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PHYSICOCHEMICAL PROPERTIES AS PREDICTORS OF ORGANIC CHEMICAL EFFECTS ON SOIL MICROBIAL RESPIRATION

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Abstract—Structure-activity analysis was used to evaluate the effects of 19 hazardous organic chemicals on microbial respiration in two slightly acidic soils (a Captina silt loam from Roane County, Tennessee, and a McLaurin sandy loam from Stone County, Mississippi), both low in organic carbon and typical of the southeastern United States. Single additions of individual chemicals were applied at 1,000 μg/g (dry weight) soil, which was moistened to 80% base saturation and incubated in the dark at 20°C. CO_2 efflux from the soils was monitored at 24-h intervals over a 6-d period. The chemicals included in the study were the following: acrylonitrile, furan, methyl ethyl ketone, tetrahydrofuran, benzene, toluene, 1,2-dichloroethane, *p*-xylene, chlorobenzene, chloroform, nitrobenzene, *trans*-1,4-dichloro-2-butene, *cis*-1,4-dichloro-2-butene, 1,2-dichlorobenzene, 1,2,3-trichloropropane, carbon tetrachloride, 1,2-dibromoethane (ethylene dibromide), 1,2,4,5-tetrachlorobenzene and hexachlorobenzene.

Seventeen of the chemicals caused temporary depressions in CO_2 efflux in at least one soil, but treatments were not significantly different from controls by the sixth day for most of these compounds, indicating the ability of the microbial community to recover from chemically induced toxicity at this concentration. Acrylonitrile, nitrobenzene, and the 1,4-dichloro-2-butenes caused sustained depressions, whereas methyl ethyl ketone and benzene increased respiration.

The octanol/water partition coefficient (K_{ow}) and molecular connectivity ($^1\chi$) gave good correlations with effects on soil respiration for a subset of benzene and the six alkyl- and chloro-substituted derivatives (i.e., toluene, *p*-xylene, chlorobenzene, 1,2-dichlorobenzene, 1,2,4,5-tetrachlorobenzene and hexachlorobenzene).

Keywords—Structure-activity relationships Soil Microorganisms Volatile organics Semivolatile organics

INTRODUCTION

Structure-activity analyses of chemicals have been enormously successful for the synthesis of drugs and agricultural chemicals to achieve maximum potency and product safety. In addition, systematic structure-activity analyses have often led to insights into mechanisms of action for biologically active molecules. These successes for drug and pesticide design promise broader applications for structure-activity relationships (SARs) in predictive ecotoxicology, wherein ultimate effects and fate are also related, in part, to the inherent toxicity of molecules and their persistence in the environment. However, the complexity of natural systems is immense and can discourage the use of SARs to predict chemical effects on populations or communities of organisms and biodegradation rates. Nonetheless, the need for this predictability is great, and much can be learned from determining what kinds of relationships hold for specific chemicals in simple systems, as well as the situations under which SARs break down.

A considerable database exists on biological degradation and structure-activity analysis of or-

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9475

ganic contaminants in aquatic systems and sludges. Many of these studies show biodegradation to be negatively correlated with K_{ow} [1–4] as well as with other physiocochemical parameters. For example, Wolfe and co-workers [5] had success correlating second-order microbial degradation rate constants (for six chemicals in natural waters) with second-order alkaline hydrolysis rate constants. In a highly ambitious study using data on the biochemical oxygen demand for the degradation of 287 chemicals, Niemi et al. [6] found molecular connectivity indices and K_{ow} to be useful structural features for predicting the relative degradability of organic chemicals in water.

In contrast to the many studies in aquatic systems, very few attempts have been made to develop SARs for either biodegradation or microbial effects of organic chemicals in soils. Alexander and Aleem [7] showed that microbial degradation of some aromatic herbicides in soil suspensions was influenced by the aliphatic side chain and the position of halogen on the ring. In addition, Alexander and Lustigman [8] reported that chloro- and nitro- groups enhanced the persistence of substituted benzenes; however, quantitative SARs were not attempted in either of these studies. Although soil degradation of propyzamide and nine of its analogues has been quantitatively correlated with substituent constants, chemical rather than biological degradation predominated in this system [9].

We measured the effects of 19 hazardous organic chemicals on soil microbial respiration in the laboratory and then analyzed the data for correlations with physicochemical properties of the chemicals by linear regression. CO_2 efflux over a 6-d period served as the index of respiration for treated and untreated soils after a single addition of each chemical. These studies were conducted as a quick, nonspecific measure of the ability of the soil microbial community to withstand chemical applications [10,11] so that subsequent determinations of biological transformation rates could be carried out at nontoxic doses. The data obtained provided an opportunity to compare SARs for chemical effects on microbial respiration in two soils of low carbon content from the southeastern United States.

MATERIALS AND METHODS

Chemicals

The following chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI): acrylonitrile, 1,2-dibromoethane (ethylene dibromide), 1,2-dichloroethane, furan, 1,2,3-trichloropropane, and *p*-xylene (all of 99+% purity); 1,2,4,5-tetrachlorobenzene (98% purity); hexachlorobenzene (97% purity); *cis*-1,4-dichloro-2-butene (95% purity); and *trans*-1,4-dichloro-2-butene (85% purity). Fisher Scientific (Pittsburgh, PA) provided certification-grade carbon tetrachloride, chlorobenzene, methyl ethyl ketone, and tetrahydrofuran. Additional suppliers were Eastman Kodak Company (Rochester, NY), for nitrobenzene (reagent-grade) and dichlorobenzene (99% pure); Mallinckrodt Company (Paris, KY), for toluene and benzene (analytical reagent-grade); and E M Science (Cherry Hill, NJ), for chloroform (99% pure).

Soils

Two soils were evaluated for chemical effects on soil microbial respiration. A Captina silt loam (Typic Fragiudult) collected from the Oak Ridge National Laboratory (ORNL) Reservation in Roane County, Tennessee, was obtained from a moderately well-drained, grassy field. The collection site, known as the 0800 area at ORNL, is flat to gently rolling bottomland of the Clinch River. Alluvial soils dominate in this area, which has been maintained in an old field successional state by occasional mowing since 1942. Before 1942, the land was used for pasture and for cultivated field crops [see ref. 12 for a detailed site description]. The soil was removed to a depth of approximately 15 cm and sieved through a gasoline-powered mechanical shaker to break up large clods and remove stones and roots before the soil was transported to the greenhouse.

A McLaurin sandy loam (Typic Paleudults) collected from Stone County, Mississippi, on the western edge of the town of Wiggins, was provided by Dr. Gary McGinnis, Mississippi State University. This soil was taken from an upland ridge with a 5% slope after scraping weeds and grasses from the surface. The soil was collected from the top 15 cm with a backhoe, packed in a metal drum, and shipped to Oak Ridge.

Both soils were immediately coarse sieved (6.3 mm) and air dried in the greenhouse, then stored in the dark at 4°C until needed. Before use, soils were fine sieved (2.0 mm) and moistened with distilled, deionized water to 80% base saturation (i.e., 190 μl/g Captina silt loam and 92 μl/g McLaurin sandy loam). No nutrient amendments were added to the soils before or during the experiments.

Soil properties were measured using standard procedures: soil saturation, pH, and sulfur were determined according to the method of Page [13]; particle size distribution was measured gravimetri-

cally. Total phosphorus and total nitrogen were analyzed according to Methods 365.4 and 351.2, respectively, of the U.S. Environmental Protection Agency [14], with slight modifications appropriate for soil samples. Cation-exchange capacities of the soils were determined using the method described by Johnson et al. [15].

Total organic carbon in the soils was measured as the amount of dissolved organic carbon plus the amount in the solid sample according to the following procedure. In the first step, carbonates were removed by the addition of 20 ml 10% (by volume) H_3PO_4 to 5-g samples in 50-ml centrifuge tubes. The tubes were vortex-mixed every 15 min and allowed to settle. This step was repeated four times. The samples were centrifuged until a clear supernatant was achieved. The supernatant was decanted repeatedly with washings of carbon-free water. The washings were combined and brought to 100 ml total volume, sparged with nitrogen gas to remove dissolved carbon dioxide, and analyzed on a Dorhman carbon analyzer using the liquid sample capability (Xertex/Dorhman, Santa Clara, CA). The remaining soil was removed from the centrifuge tubes, dried in polytetrafluoroethylene dishes, then pulverized by mortar and pestle. Triplicate subsamples were analyzed for remaining carbon on a Leco WR-12 solid sample analyzer (Leco Corporation, St. Joseph, MI).

Soil microbial respiration

Individual chemicals were added directly as undiluted liquid or solid near the surface of 50-g soil samples contained in stoppered, 8 × 5-cm glass jars at a concentration of 1,000 μg/g soil (dry weight). Soils were moistened with distilled, deionized water to 80% base saturation (as specified above) before addition of test chemicals; however, soils were not mixed after addition of the chemicals so that increased losses from volatilization could be avoided. Triplicate treatments with matched controls for each chemical and soil were incubated at 20 ± 0.4°C in the dark. CO_2 efflux was measured on an infrared gas analyzer (Model 300, Mine Safety Appliances Co., Pittsburgh, PA) at 24-h intervals over a 7-d incubation period by purging the headspace with moist, CO_2-free air [16].

Initially, day 4 respiration data were selected for SAR analysis based on the observation that many chemicals showed a delayed effect on microbial respiration, yet respiration effects were not different from control conditions by the end of the experiment (day 6). Thus, day 4 data provided enough time for the effect to be exerted, but not so much time that the effect disappeared. These data were expressed two ways for each chemical: (a) as the difference between treatments and matched controls on day 4 (i.e., CO_2 EFFLUX$_{control}$ − CO_2 EFFLUX$_{treatment}$) and (b) as the $\log_{10}$ of the ratio of CO_2 efflux in the treatment to that of the control, that is,

respiratory effect

$$= \log_{10}\left(\frac{(CO_2\ \mathrm{EFFLUX}_{treatment}}{CO_2\ \mathrm{EFFLUX}_{control}}\right) \quad (1)$$

This transformation permitted log-linear relationships between biological activity and chemical structure to be examined, a process that could not be performed for the data set based on differences because of the presence of negative numbers.

Physicochemical and toxicological properties

Chemical-specific data on water solubility, vapor pressure, Henry's law constant, octanol/water partition coefficient and acute oral rat toxicity were compiled from refereed citations in the National Library of Medicine Hazardous Substance Data Bank and supplemented by searches of other sources. The ranges of these parameters for the test chemicals are shown in Table 1. In the few instances in which data were not found in the literature, estimates were made according to methods in Lyman et al. [17]; namely, partition coefficients (K_{ow}) were obtained using fragment constants, and Henry's law constants were calculated from the equation:

$$H = P_{vp}/S \quad (2)$$

where P_{vp} is vapor pressure in torr, S is aqueous solubility in moles per liter, and H is in torr per mole per liter.

Table 1. Property ranges for test chemicals used in SAR analyses

Property	Range
$\mathrm{Log}_{10}K_{ow}$	0.12–6.35
Vapor pressure (torr)	1.09×10^{-5}–700 at 20°C
Water solubility (M)	1.09×10^{-6}–4.17
Henry's constant (torr/M)	9.7–1.8×10^{4}
Rat oral LD50 (mmol/kg)	0.047–63

RESULTS

Soil characterization and soil microbial respiration

Although the soils used in these experiments were both slightly acidic and low in organic carbon, they differed in a number of important ways (Table 2). Notably, the Captina silt loam had a higher organic carbon content (1.49 ± 0.06%) than the McLaurin sandy loam (0.66 ± 0.04%), as well as a higher cation-exchange capacity (10.15 ± 0.44 meq/100 g silt loam vs. 1.15 ± 0.17 meq/100 g sandy loam). Most cations extractable in NH_4NO_3 and NH_4Cl were more abundant in the silt loam; however, the sandy loam was richer in nitrogen, phosphorus and sulfur. A more detailed account of the exchangeable cations has been published elsewhere [18].

The bulk density, the ratio of the mass to the macroscopic volume (bulk) of soil particles plus pore spaces, was lower under experimental conditions for the Captina silt loam (0.89 ± 0.005 g/ml) than for the McLaurin sandy loam (1.28 ± 0.034 g/ml).

Overall, there was a higher microbial respiration rate for the soil with the higher organic carbon content (i.e., the Captina silt loam, with organic carbon content of 1.49%, had a higher rate than the McLaurin sandy loam, with organic carbon content of 0.66%). Control soils of both types showed a gradual decline in CO_2 efflux over the 7-d incubation period (Fig. 1), that is, 50% decline for the Captina silt loam and 39% decline for the McLaurin sandy loam. Twenty-four-hour respiration rates for the sandy loam were approximately one-half those for the silt loam, with the exception of the first day's readings, when respiration in the former soil was typically one-third that of the other (Fig. 1). No statistically significant difference (Student's t test at $p \geq 0.05$) in respiration was observed for controls of either soil type during the months that the experiments were conducted; thus, the rates shown in Figure 1 represent the performance of the controls for the entire study period.

Most chemicals depressed CO_2 efflux initially,

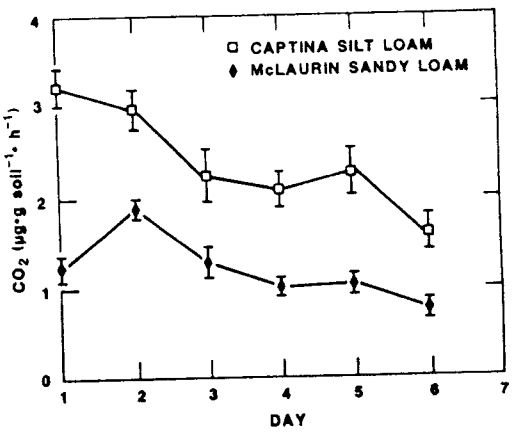

Fig. 1. CO_2 efflux from control soils moistened to 80% saturation and held in the dark at 20 ± 0.4°C (mean of 19 chemical treatments each with triplicate determinations ± 1 SE).

Table 2. Selected physical and chemical properties of test soils

Parameter	Test soil[a]	
	Captina silt loam (Roane County, TN)	McLaurin sandy loam (Stone County, MS)
$pH_{distilled\ water}$	5.33 ± 0.03	4.92 ± 0.08
pH_{CaCl_2}	4.97 ± 0.08	4.43 ± 0.03
Total organic carbon (%)	1.49 ± 0.06	0.66 ± 0.04
Sand (%)	7.7 ± 0.7	74.9 ± 0.6
Silt (%)	62.5 ± 2.4	20.4 ± 1.7
Clay (%)	29.9 ± 2.4	4.7 ± 1.2
Nitrogen (mg/g)	0.18[b]	1.3[b]
Phosphorus (mg/g)	0.04 ± 0.00	0.49 ± 0.02
Sulfur (mg/g)	0.084[b]	0.186[b]
Cation-exchange capacity[c]		
NH_4NO_3 extraction (meq/100 g)	1.15 ± 0.17	10.15 ± 0.44
NH_4Cl extraction (meq/100 g)	0.65[b]	10.05 ± 0.83

[a]All numeric values are reported as the mean ± 1 SD for three samples unless indicated otherwise.
[b]Value reported is based on one sample.
[c]Determined according to Johnson et al. [15].

but by the final day of the experiment (day 6) had no effect (e.g., dichlorobenzene) (Fig. 2). Tetrachlorobenzene was the only chemical of the 19 included in the study that did not affect soil microbial respiration when added to soil at 1,000 μg/g. Effects for all 19 chemicals are summarized in Table 3. In addition, specific examples of the types of responses observed are provided below. For the full data set for all chemicals, see Walton et al. [18].

The data for dichlorobenzene are typical of a temporary depression in respiration due to chemical treatment. On days 1, 2 and 3, CO_2 evolution was significantly depressed for the sandy loam, but the depression was not significant for the silt loam (Fig. 2). Measurements that did not overlap at 1 SD from the mean for triplicate treatments were considered significantly different. Evolution of CO_2 was not significantly different from matched controls on days 4, 5 and 6 for either soil type. Such a response is noted as "−" for all days and "0" for day 6 in Table 3, to indicate that a depression occurred during the experiment but disappeared by the final day. By comparison, acrylonitrile depressed respiration at 1,000 μg/g in both soils (Fig. 3) and remained significantly depressed ("−" for all days and "−" for day 6 in Table 3), a pattern also observed for nitrobenzene, *trans*-1,4-dichloro-2-butene and *cis*-1,4-dichloro-2-butene in the sandy loam. Only acrylonitrile and *trans*-1,4-dichloro-2-butene showed this effect in the silt loam.

Another type of response, indicated by "+" for all days and "+" for day 6, is that of methyl ethyl

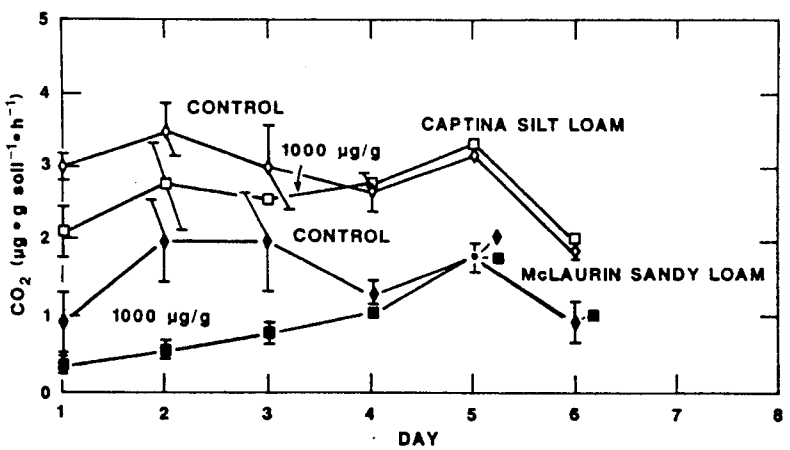

Fig. 2. A single addition of dichlorobenzene at 1,000 μg/g soil (dry wt.) on day 0 caused a temporary depression in CO_2 efflux. Each datum point is the mean ± 1 SE for triplicate experiments.

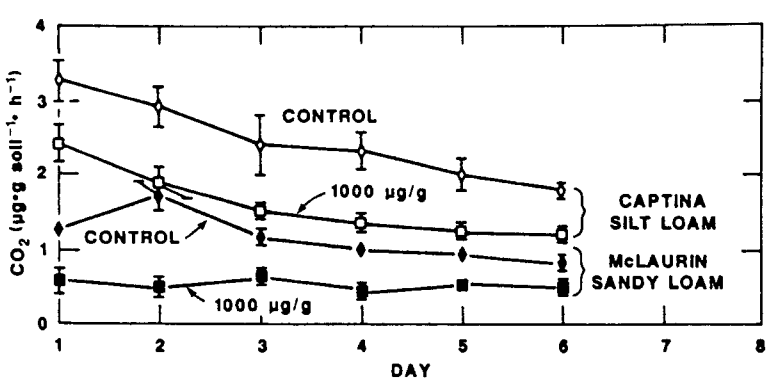

Fig. 3. Acrylonitrile depressed microbial respiration in both soils after a single addition at 1,000 μg/g soil (dry wt.) on day 0. Each datum point is the mean ± 1 SE for triplicate experiments.

Table 3. Effects of individual chemicals on CO_2 efflux from soils

Chemical	Effect on CO_2 efflux[a]			
	Captina silt loam		McLaurin sandy loam	
	All days	Day 6	All days	Day 6
Acrylonitrile	−	−	−	−
Furan	−	0	0	0
Methyl ethyl ketone	+	+	−, +	0
Tetrahydrofuran	+	0	−	0
Benzene	0	0	−, +	+
Toluene	+	0	−	0
1,2-Dichloroethane	−	0	−	0
p-Xylene	0	0	−, +	0
Chlorobenzene	+	0	+	0
Chloroform	+	0	−, +	0
Nitrobenzene	−	0	−	−
trans-1,4-Dichloro-2-butene	−	−	−	−
cis-1,4-Dichloro-2-butene	−	0	−	−
1,2-Dichlorobenzene	−	0	−	0
1,2,3-Trichloropropane	−	0	−	0
Carbon tetrachloride	0	0	−	0
Ethylene dibromide	−	0	−	0
1,2,4,5-Tetrachlorobenzene	0	0	0	0
Hexachlorobenzene	−	0	−	0

The table summarizes differences between treated soils and matched controls occurring at any time during the course of the experiment (all days) and on the final day of the experiment (day 6).
[a]Values for CO_2 efflux in treatment relative to the control are as follows: increase (+), decrease (−), no significant difference (0), and initial decrease followed by a later increase (−,+).

ketone in the silt loam, where CO_2 efflux was significantly increased by the third day and every day thereafter. This experiment was extended an additional 24 h, after which time a 6.3-fold increase over the control was observed (Fig. 4). The elevated respiration rates observed for methyl ethyl ketone and benzene suggest that these chemicals were readily degraded biologically.

Soil microbial function is not likely to be grossly impaired by application of these substances individually at 1,000 μg/g, even in soils of relatively low carbon content. Because soil microbial respiration did not return to control levels after addition of acrylonitrile, nitrobenzene and the 1,4-dichloro-2-butene at 1,000 μg/g, further toxicity tests were run and it was established that each chemical could be added to the two soils at 500 μg/g without irreversibly suppressing microbial respiration [18]. Sixteen of the chemicals caused no depression or only temporary depressions in CO_2 efflux, such that microbial respiration was not different from control rates by the sixth and final day of the experiments. Because a loading rate of 1,000 μg/g exceeds standard application rates for chemicals during land application (J. Matthews, U.S. Environmental Protection Agency, Ada, OK, personal communication), higher concentrations of chemicals were not investigated in this study.

Structure-activity analyses

The effects of individual chemicals on CO_2 efflux from soils are shown in Table 4 for single additions of 1,000 μg/g soil (dry weight). Attempts to correlate chemical effects on soil microbial respiration with $\log_{10}$ of *n*-octanol/water partition coefficients (K_{ow}), aqueous solubilities (mole per liter), vapor pressures (torr), and Henry's law constants (torr per mole per liter) failed to show significant linear correlations for the group of 19 chemicals for which complete data were available. The lack of correlation between the endpoint and either vapor pressure or Henry's law constant indicated that loss of chemicals from the incubation jars due to periodic flushing of the headspace was not a critical factor affecting toxicity. That is, highly volatile compounds may have proved less toxic simply because they were removed from the system more quickly by volatilization; however, this apparently was not an important determinant of the effect on soil microbial respiration. Because the sorption partition coefficients (K_p) and partition coefficients for organic carbon in soil (K_{oc})

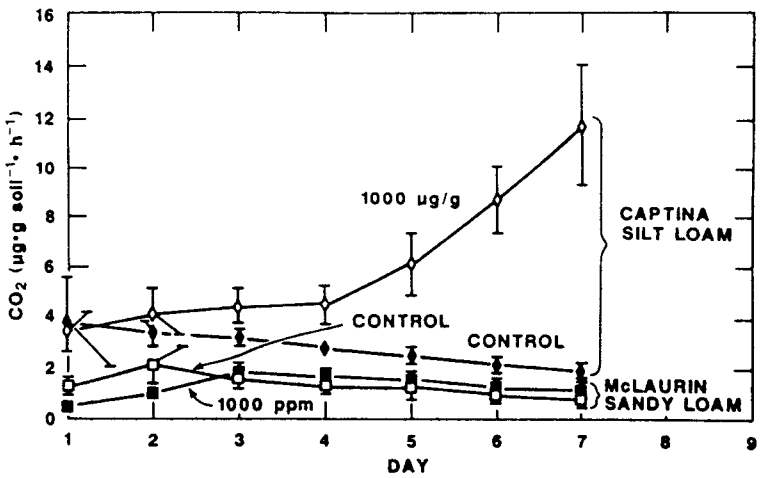

Fig. 4. Methyl ethyl ketone added to soil at 1,000 µg/g soil (dry wt.) on day 0 stimulated CO_2 efflux in the Captina silt loam; however, respiration was not enhanced in the McLaurin sandy loam under similar treatment conditions. Each datum point is the mean ± 1 SE for triplicate experiments.

Table 4. Effects of individual chemicals on CO_2 efflux from soils on day 4 at 1,000 µg/g soil (dry weight)

	Effect on CO_2 efflux (day 4)			
	Captina silt loam		McLaurin sandy loam	
Chemical	Difference[a] (µg g soil^{-1} h^{-1})	Ratio[b]	Difference[a] (µg g soil^{-1} h^{-1})	Ratio[b]
Acrylonitrile	−0.96	−0.230	−0.59	−0.387
Furan	−0.22	−0.040	0.27	0.092
Methyl ethyl ketone	1.77	0.212	0.36	0.108
Tetrahydrofuran	0.22	0.081	0.10	0.050
Benzene	0.52	0.086	2.48	0.492
Toluene	0.36	0.055	0.44	0.154
1,2-Dichloroethane	−0.04	0.018	0.18	0.062
p-Xylene	0.70	0.091	0.59	0.177
Chlorobenzene	0.29	0.054	0.71	0.248
Chloroform	0.97	0.143	0.90	0.249
Nitrobenzene	−0.55	−0.109	−0.61	−0.409
trans-1,4-Dichloro-2-butene	−0.67	−0.375	−0.40	−0.248
cis-1,4-Dichloro-2-butene	0.40	−0.248	0.45	−0.283
1,2-Dichlorobenzene	0.12	0.019	−0.22	−0.082
1,2,3-Trichloropropane	0.09	0.035	0.18	0.084
Carbon tetrachloride	0.21	0.034	−0.34	−0.102
Ethylene dibromide	0.94	0.148	0.50	0.173
1,2,4,5-Tetrachlorobenzene	−0.15	−0.037	0.06	0.048
Hexachlorobenzene	−0.73	−0.202	0.21	0.158

[a]Respiratory effect is expressed as the difference between treatments and matched controls on day 4 (i.e., CO_2 $EFFLUX_{control}$ − CO_2 $EFFLUX_{treatment}$).
[b]Respiratory effect is expressed as the $\log_{10}$ of the ratio of CO_2 efflux in the treatment to that of the control, (i.e., respiratory effect = $\log_{10}$ (CO_2 $EFFLUX_{treatment}$/CO_2 $EFFLUX_{control}$).

are highly correlated with K_{ow}, these parameters were not included in the analysis because of their redundancy.

Linear regression analyses for physicochemical properties and respiratory effects using data other than that for day 2 and data from the day with the greatest deviation from the control for each test chemical gave the same results as the day 4 data.

Table 5. Correlation coefficients (r) for physicochemical properties of 16 organic chemicals when correlated with their effect on soil microbial respiration

Property	Correlation coefficient (r)	
	Captina silt loam	McLaurin sandy loam
Log_{10} K_{ow}	0.19	0.24
Log_{10} vapor pressure (torr)	0.03	0.03
Log_{10} water solubility (M)	0.04	0.10
Log_{10} Henry's constant (torr/M)	0.25	0.02
Log_{10} rat oral LD50 (mmol/kg)	0.13	0.04

None of the correlations was significant using Student's t test ($p \geq 0.05$). Microbial respiration was expressed as $\log_{10}$ of the ratio CO_2 $\text{EFFLUX}_{\text{treatment}}/CO_2$ $\text{EFFLUX}_{\text{control}}$ in a Captina silt loam and a McLaurin sandy loam.

In each case, no marked improvement in correlations was observed as compared with the results obtained with the day 4 numbers—a finding that appeared to justify the reasoning behind use of day 4 data (described under Soil Microbial Respiration in the Materials and Methods section).

In addition to regressions of respiratory effects against nonbiological properties of the molecules, the day 4 data were also compared with the rat oral LD50 (mmol/kg) and the $\log_{10}$ of the LD50 for each chemical. LD50 data were found for all of the chemicals except furan and tetrahydrofuran, and ranged from 0.047 mmol/kg (3.4 mg/kg) for methyl ethyl ketone to 63 mmol/kg (4.9 g/kg) for benzene. Correlation coefficients for these regressions were also extremely poor (Table 5). The results of these regressions indicate that acute, lethal toxicity in rats was of little value for predicting even relative toxicity of the chemicals to soil microorganisms.

The SAR analyses for the group of 19 chemicals showed neither $\log_{10}$ K_{ow}, water solubility, vapor pressure, Henry's law constant, nor rat acute toxicity to be correlated with chemical effects on soil microbial respiration. However, analysis of a subset of chemicals of relatively similar structures yielded good correlations of respiratory effects with $\log_{10}$ K_{ow} and molecular connectivity for the Captina silt loam. This subset was benzene, toluene, p-xylene, chlorobenzene, dichlorobenzene, tetrachlorobenzene and hexachlorobenzene. Table 6 shows the physicochemical properties used for these compounds.

The relationship of respiration effect to K_{ow} is described by the following equation for the Captina silt loam:

$$y = 0.239 - 0.064x \qquad N = 7,\ r = 0.91 \qquad (3)$$

where y is $\log_{10}$ (CO_2 $\text{EFFLUX}_{\text{treatment}}/CO_2$ $\text{EFFLUX}_{\text{control}}$), x is $\log_{10}$ K_{ow}, N is the number of chemicals and r is the correlation coefficient. This correlation was slightly improved by using molecular connectivity ($^1\chi$) instead of $\log_{10}$ K_{ow} (Table 7):

$$y = 0.456 - 0.114x \qquad N = 7,\ r = 0.94 \qquad (4)$$

where x is $^1\chi$ (Fig. 5). Correlations were considerably lower for the McLaurin sandy loam (Table 7), which may be due to the lower organic carbon content of the soil such that nonbiological factors other than partitioning predominated [19].

DISCUSSION

Most chemicals depressed carbon dioxide efflux in the two test soils when applied at 1,000 μg/g soil

Table 6. N-Octanol/water partition coefficients (K_{ow}) and molecular connectivity indices ($^1\chi$) for benzene, alkylbenzenes and chlorobenzenes

Chemical	$\log_{10}$ K_{ow}[a]	$^1\chi$[b]
Benzene	1.95 (1.56,2.15)[c]	3.000
Toluene	2.50 (2.69,2.15)[c]	3.394
p-Xylene	3.15	3.788
Chlorobenzene	2.83	3.394
1,2-Dichlorobenzene	3.38	3.805
1,2,4,5-Tetrachlorobenzene	4.93	4.609
Hexachlorobenzene	6.35[d]	5.464

[a]Obtained from the U.S. Department of Health and Human Services [24].
[b]Obtained from Sabljić [25].
[c]Calculated as the arithmetical mean of the values in parentheses reported in reference 24.
[d]Calculated according to the fragment method of Lyman et al. [17].

Table 7. Correlation coefficients (r) for physicochemical properties of benzene, alkylbenzenes and chlorobenzenes[a] when correlated with their effect on soil microbial respiration

Property	Correlation coefficient (r)	
	Captina silt loam	McLaurin sandy loam
$\text{Log}_{10}\ K_{ow}$	0.91[b]	0.45[d]
Molecular connectivity ($^1\chi$)	0.94[b]	0.44[d]
Log_{10} vapor pressure (torr)	0.84[c]	0.64[d]
Log_{10} water solubility (M)	0.84[c]	0.64[d]
Log_{10} Henry's constant (torr/M)	0.85[c]	0.79[c]
Log_{10} rat oral LD50 (mmol/kg)	0.18[d]	0.79[c]

Microbial respiration was expressed as the $\log_{10}$ of the ratio CO_2 EFFLUX$_{treatment}$/CO_2 EFFLUX$_{control}$ in a Captina silt loam and a McLaurin sandy loam.
[a]Benzene, toluene, p-xylene, chlorobenzene, 1,2-dichlorobenzene, 1,2,4,5-tetrachlorobenzene, and hexachlorobenzene.
[b]Significant at the 99% level (student's t test).
[c]Significant at the 95% level.
[d]Not significant at the 95% level.

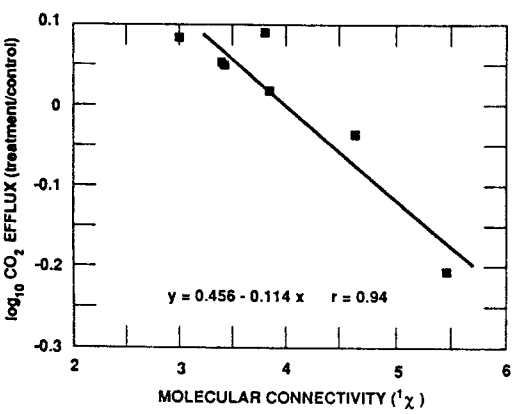

Fig. 5. Regression of soil microbial respiration [$\log_{10}CO_2$ efflux (treatment/control)] on molecular connectivity indices ($^1\chi$) of benzene and six chloro- and alkyl- derivatives.

dry weight. However, this effect disappeared within a few days. Tetrachlorobenzene was the only compound of the 19 tested that did not affect soil microbial respiration at the 1,000 μg/g loading rate. Although acrylonitrile, nitrobenzene, and the 1,4-dichloro-2-butenes were toxic at 1,000 μg/g, depression of CO_2 was temporary at treatments of 500 μg/g for each substance. Thus, none of the chemicals evaluated showed a high potential for adverse effects on soil microbial communities.

Increases in microbial respiration, measured as either biochemical oxygen demand or CO_2 evolution, are often used as an indication of biological transformation of organic substances in soil and water [6,20–22]. The respiration data were not used to calculate theoretical increases in CO_2 efflux that would result from biological degradation of the test chemicals in the present study because separate studies were conducted for the specific purpose of determining degradation rates [18]. Nonetheless, the elevated respiration rates observed for methyl ethyl ketone and benzene suggest that these chemicals were readily degraded.

The biological data obtained in the respiration experiments are not typical of those used in whole-organism SAR studies, in which toxicity is reported as a lethal dose to a given percentage of the treated population (e.g., LD50), as an effective concentration (e.g., EC50) or as a lethal concentration (e.g., LC50). Experiments designed to express toxicity as an effective concentration for the present study (i.e., the amount required to reduce respiration by a given percentage) would have been impractical for a number of reasons (including the following) and thus were not attempted. (a) Many of the chemicals are solvents of low toxicity to microorganisms and are likely to affect respiration only at high concentrations. Therefore, increases in chemical loading until an effective concentration is observed may simply decrease oxygen diffusion in the soil, rather than produce direct toxicity to the microorganisms. Such data are likely to detract from meaningful SAR correlations. (b) Individual chemical loading rates on soils in excess of 1,000 μg/g are not realistic in the context of environmentally acceptable application of waste chemical mixtures in soils (J. Matthews, U.S. Environmental Protection Agency, Ada, OK, personal communication). Thus, the approach of comparing

toxicological effects resulting from treatment at a fixed dose (e.g., 1,000 μg/g), rather than basing analysis on the concentration of chemical that produces a fixed response (such as a 50% decrease in microbial respiration rate), is a reasonable alternative and was employed in this study.

The results obtained in the described experiments are valid only in the context of the experimental conditions used. For example, no nutrient amendments were made to the soils in these experiments because the data were gathered for modeling chemical fate under conditions of land application, when site management may be less than ideal. Thus, the chemical effects observed on soil respiration can be viewed as conservative estimates of microbial responses because of nonoptimal nutrient conditions.

Other experimental conditions used also limit direct applicability of the results to field conditions. For example, soils were sieved, air dried and stored for later use rather than tested fresh from the field. The limitations and artifacts associated with these manipulations were accepted in light of the need to conduct experiments over a period of months without frequent soil collection and characterization. However, because land application of waste chemicals typically involves plowing and discing, disruption of the soil structure in the laboratory is not as great a source of error for these studies, which were designed with land treatment in mind, as it would be if the studies were conducted to predict chemical effects on microbial populations associated with undisturbed soils. Thus, the techniques used compromised environmental reality somewhat, but ensured minimal experimental variability and permitted comparisons of individual chemical effects on soils even though the experiments were conducted over a number of months.

Linear correlations for physicochemical parameters with chemical effects on soil respiration were poor for the complete data set; however, they were good for a subset of benzene and its chloro- and alkyl- derivatives. This finding suggests that multiple SARs are likely to be required to predict biologically mediated processes from large data sets that include structurally dissimilar compounds. Nonetheless, the findings herein provide encouraging evidence that quantitative structure-activity analyses may be used to predict the biodegradation and effects of organic chemicals in matrices as complex as those of soil if the limitations of data extrapolation are defined [23].

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REFERENCES

1. **Beltrame, P., P.L. Beltrame** and **P. Carniti.** 1984. Inhibiting action of chloro- and nitro-phenols on biodegradation of phenol: A structure-toxicity relationship. *Chemosphere* **13**:3–9.
2. **Pitter, P.** 1985. Correlation of microbial degradation rates with the chemical structure. *Acta Hydrochim. Hydrobiol.* **13**:453–460.
3. **Pitter, P.** 1976. Determination of biological degradability of organic substances. *Water Res.* **10**:231–235.
4. **Vaishnav, D.D., R.S. Boethling** and **L. Babeu.** 1987. Quantitative structure-biodegradability relationships for alcohols, ketones and alicyclic compounds. *Chemosphere* **16**:695–703.
5. **Wolfe, N.L., D.F. Paris, W.C. Steen** and **G.L. Baughman.** 1980. Correlation of microbial degradation rates with chemical structure. *Environ. Sci. Technol.* **14**:1143–1144.
6. **Niemi, G.J., G.D. Veith, R.R. Regal** and **D.D. Vaishnav.** 1987. Structural features associated with degradable and persistent chemicals. *Environ. Toxicol. Chem.* **6**:515–527.
7. **Alexander, M.** and **M.I.H. Aleem.** 1961. Effect of chemical structure on microbial decomposition of aromatic herbicides. *J. Agric. Food Chem.* **9**:44–47.
8. **Alexander, M.** and **B.K. Lustigman.** 1966. Effect of chemical structure on microbial degradation of substituted benzenes. *J. Agric. Food Chem.* **14**:410–413.
9. **Cantier, J.M., J. Bastide** and **C. Coste.** 1986. Structure-degradability relationships for propyzamide analogues in soils. *Pestic. Sci.* **17**:235–241.
10. **Atlas, R.M.** and **R. Bartha.** 1987. *Microbial Ecology: Fundamentals and Applications.* Benjamin/Cumminds Publishing Co., Menlo Park, CA.
11. **Pramer, R.** and **R. Bartha.** 1972. Preparation and processing of soil samples for biodegradation studies. *Environ. Lett.* **2**:217–224.
12. **Ward, M.H., G.S. Saylor** and **R.J. Luxmoore.** 1984. Synthetic fuel oil effects on microbial activity and nitrogen transformations in soil. ORNL/TM-8591. Oak Ridge National Laboratory, Oak Ridge, TN.
13. **Page, A.L.** 1982. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 2nd ed. Agronomy Monograph No. 9. American Society of Agronomy and Soil Science Society of America, Madison, WI.

14. **U.S. Environmental Protection Agency.** 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. Environmental Monitoring and Support Laboratory, Cincinnati, OH.
15. **Johnson, D.W., D.D. Richter, G.M. Lovett** and **S.E. Lindberg.** 1985. The effects of atmospheric deposition on potassium, calcium, and magnesium cycling in two deciduous forests. *Can. J. For. Res.* **15**:774–782.
16. **Edwards, N.T.** 1982. A time-saving technique for measuring respiration rates in incubated soil samples. *Soil Sci. Soc. Am. J.* **46**:1114–1116.
17. **Lyman, W.J., W.F. Reehl** and **D.H. Rosenblatt,** eds. 1982. *Handbook of Chemical Property Estimation Methods: Environmental Behavior of Organic Compounds.* McGraw-Hill, New York, NY.
18. **Walton, B.T., M.S. Hendricks, T.A. Anderson** and **S.S. Talmage.** 1988. Treatability of hazardous chemicals in soils: Volatile and semivolatile organics. ORNL-6451. Oak Ridge National Laboratory, Oak Ridge, TN (in press).
19. **Zelles, L., I. Scheunert** and **F. Korte.** 1986. Comparison of methods to test chemicals for side effects on soil microorganisms. *Ecotoxicol. Environ. Safety* **12**: 53–69.
20. **Howard, P.H., H.C. Sikka** and **S. Banerjee.** 1981. Test methods for determining the biodegradation of organic chemicals in the aquatic environment. In G. Zweig and M. Beroza, eds., *Test Protocols for Environmental Fate and Movement of Toxicants.* Association of Official Analytical Chemists, Arlington, VA, pp. 150–167.
21. **Scow, K.M.** 1982. Rate of biodegradation. In W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds., *Handbook of Chemical Property Estimation Methods: Environmental Behavior of Organic Compounds.* McGraw-Hill, New York, NY, pp. 9-1 to 9-85.
22. **Boatman, R.J., S.L. Cunningham** and **D.A. Ziegler.** 1986. A method for measuring the biodegradation of organic chemicals. *Environ. Toxicol. Chem.* **5**:233–243.
23. **Walton, B.T.** and **T.A. Anderson.** 1988. Structural properties of organic chemicals as predictors of biodegradation and microbial toxicity in soils. *Chemosphere* (in press).
24. **U.S. Department of Health and Human Services.** 1987. Hazardous Substances Data Bank. National Library of Medicine Toxnet File. National Institutes of Health, Bethesda, MD.
25. **Sabljić, A.** 1987. On the prediction of soil sorption coefficients of organic pollutants from molecular structure: Application of molecular topology model. *Environ. Sci. Technol.* **21**:358–366.