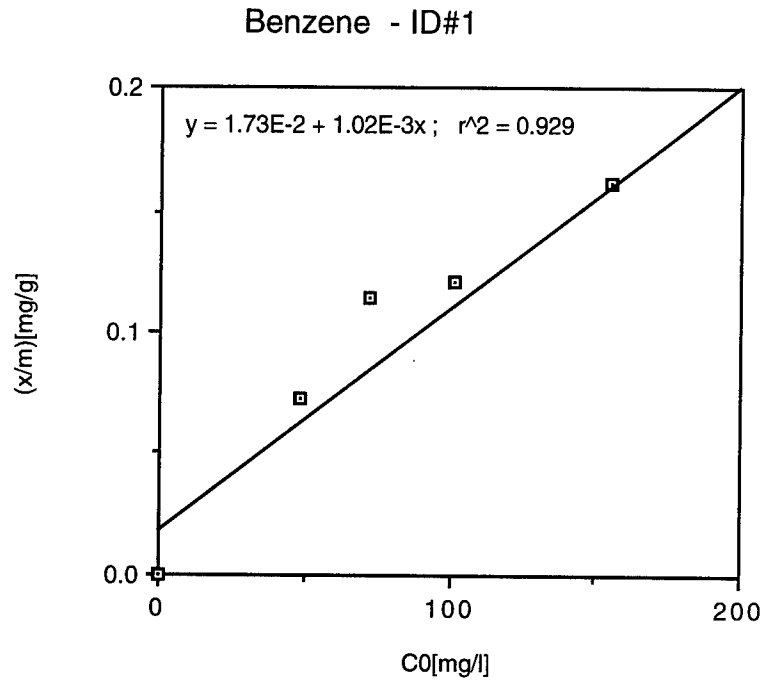
Mixture Nº	Chemical Nº	Chemical Name	Results
10-5	6	1,2 Dichlorobenzene	r^2 = 0.954; TU = 0.10
	2	Toluene	AI = 0.01; MTI = 1.00
	11	Dibromomethane	
	18	Bromodichloromethane	
	29	Isobutyl acetate	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
10-6	7	1,3 Dichlorobenzene	r^2 = 0.956; TU = 0.11
	2	Toluene	AI = -0.05; MTI = 0.98
	11	Dibromomethane	
	18	Bromodichloromethane	
	30	N - Amyl acetate	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
10-7	1	Benzene	r^2 = 0.890; TU = 0.10
	14	1,1,1 Trichloroethane	AI = 0.00; MTI = 1.00
	9	2,4 Dimethyl phenol	
	18	Bromodichloromethane	
	2	Toluene	
	17	Bromochloromethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
10-8	1	Benzene	r^2 = 0.887; TU = 0.10
	10	Dichloromethane	AI = 0.02; MTI = 1.01
	9	2,4 Dimethyl phenol	
	18	Bromodichloromethane	
	2	Toluene	
	16	1,2 Dichloropropane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	

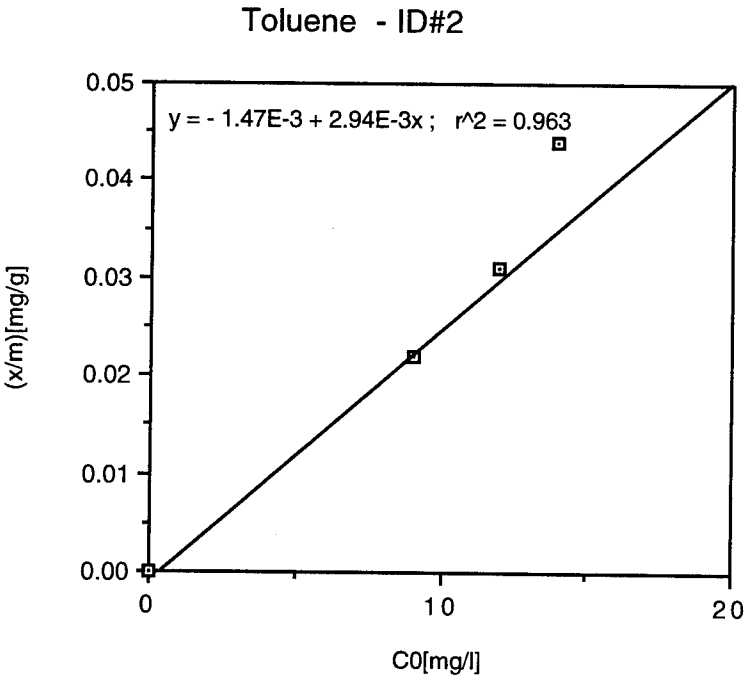
Mixture Nº	Chemical Nº	Chemical Name	Results
10-9	5	Chlorobenzene	r^2 = 0.900; TU = 0.09
	3	O - Xylene	AI = 0.08; MTI = 1.03
	11	Dibromomethane	
	18	Bromodichloromethane	
	2	Toluene	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	34	Methyl N - propyl ketone	
	33	Methyl isobutyl ketone	
	1	Benzene	
10-10	5	Chlorobenzene	r^2 = 0.895; TU = 0.08
	1	Benzene	AI = 0.22 ; MTI = 1.09
	12	Carbontetrachloride	
	2	Toluene	
	20	Ethylene dibromide	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	30	N - Amyl acetate	
	29	Isobutyl acetate	
	27	Octanol	
10-11	6	1,2 Dichlorobenzene	r^2 = 0.879; TU = 0.10
	2	Toluene	AI = 0.00; MTI = 1.00
	11	Dibromomethane	
	28	N - Butyl acetate	
	1	Benzene	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	30	N - Amyl acetate	
	29	Isobutyl acetate	
	27	Octanol	
10-12	6	1, 2 Dichlorobenzene	r^2 = 0.969; TU = 0.10
	2	Toluene	AI = 0.02; MTI = 1.01
	11	Dibromomethane	
	1	Benzene	
	29	Isobutyl acetate	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	

Mixture Nº	Chemical Nº	Chemical Name	Results
10-13	7	1,3 Dichlorobenzene	r^2 = 0.945; TU = 0.10
	2	Toluene	AI = 0.05; MTI = 1.02
	11	Dibromomethane	
	1	Benzene	
	30	N - Amyl acetate	
	13	1,2 Dichloroethane	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
10-14	7	1,3 Dichlorobenzene	r^2 = 0.955; TU = 0.10
	2	Toluene	AI = 0.02; MTI = 1.01
	11	Dibromomethane	
	1	Benzene	
	20	Ethylene dibromide	
	31	Ethyl acetate	
	22	Trichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	27	Octanol	
10-15	8	1,2,4 Trichlorobenzene	r^2 = 0.971; TU = 0.09
	2	Toluene	AI = 0.11; MTI = 1.05
	11	Dibromomethane	
	1	Benzene	
	22	Trichloroethylene	
	13	1,2 Dichloroethane	
	35	Cyclohexanone	
	5	Chlorobenzene	
	33	Methyl isobutyl ketone	
	29	Isobutyl acetate	
10-16	8	1,2,4 Trichlorobenzene	r^2 = 0.957; TU = 0.11
	2	Toluene	AI = -0.06' MTI = 0.98
	11	Dibromomethane	
	22	Trichloroethylene	
	1	Benzene	
	13	1,2 Dichloroethane	
	21	*cis* - 1,2 Dichloroethylene	
	35	Cyclohexanone	
	33	Methyl isobutyl ketone	
	29	Isobutyl acetate	

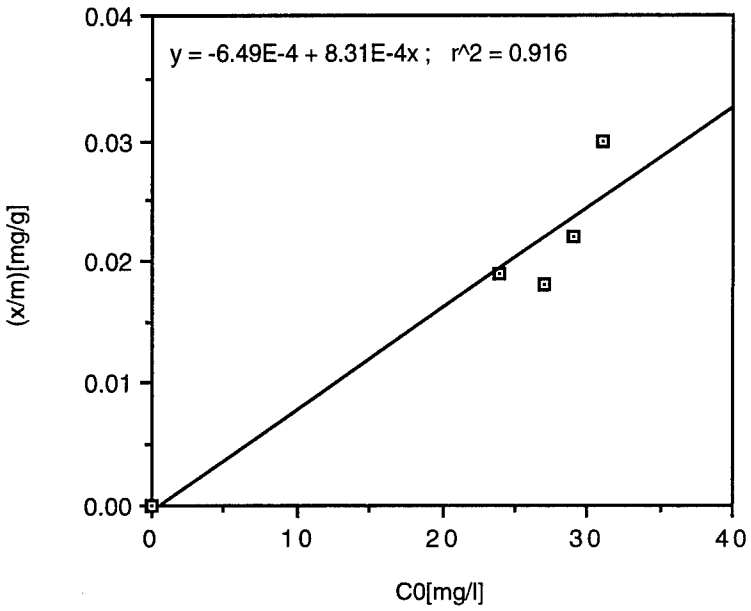
APPENDIX VII

APPENDIX VII
Results of adsorption isotherms with soils

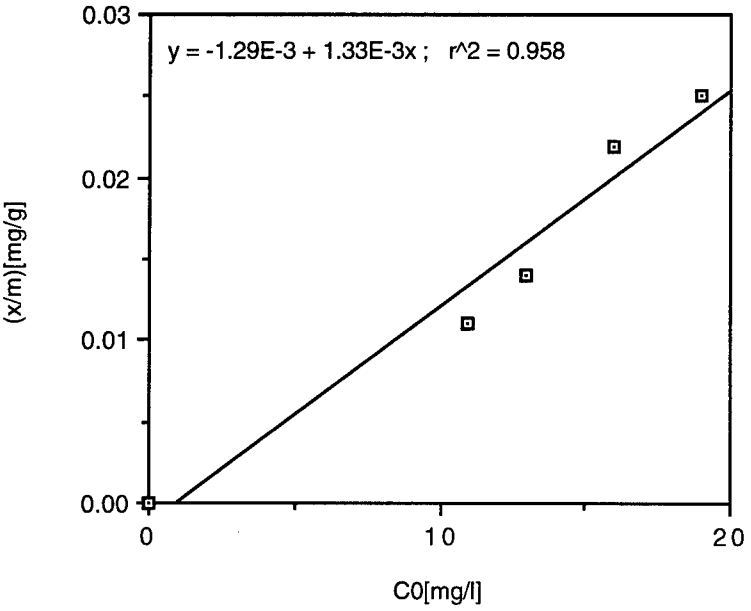




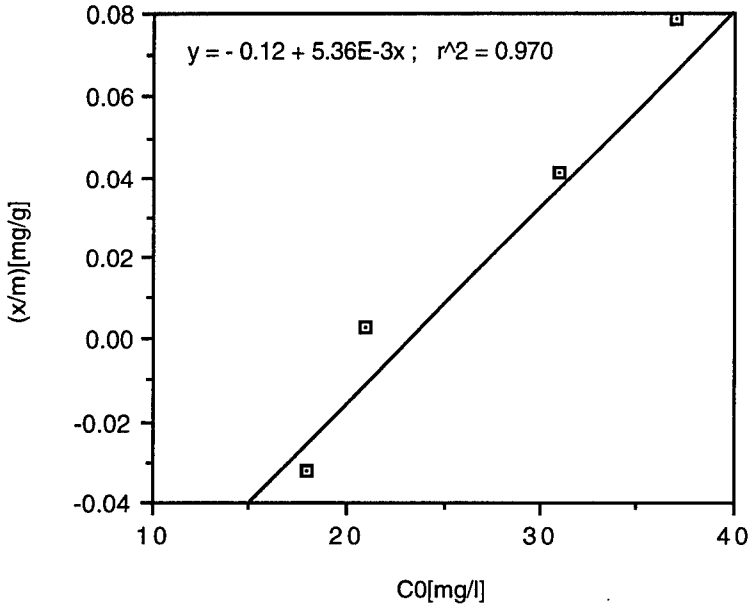
O - Xylene - ID#3



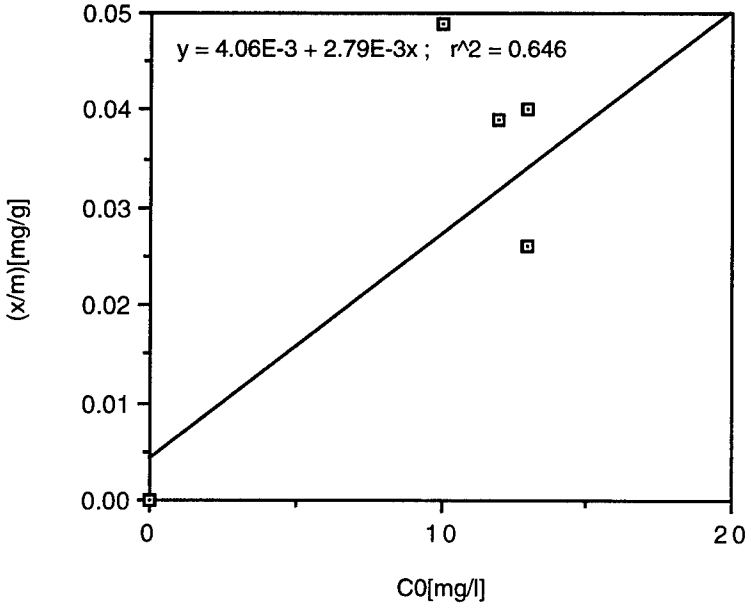
ERthjylbenzene - ID#4



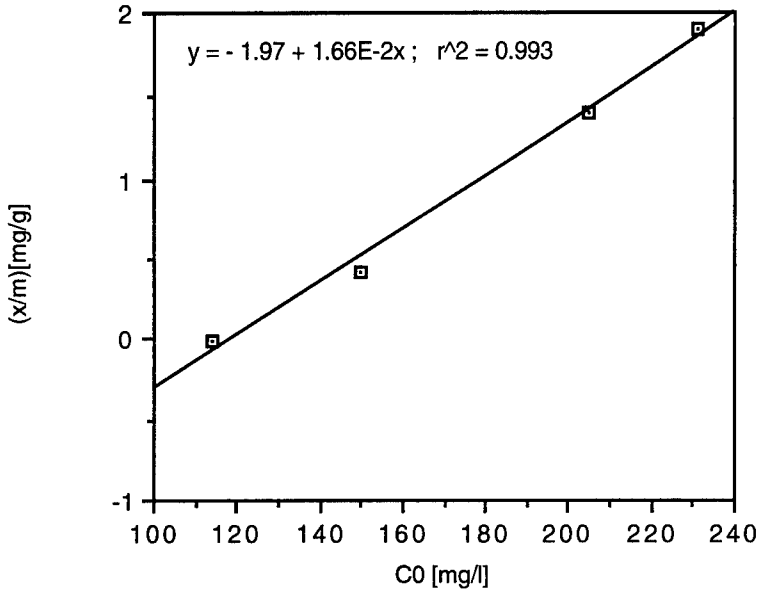
Chlorobenzene - ID#5



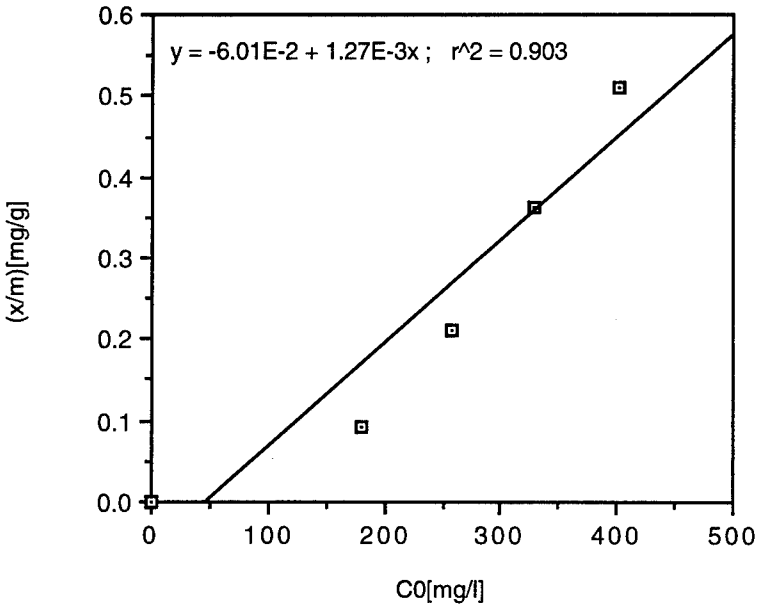
1,2 Dichlorobenzene - ID#6



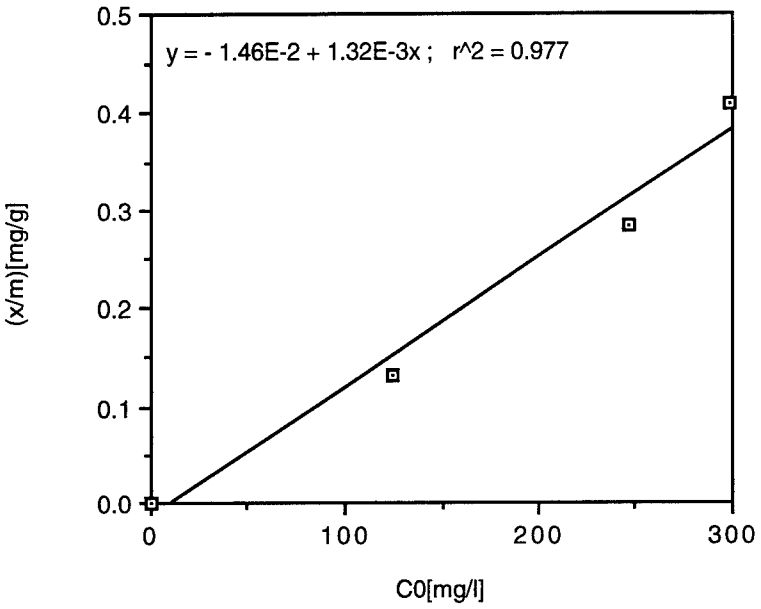
Dichloromethane - ID#10



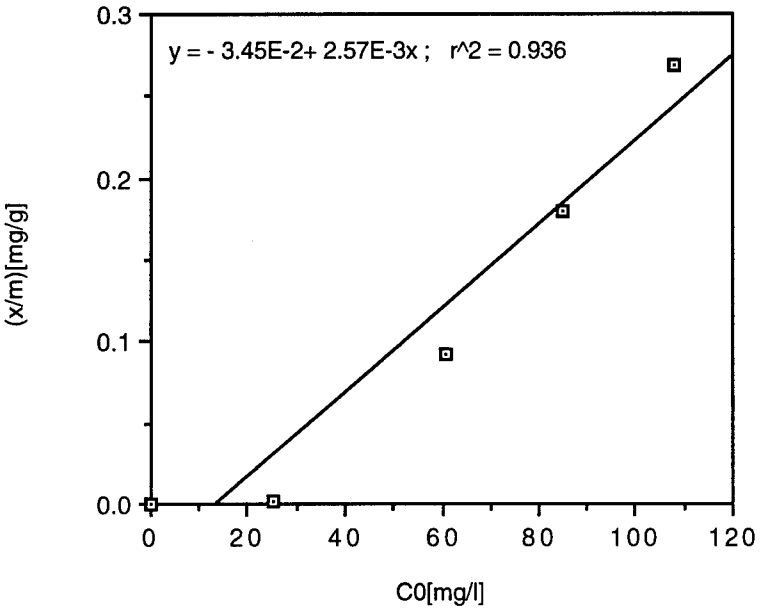
Dibromomethane - ID#11



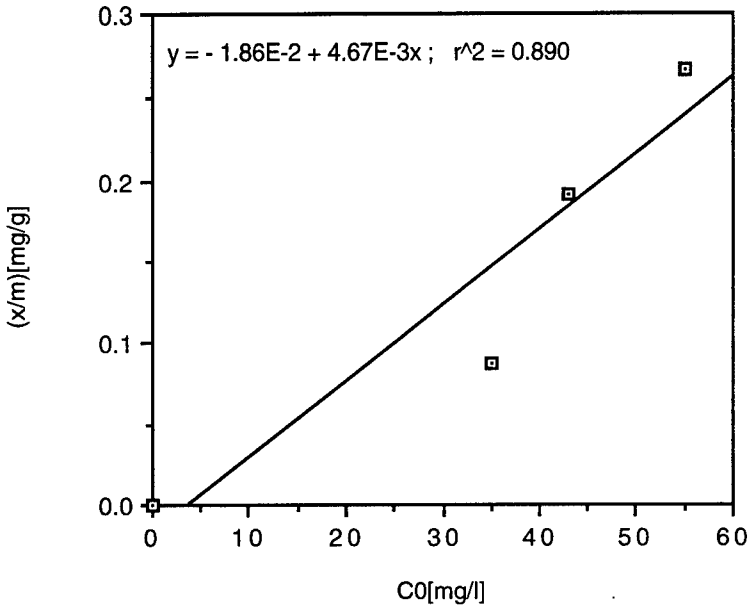
1,2 Dichloroethane - ID#13



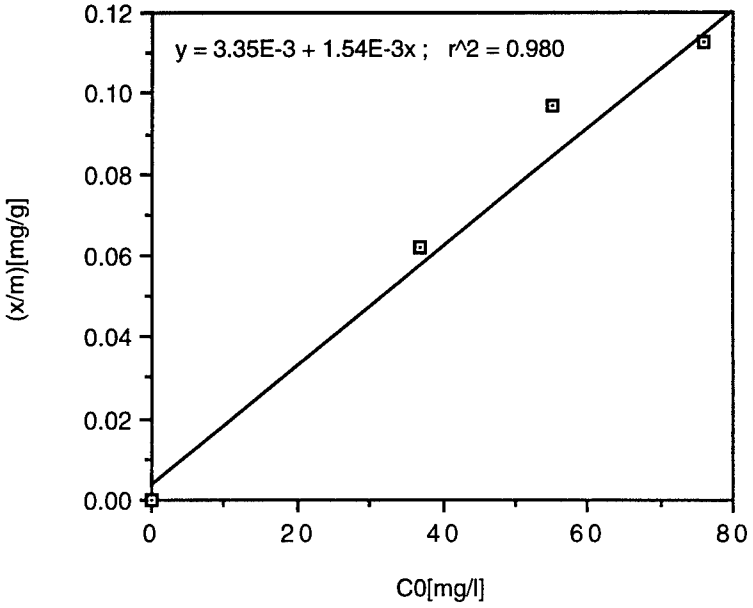
1,1,1 Trichloroethane - ID#14



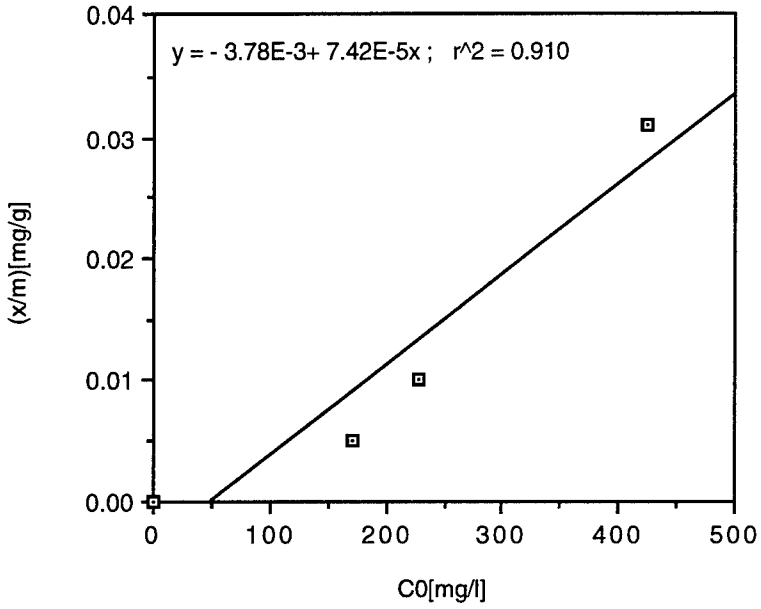
1,1,2,2 Tetrachloroethane - ID#15



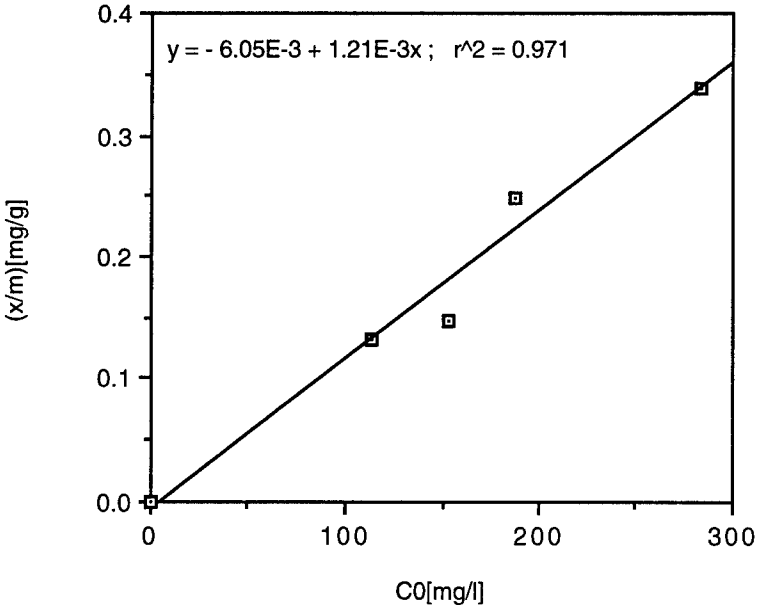
1,2 Dichloropropane - ID#16



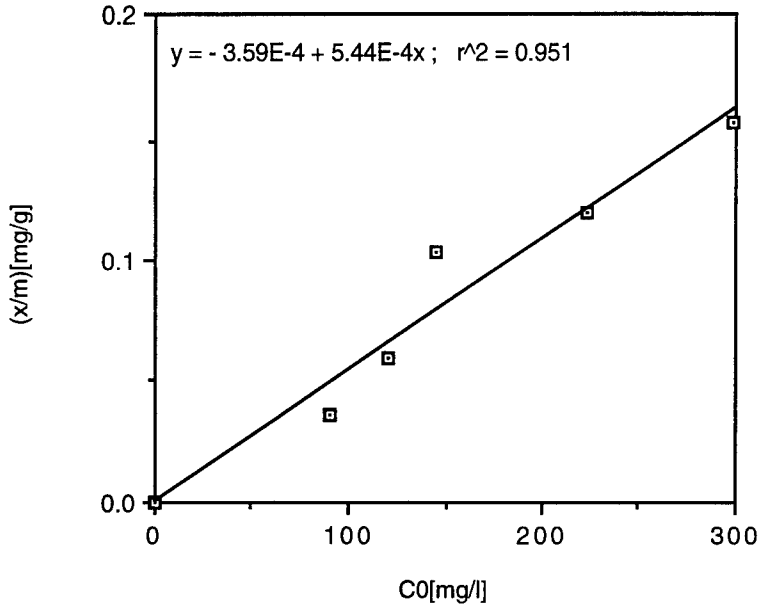
Bromodichloromethane - ID#18



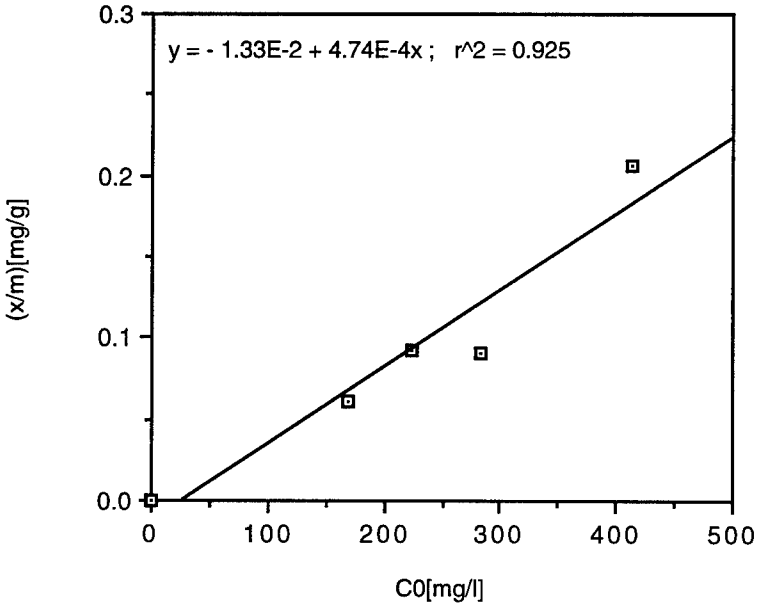
Chlorodibromomethane - ID#19



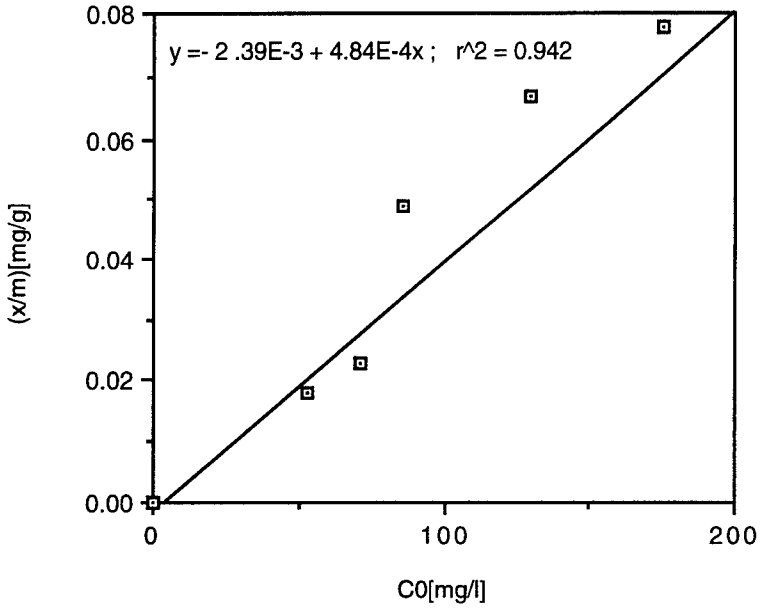
Ethylene dibromide - ID#20



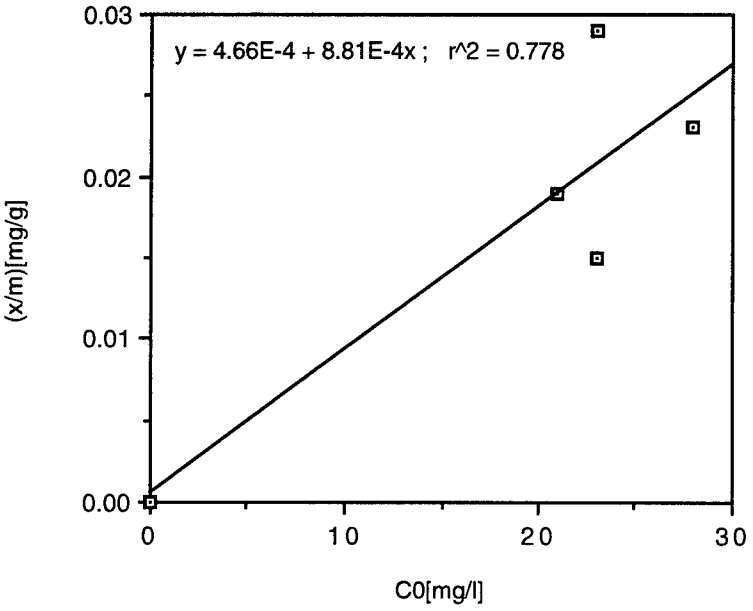
cis 1,2 Dichloroethylene - ID#21



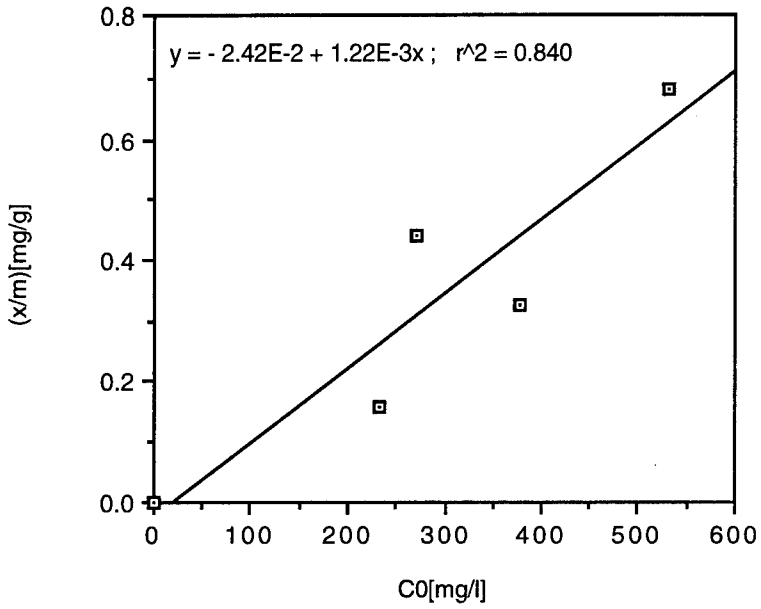
Trichloroethylene - ID#22



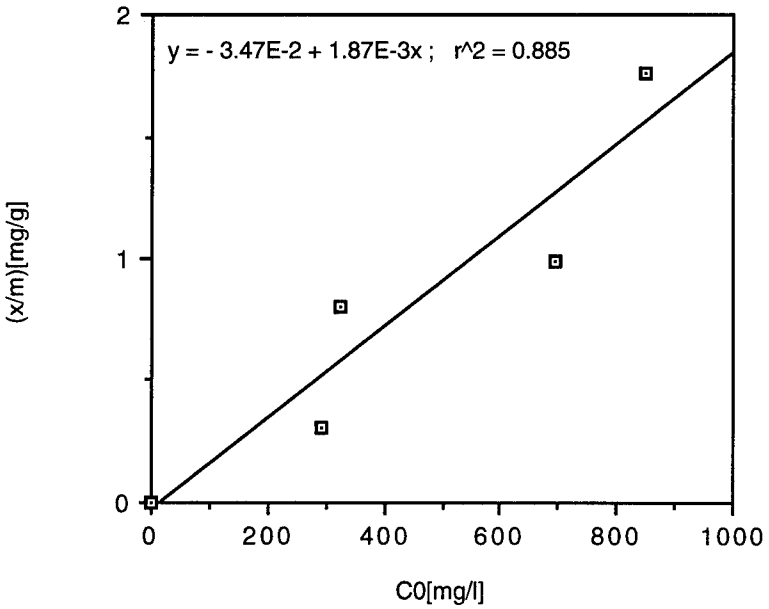
Tetrachloroethylene - ID#23



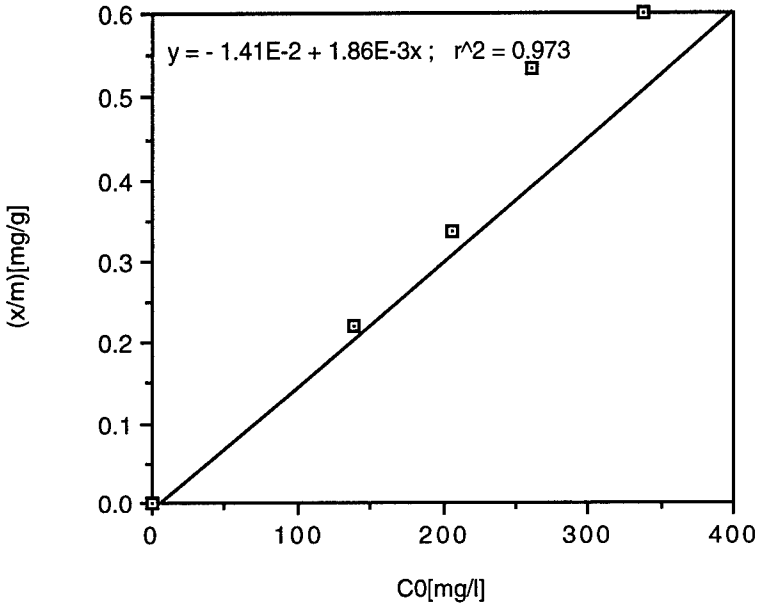
N - Butyl acetate - ID#28



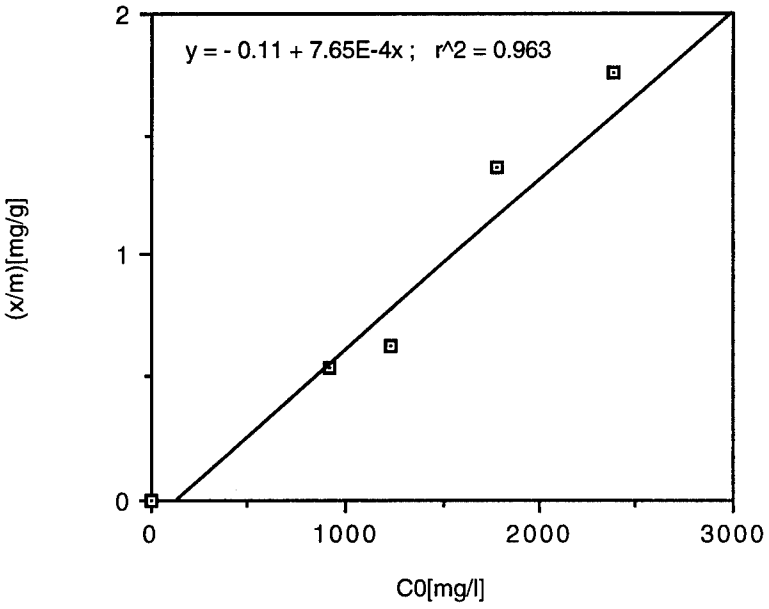
Isobutyl acetate - ID#29



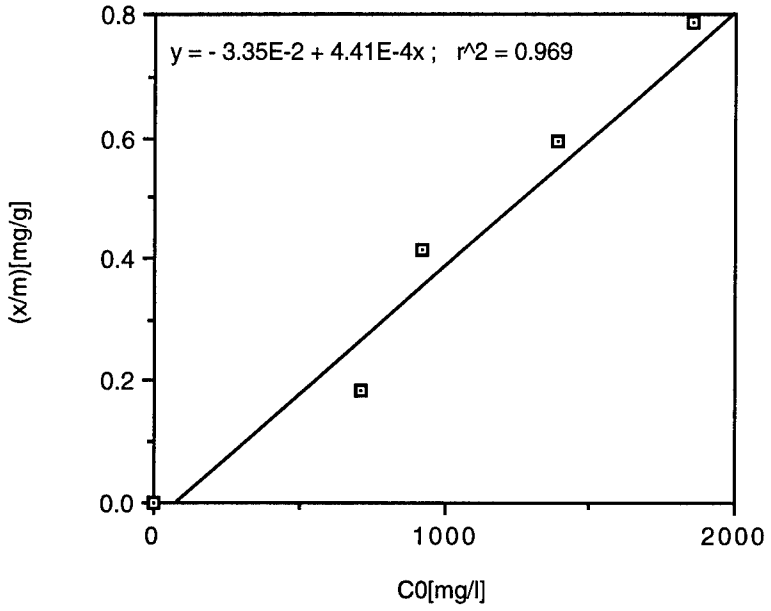
N - Amyl acetate - ID#30



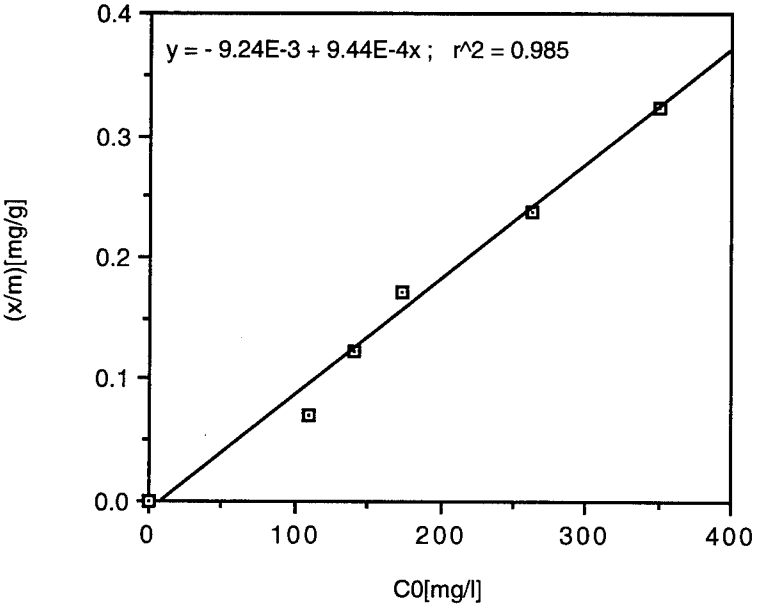
Ethyl acetate - ID#31



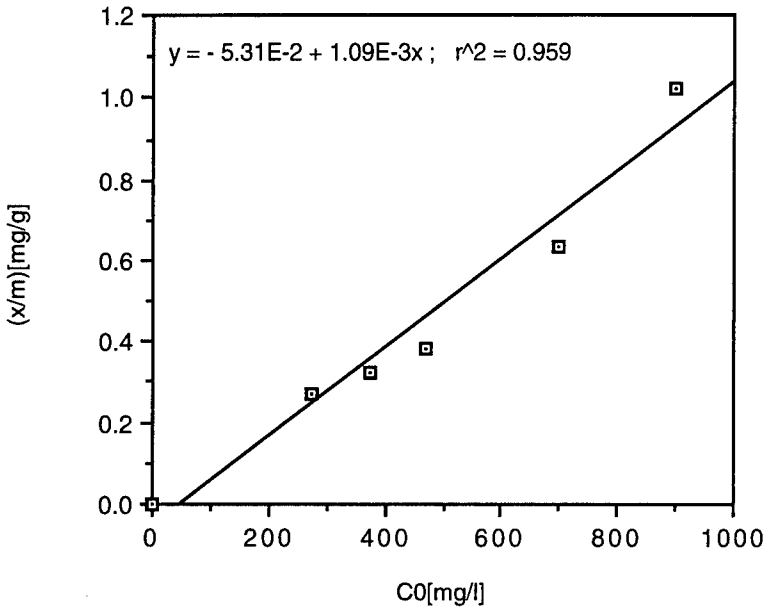
Acetone - ID#32



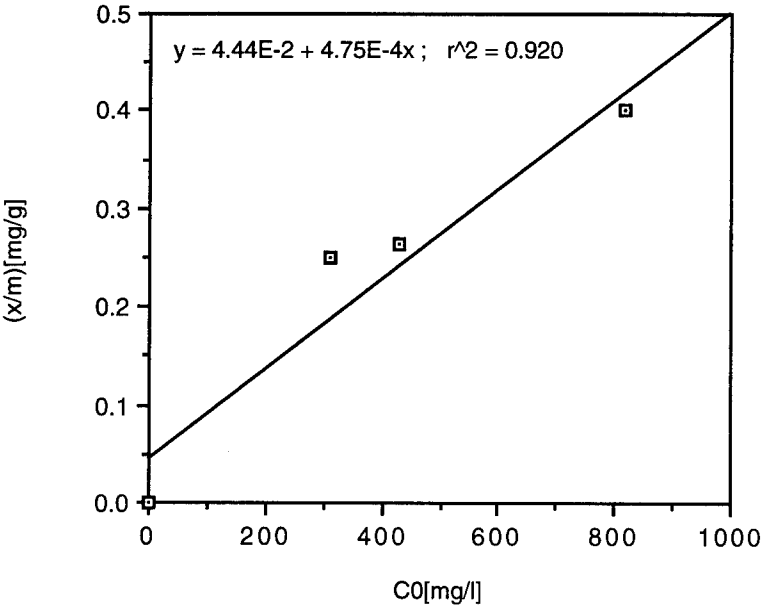
Methyl isobutyl ketone - ID#33



Methyl - N- propyl ketone - ID#34



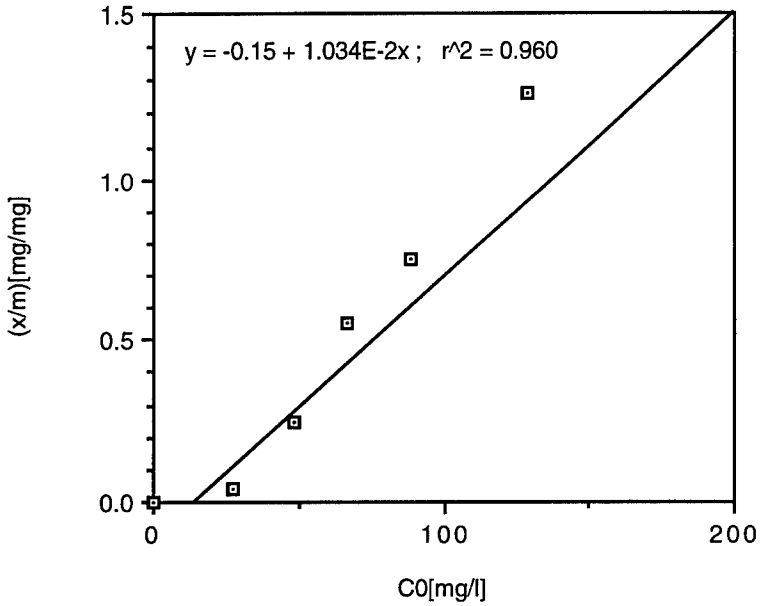
Cyclohexanone - ID#35



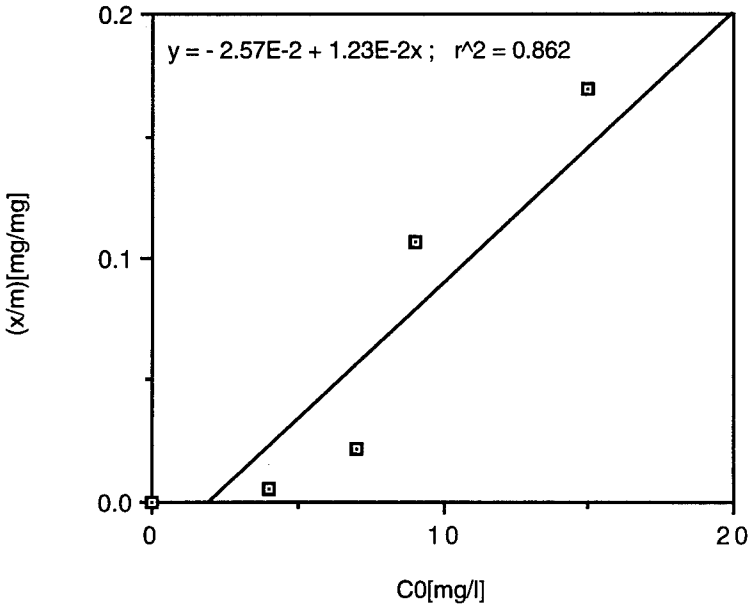
APPENDIX VIII

APPENDIX VIII
Results of biosorption isotherms with Polytox microbial cells

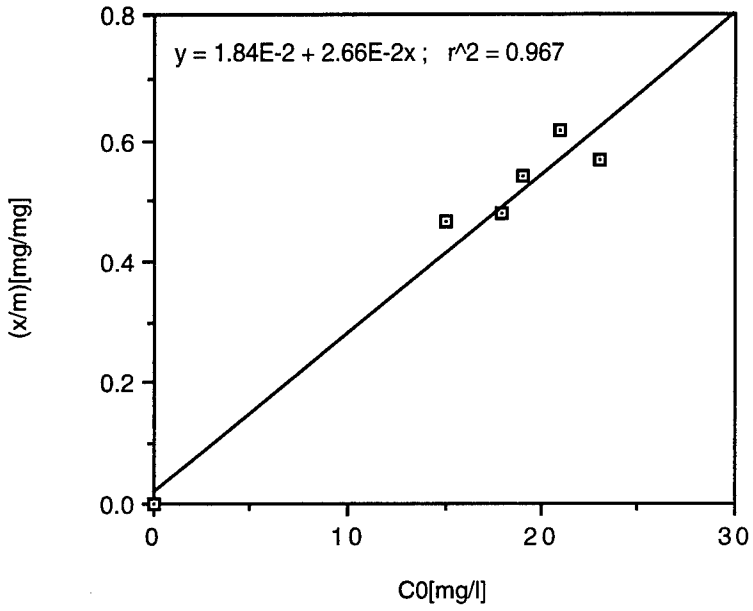
Benzene - ID#1



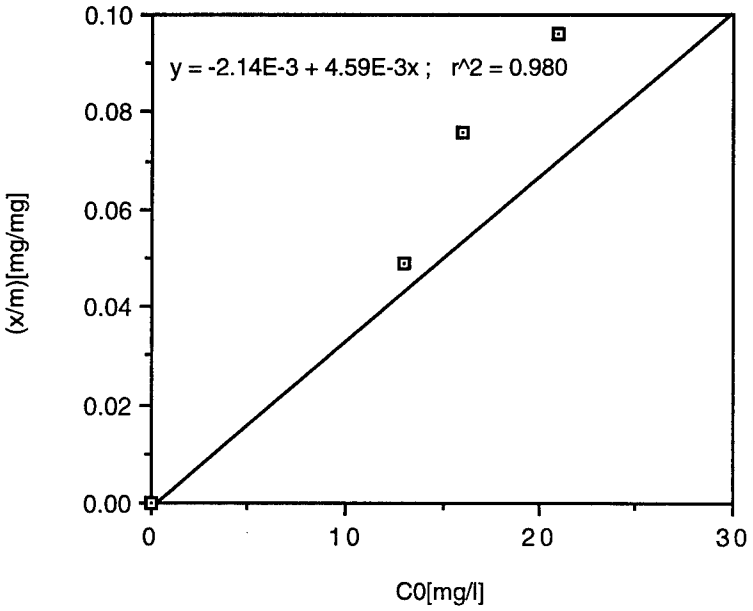
Toluene - ID#2



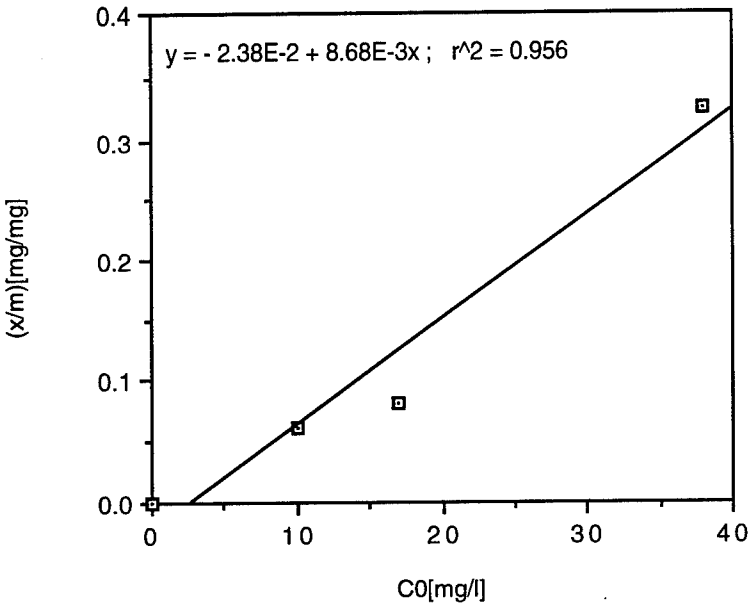
O - Xylene - ID#3



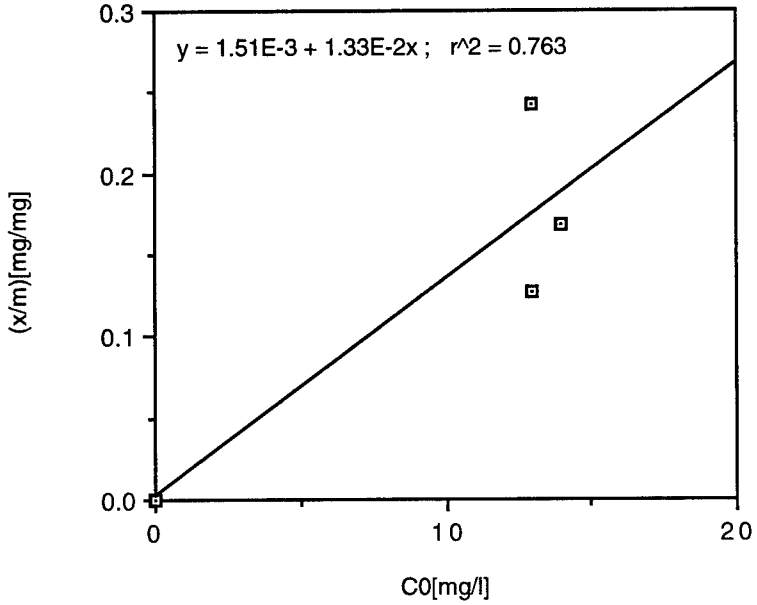
Ethylbenzene - ID#4



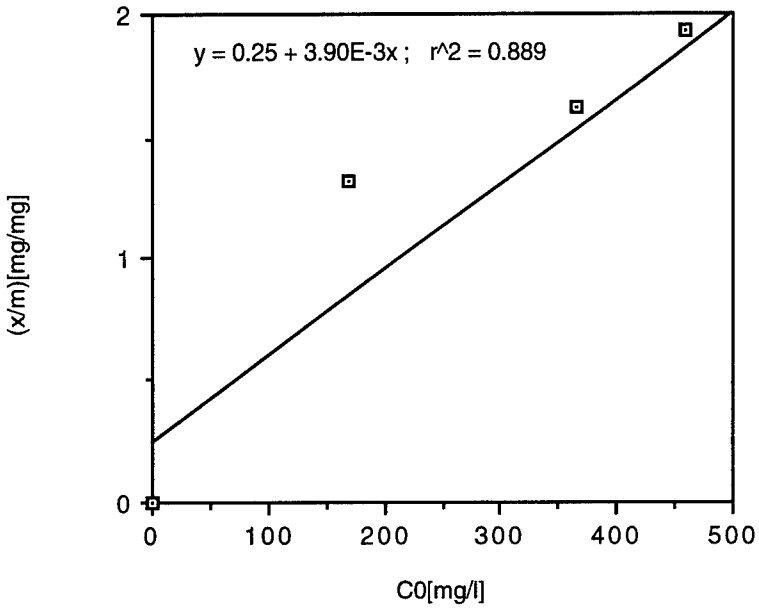
Chlorobenzene - ID#5



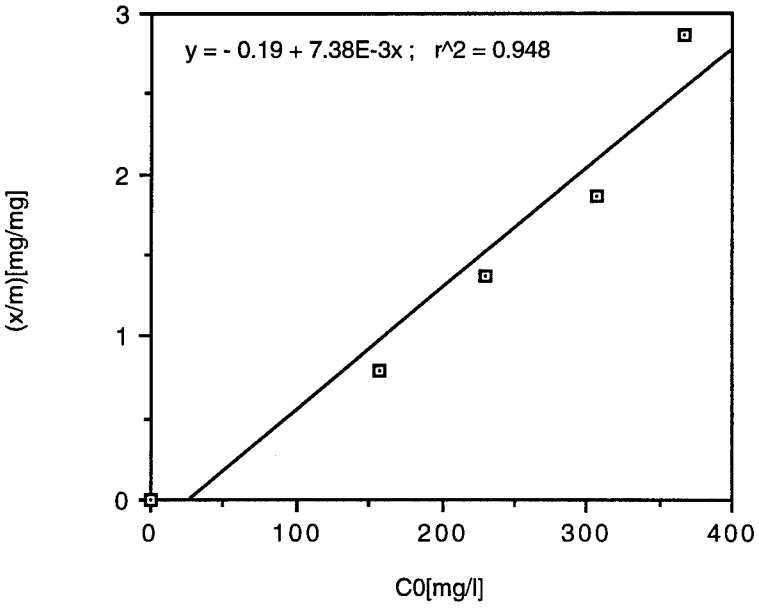
1,2 Dichlorobenzene - ID#6



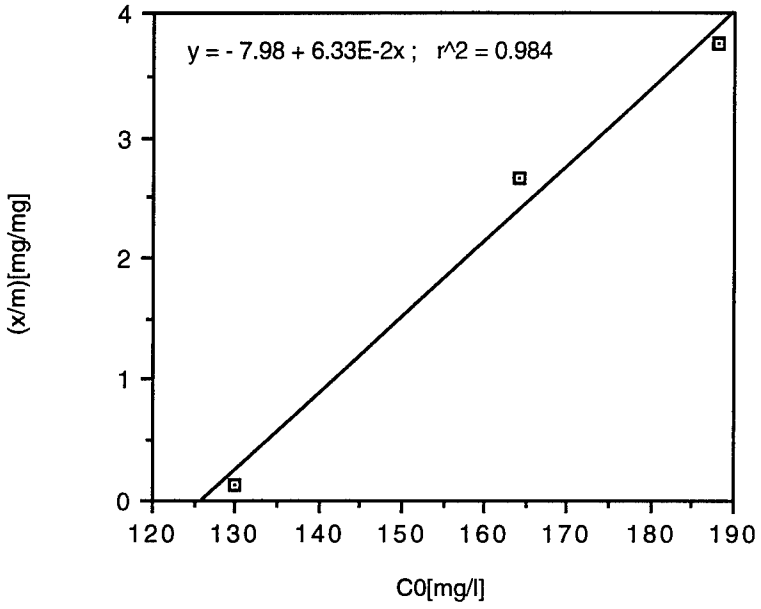
Dichloromethane - ID#10



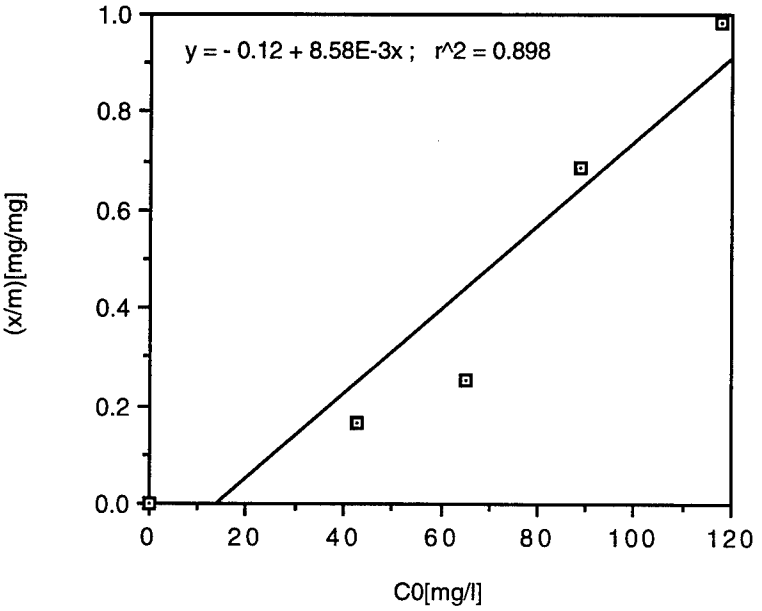
Dibromomethane - ID#11



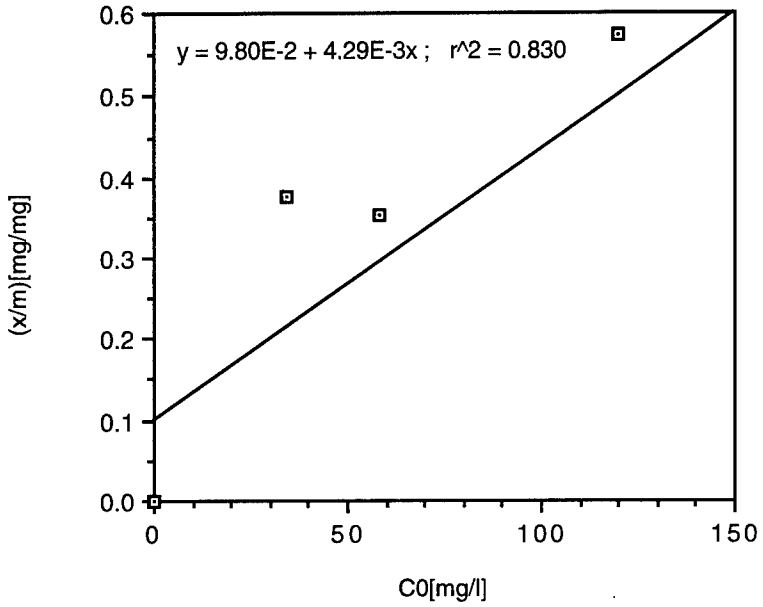
1,2 Dichloroethane - ID#13



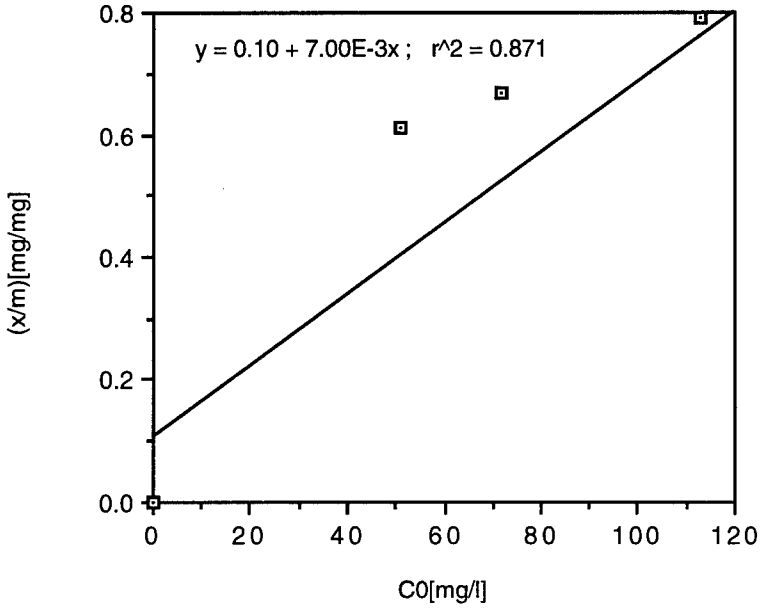
1,1,1 Trichloroethane - ID#14



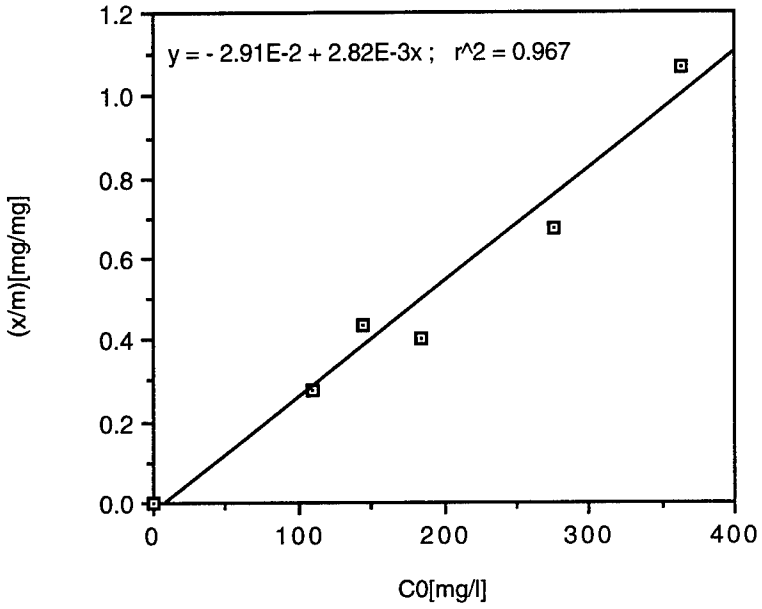
1,1,2,2 Tetrachloroethane - ID#15



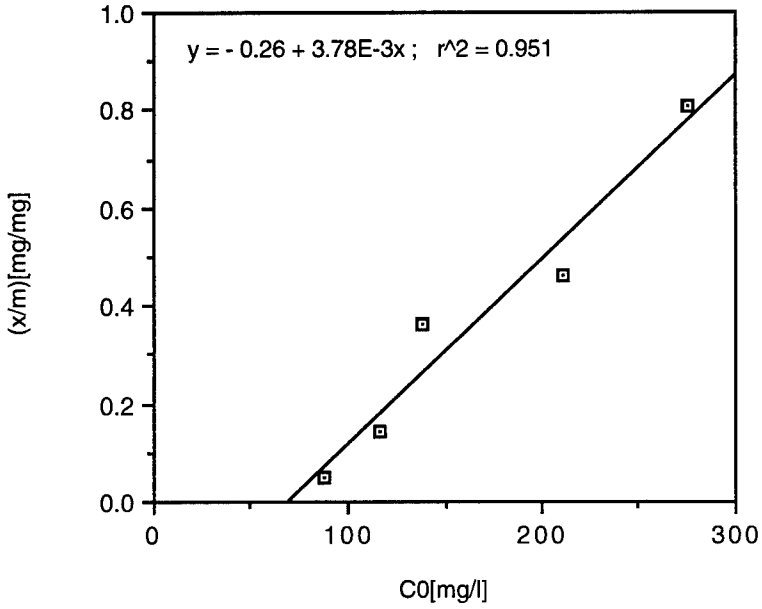
1,2 Dichloropropane - ID#16



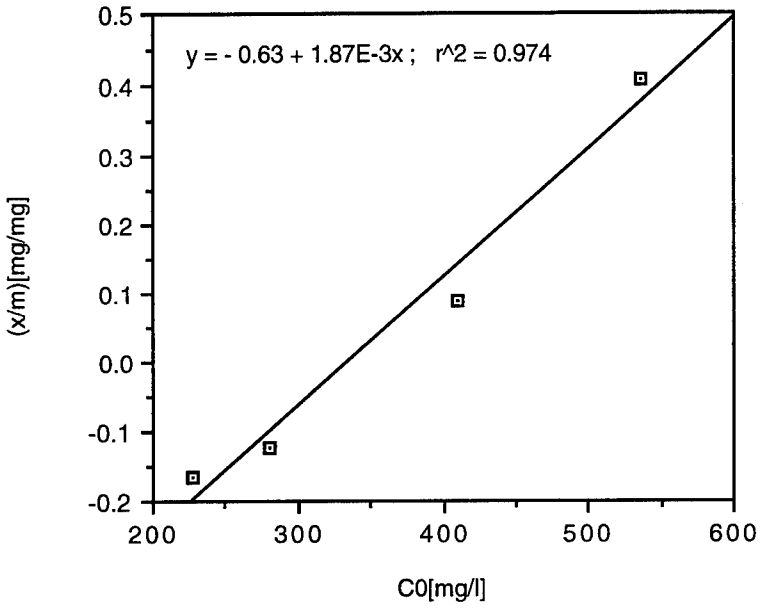
Chlorodibromomethane - ID#19



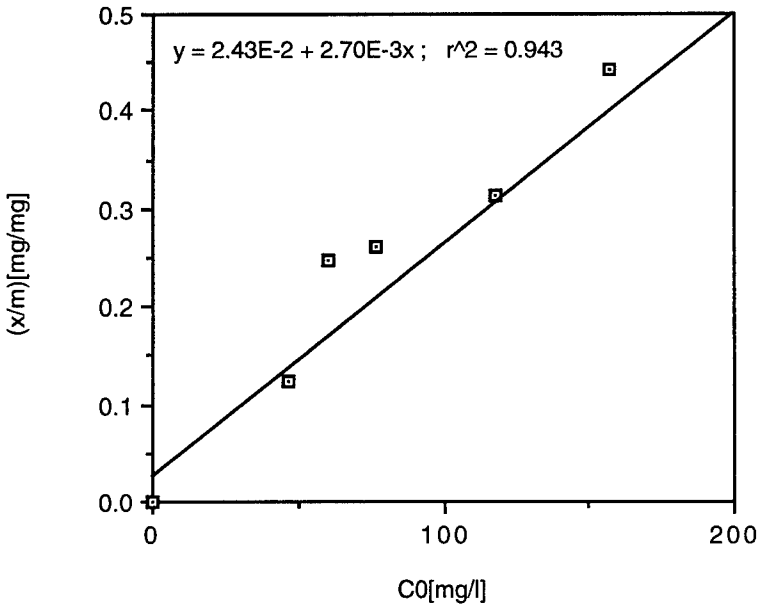
Ethylene dibromide - ID#20



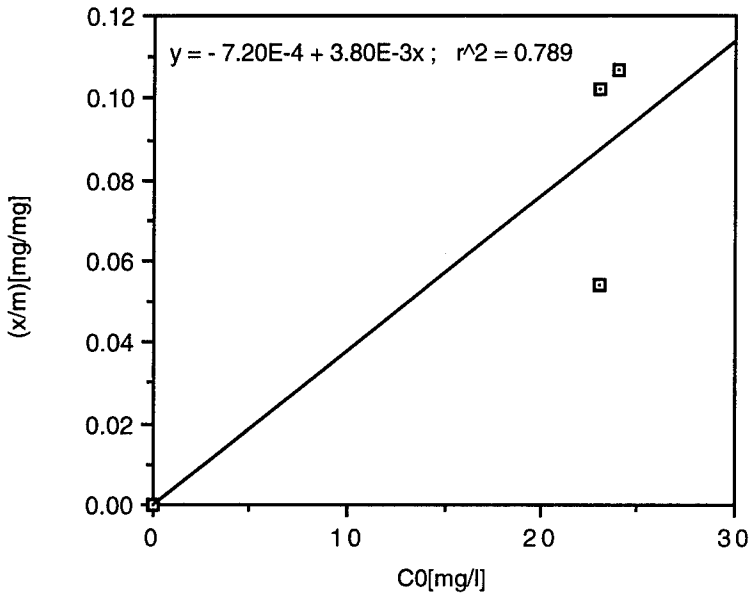
cis 1,2 Dichloroethylene - ID#21



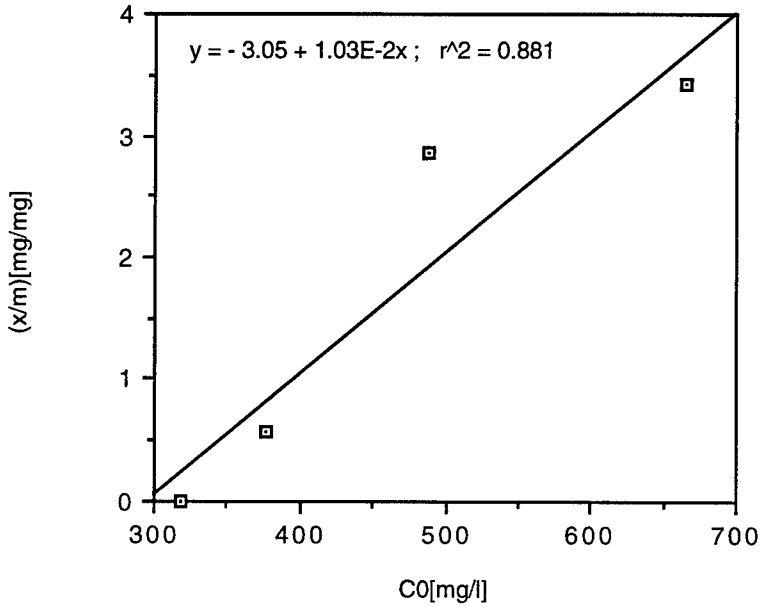
Trichloroethylene - ID#22



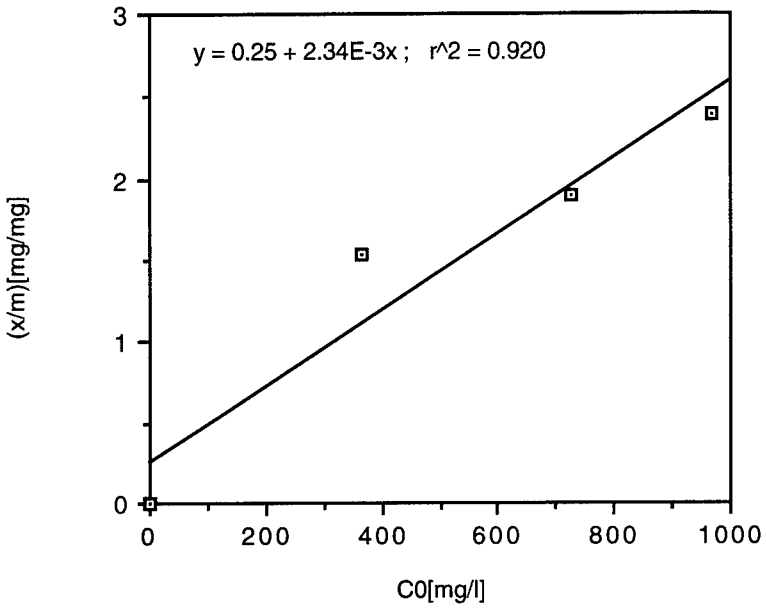
Tetrachloroethylene - ID#23



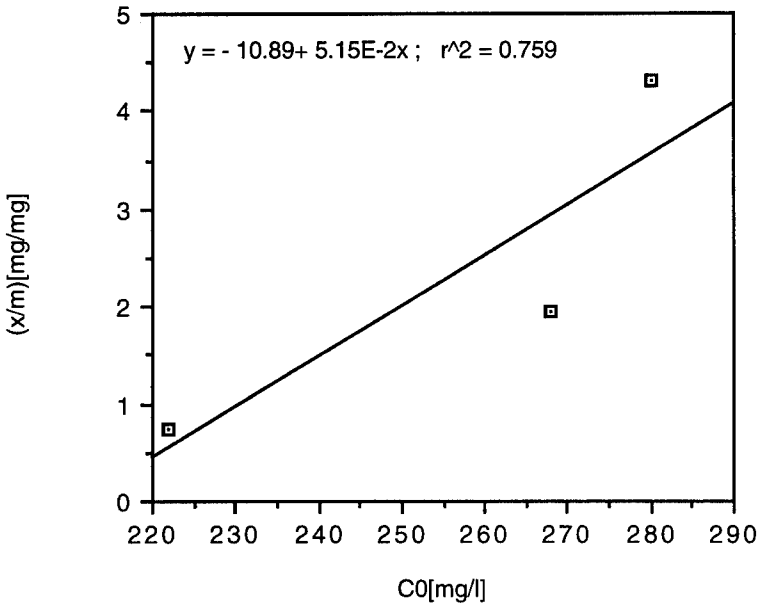
N - Butyl acetate - ID#28



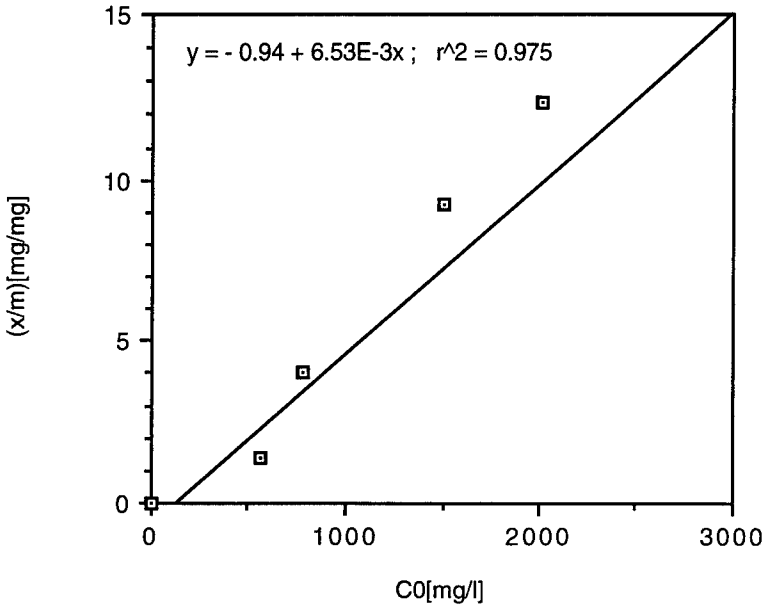
Isobutyl acetate - ID#29



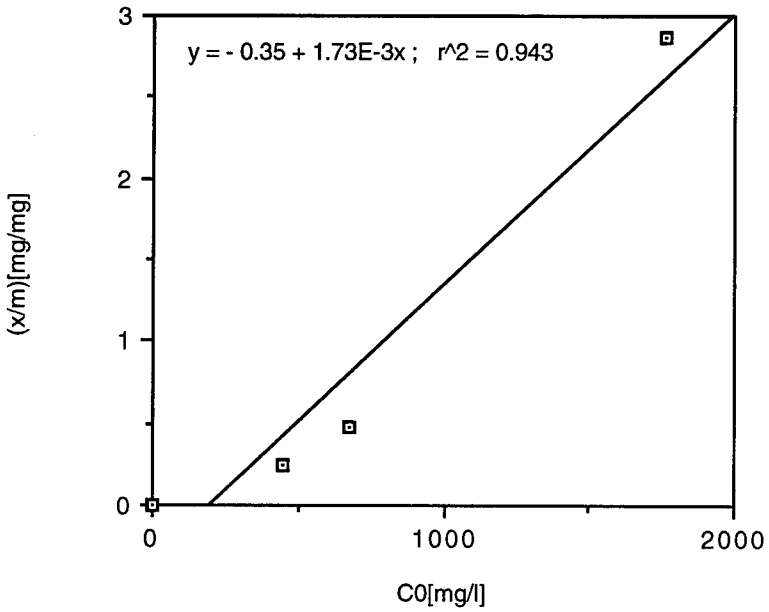
N - Amylacetate - ID#30



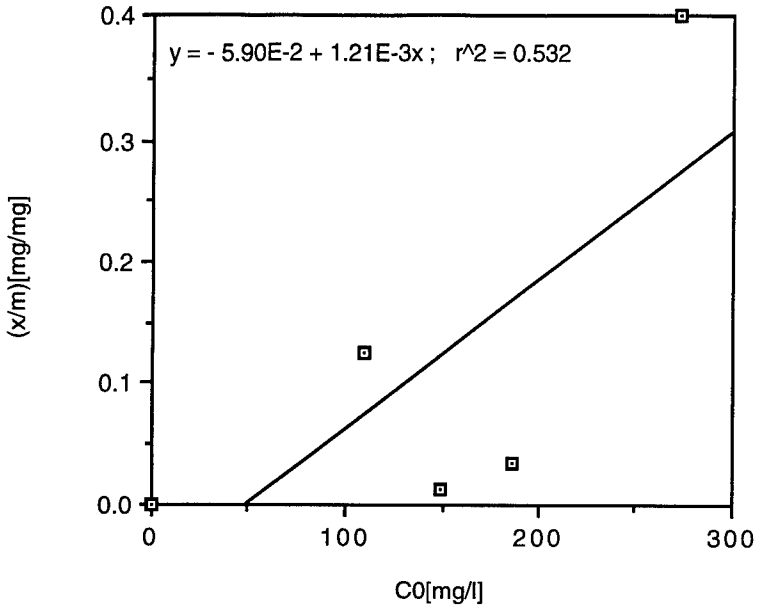
Ethyl acetate - ID#31



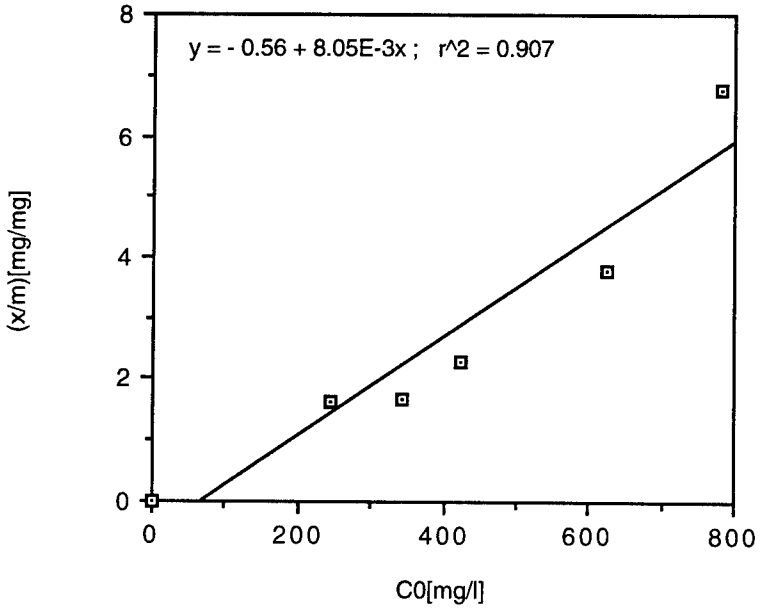
Acetone - ID#32



Methyl isobutyl ketone - ID#33



Methyl - N - propyl ketone - ID#34



APPENDIX IX

Table IX - I. ISOTHERM RESULTS ON ADSORPTION OF CHEMICALS TO SOIL

ID #	Chemical Name	K_d[l/g]	r^2	conf int on n values 95%L	95%U
1	Benzene	0.001	0.929	0.13	1.14
2	Toluene	0.003	0.963	-0.98	4.19
3	O-Xylene	0.001	0.916	-6.00	13.17
4	Ethylbenzene	0.001	0.958	0.68	2.64
5	Chlorobenzene	0.005	0.970	-2.38	13.73
6	1,2 Dichlorobenzene	0.003	0.646	-0.037	0.045
7	1,3 Dichlorobenzene	N/A	N/A	N/A	N/A
8	1,2,4 Trichlorobenzene	N/A	N/A	N/A	N/A
9	2,4 Dimethyl phenol	N/A	N/A	N/A	N/A
10	Dichloromethane	0.017	0.993	0.25	6.82
11	Dibromomethane	0.001	0.903	1.79	2.53
12	Carbontetrachloride	N/A	N/A	N/A	N/A
13	1,2 Dichloroethane	0.001	0.977	-0.82	2.31
14	1,1,1 Trichloroethane	0.003	0.936	1.23	5.56
15	1,1,2,2 Tetrachloroethane	0.005	0.890	-7.50	12.33
16	1,2 Dichloropropane	0.002	0.980	-0.12	1.27
17	Bromochloromethane	N/A	N/A	N/A	N/A
18	Bromodichloromethane	7.42E-5	0.910	2.19	4.93
19	Chlorodibromomethane	0.001	0.971	0.09	2.14
20	Ethylene dibromide	0.001	0.951	0.78	1.67
21	*cis* - 1,2 Dichloroethylene	5.00E-4	0.925	-11.88	22.52
22	Trichloroethylene	4.84E-4	0.942	0.69	2.01
23	Tetrachloroethylene	0.001	0.778	-16.86	27.50
24	Ethanol	N/A	N/A	N/A	N/A
25	Propanol	N/A	N/A	N/A	N/A
26	Pentanol	N/A	N/A	N/A	N/A
27	Octanol	N/A	N/A	N/A	N/A
28	N- Butyl acetate	0.001	0.840	-0.23	4.85
29	Isobutyl acetate	0.002	0.885	-1.45	4.51
30	N- Amyl acetate	0.002	0.973	0.46	1.92
31	Ethyl acetate	0.001	0.963	0.42	2.28
32	Acetone	4.00E-4	0.969	0.04	2.77
33	Methyl isobutyl ketone	0.001	0.985	0.73	1.72
34	Methyl N- propyl ketone	0.001	0.959	0.65	1.58
35	Cyclohexanone	4.75E-4	0.920	-1.01	2.02

Table IX - II. ISOTHERM RESULTS ON BIOSORPTION OF CHEMICALS TO MICROBIAL CELLS

				conf int on n values	
ID #	Chemical Name	K_p[l/mg]	r^2	95%L	95%U
1	Benzene	0.010	0.960	-1.31	3.18
2	Toluene	0.012	0.862	0.18	4.31
3	O-Xylene	0.027	0.967	-0.03	1.11
4	Ethylbenzene	0.005	0.980	-3.85	6.53
5	Chlorobenzene	0.009	0.956	0.21	1.72
6	1,2 Dichlorobenzene	0.013	0.763	-0.259	0.262
7	1,3 Dichlorobenzene	N/A	N/A	N/A	N/A
8	1,2,4 Trichlorobenzene	N/A	N/A	N/A	N/A
9	2,4 Dimethyl phenol	N/A	N/A	N/A	N/A
10	Dichloromethane	0.004	0.889	-0.80	1.51
11	Dibromomethane	0.007	0.948	0.89	2.01
12	Carbontetrachloride	N/A	N/A	N/A	N/A
13	1,2 Dichloroethane	0.063	0.984	-24.58	40.89
14	1,1,1 Trichloroethane	0.009	0.898	0.67	3.15
15	1,1,2,2 Tetrachloroethane	0.004	0.830	-0.15	1.09
16	1,2 Dichloropropane	0.007	0.871	-0.17	0.81
17	Bromochloromethane	N/A	N/A	N/A	N/A
18	Bromodichloromethane	N/A	N/A	N/A	N/A
19	Chlorodibromomethane	0.003	0.967	0.55	1.54
20	Ethylene dibromide	0.004	0.951	1.31	3.35
21	*cis* - 1,2 Dichloroethylene	0.002	0.974	-0.44	4.19
22	Trichloroethylene	0.003	0.943	0.29	1.73
23	Tetrachloroethylene	0.004	0.789	N/A	N/A
24	Ethanol	N/A	N/A	N/A	N/A
25	Propanol	N/A	N/A	N/A	N/A
26	Pentanol	N/A	N/A	N/A	N/A
27	Octanol	N/A	N/A	N/A	N/A
28	N- Butyl acetate	0.010	0.881	-4.59	10.41
29	Isobutyl acetate	0.002	0.920	-5.21	11.08
30	N- Amyl acetate	0.052	0.759	-18.66	32.15
31	Ethyl acetate	0.007	0.975	0.41	3.15
32	Acetone	0.002	0.943	0.74	6.50
33	Methyl isobutyl ketone	0.001	0.532	-0.53	12.09
34	Methyl N- propyl ketone	0.008	0.907	0.85	2.41
35	Cyclohexanone	N/A	N/A	N/A	N/A

Table IX - III. HENRY'S CONSTANTS USED IN THIS STUDY

ID #	Chemical Name	log H Non dimen.	H
1	Benzene	-0.73	0.186
2	Toluene	-0.59	0.257
3	O-Xylene	-0.45	0.355
4	Ethylbenzene	-0.52	0.302
5	Chlorobenzene	-1.02	0.095
6	1,2 Dichlorobenzene	-1.32	0.048
7	1,3 Dichlorobenzene	-1.32	0.048
8	1,2,4 Trichlorobenzene	-1.61	0.025
9	2,4 Dimethyl phenol	-1.22	0.060
10	Dichloromethane	-0.74	0.182
11	Dibromomethane	-1.37	0.043
12	Carbontetrachloride	0.06	1.148
13	1,2 Dichloroethane	-0.64	0.229
14	1,1,1 Trichloroethane	-0.76	0.174
15	1,1,2,2 Tetrachloroethane	-1.18	0.066
16	1,2 Dichloropropane	-0.47	0.339
17	Bromochloromethane	-1.07	0.085
18	Bromodichloromethane	-1.25	0.056
19	Chlorodibromomethane	-1.51	0.031
20	Ethylene dibromide	-0.82	0.151
21	*cis* - 1,2 Dichloroethylene	-1.92	0.012
22	Trichloroethylene	-1.32	0.048
23	Tetrachloroethylene	-0.34	0.457
24	Ethanol	-3.59	2.57E-4
25	Propanol	-3.49	3.24E-4
26	Pentanol	-3.29	0.001
27	Octanol	-2.99	0.001
28	N- Butyl acetate	-1.81	0.015
29	Isobutyl acetate	-1.74	0.018
30	N- Amyl acetate	-1.71	0.019
31	Ethyl acetate	-2.01	0.010
32	Acetone	-1.21	0.062
33	Methyl isobutyl ketone	-0.85	0.141
34	Methyl N- propyl ketone	-1.02	0.095
35	Cyclohexanone	-1.47	0.034

Table IX - IV. AQUEOUS SOLUBILITIES USED IN THIS STUDY

ID #	Chemical Name	Solubility (mg/l)
1	Benzene	1782
2	Toluene	515
3	O-Xylene	175
4	Ethylbenzene	152
5	Chlorobenzene	497
6	1,2 Dichlorobenzene	92
7	1,3 Dichlorobenzene	124
8	1,2,4 Trichlorobenzene	30
9	2,4 Dimethyl phenol	3296
10	Dichloromethane	13032
11	Dibromomethane	11429
12	Carbontetrachloride	791
13	1,2 Dichloroethane	8610
14	1,1,1 Trichloroethane	1500
15	1,1,2,2 Tetrachloroethane	2958
16	1,2 Dichloropropane	2799
17	Bromochloromethane	14791
18	Bromodichloromethane	3357
19	Chlorodibromomethane	2438
20	Ethylene dibromide	1901
21	*cis* - 1,2 Dichloroethylene	3499
22	Trichloroethylene	1099
23	Tetrachloroethylene	150
24	Ethanol	576766
25	Propanol	254683
26	Pentanol	21577
27	Octanol	555
28	N- Butyl acetate	5058
29	Isobutyl acetate	6714
30	N- Amyl acetate	2061
31	Ethyl acetate	78343
32	Acetone	58076
33	Methyl isobutyl ketone	2371
34	Methyl N- propyl ketone	6281
35	Cyclohexanone	6902