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Structure-Activity Relationships for the Degradation of a Mixture of Organic Chemicals in Soil

T. A. Anderson
B. T. Walton

Environmental Sciences Division
Publication No. 3265

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ORNL/TM-11108

ENVIRONMENTAL SCIENCES DIVISION

STRUCTURE-ACTIVITY RELATIONSHIPS FOR THE DEGRADATION OF A MIXTURE OF ORGANIC CHEMICALS IN SOIL[1]

T. A. Anderson[2]
B. T. Walton

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[1]Submitted as a thesis by T. A. Anderson to the Graduate Council of the University of Tennessee in partial fulfillment of the requirements for the degree of Master of Science.

[2]The University of Tennessee, Knoxville.

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ABSTRACT

ANDERSON, T. A. and B. T. WALTON. 1989. Structure-activity relationships for the degradation of a mixture of organic chemicals in soil. ORNL/TM-11108. Oak Ridge National Laboratory, Oak Ridge, Tennessee. 77 pp.

The degradation rates of a mixture of volatile and semi-volatile organic compounds (N = 16) were determined by monitoring the removal of parent chemicals from two different soil types, a Captina silt loam (Typic Fragiudult) and a McLaurin sandy loam (Typic Paleudults), over a 7-day period. The compounds were applied to the soil in a mixture such that the concentration of each individual chemical was 100 μg/g soil (dry weight). Soil samples, along with sterile (autoclaved) controls, were incubated in the dark at 20° C in 8 x 5-cm glass jars equipped with teflon-lined stoppers and charcoal traps. Samples were disassembled, extracted with methanol, and analyzed on days 0, 2, 3, 6, and 7 of the experiment. Linear regression analysis was used to model the disappearance of the compounds from the soils. First-order degradation rate constants and half-lives were calculated. Half-lives for the 16 organic compounds were relatively short (<23 days). Correlations between physicochemical parameters and half-lives were poor ($r \leq 0.63$) for the entire data set (N = 16), however, a group of structurally related benzene derivatives (N = 7) gave good correlations in both soil types with $\log_{10}$ octanol-water partition coefficient ($r \geq 0.89$), $\log_{10}$ molecular weight ($r \geq 0.84$), $\log_{10}$ water solubility ($r \geq 0.77$), and molecular connectivity ($r \geq 0.83$). The present study illustrates the possibility of using structure-activity relationships for

predicting the degradation of organic compounds in complex systems such as soil and provides data on the degradation of a mixture of volatile and semi-volatile organic compounds under defined experimental conditions.

CHAPTER I

INTRODUCTION

1.1 Background on Land Application of Hazardous Chemicals

Soil microbiota have the potential to decompose and detoxify a wide range of organic substances. The application of organic chemicals on soil can be an effective strategy for disposal, provided that these substances are evaluated to determine which ones are suitable for safe application. Traditionally, land application of wastes has been used as a method for supplementing plant nutrients or improving soil properties. Increases in hazardous waste generation and restrictions on other disposal methods have spurred interest in land application of wastes not previously considered appropriate as soil amendments (Matthews, 1986). Parallel to this increased need for safe and economical methods of disposal is a regulatory requirement for data on the toxicity, bioaccumulation, sorption, and degradation of organic chemicals in soils (Resource Conservation and Recovery Act, see Walton and Edwards, 1986). These detailed, waste-specific investigations can help maximize the effectiveness of a land application operation and can also provide the necessary technology for in situ restoration of spills.

1.2 Rationale

The primary concern for application of wastes to soils is the presence of hazardous chemicals having the potential to adversely affect human health and the environment by their toxicity, migration,

or biological accumulation. In order for regulatory agencies to make appropriate decisions on these chemicals, they must have data on their potential environmental impact. The primary objective of this study was to measure the relative rates of disappearance, in two soil types, of a mixture of 16 organic compounds commonly found in hazardous waste streams. Ultimately, the data generated in this research is to be used by EPA personnel (Robert S. Kerr Environmental Research Laboratory) to model the environmental fate of organic contaminants that are candidates for application to soil.

1.3 Application of a Prebiological Screen and QSAR to Biodegradation Data

In addition to generating data for use by EPA, this study also provided an opportunity to (1) evaluate the data for predictive relationships between chemical structure and degradation rates and (2) to evaluate the data using a prebiological screen for ecotoxicologic effects. The use of quantitative structure-activity relationships (QSAR) to predict half-lives of organic pollutants has been used in aquatic environments (e.g., Niemi et al., 1987; Mackay et al., 1985) and could have applications to soil systems as well (Walton and Anderson, in press). Because soils are more complex than most aquatic test systems in which QSARs for biodegradation have been described, existing structure-activity relationships may be marked. Nonetheless, many applications exist for predictive equations describing chemical degradation in soil (e.g., environmental risk assessments), thus the potential benefits that may accrue provide a rationale for exploring

this strategy. Also, QSARs can help to identify the critical processes that determine degradation rates in soil (Parsons et al., 1987; Pitter, 1985). Gillett (1983) developed a prebiological screen (PBS) which uses degradation half-lives, octanol-water partition coefficients, and Henry's Law constants to make inferences on the probability that a given compound will have an adverse environmental impact. The PBS can be used to identify effects of chemicals on populations and help indicate informational needs for chemicals at the pre-manufacturing stage (Gillett, 1983).

CHAPTER II

LITERATURE REVIEW

2.1 Microbial Degradation

There is an enormous amount of data on the biodegradation of organic compounds, due largely to the impact of the pesticide industry. Excellent reviews are available elsewhere on organic compounds (Meikle, 1972), pesticides (Kaufman, 1974; Sanborn et al., 1977), petroleum hydrocarbons (Gutnick and Rosenberg, 1977), herbicides (Cripps and Roberts, 1978), insecticides (Matsumura and Benezet, 1978), and chlorinated aromatic compounds (Rochkind et al., 1986). However, brief reviews of microbial degradation of xenobiotics in the environment, transformation pathways used by microorganisms, and chemical structure effects on degradation will be provided, especially for those compounds used in this study.

Microorganisms are capable of carrying out hydrolytic, oxidative, reductive, or conjugative degradation reactions (Hill, 1978). Hydrolytic reactions involve the hydrolyses of organic esters, amides, nitriles, and halogenated aliphatic compounds (e.g., 1,2,3--trichloropropane, chloroform, carbon tetrachloride). Hydrolysis of the ether linkage between the aromatic ring and the fatty acid side chain is the first step in the degradation of the herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) (Tiedje and Alexander, 1969). This is followed by beta-oxidation of the aliphatic side chain and hydroxylation of the aromatic ring. Hydrolytic dehalogenation involves

removal of the halogen(s) from the aliphatic group with the subsequent formation, via beta-oxidation, of tricarboxylic acid cycle precursors (Hill, 1978; Kearney et al., 1964). The herbicides TCA (trichloroacetic acid) and Dalapon (2,2-dichloropropionic acid) are excellent examples.

Oxidative reactions can involve aromatic hydrocarbon hydroxylation (to phenols), aliphatic hydrocarbon oxidation (to alcohols, ketones, and acids), N, S, and O dealkylation, epoxidation, N and S oxidation, amine oxidation (to aldehydes and ketones) and alcohol and aldehyde oxidations (to aldehydes and acids). Microbial attack on benzene involves hydroxylation of the aromatic ring by bacterial dioxygenase enzymes to catechol (1,2-dihydroxybenzene) (Rochkind et al., 1986). Substituted benzenes (e.g., toluene) (Nozaka and Kusonose, 1968; 1969) and simple polynuclear aromatic hydrocarbons (e.g., naphthalene) (Davies and Evans, 1964) are similarly oxidized to substituted catechols (cis-dihydrodiols), however, the substituent can be oxidized before or after hydroxylation of the aromatic ring. Further ring cleavage of the catechols forms acetyl-CoA, acetaldehyde, succinic acid, and pyruvic acid (Dagley and Gibson, 1965); all of which are intermediates in metabolic energy production. The degradation of mono- through tetra-chlorobenzenes, via aromatic ring hydroxylation, produces chlorophenols (Rochkind et al., 1986) which can be methylated by microbes to form chloroanisoles (Neilson et al., 1983) or transformed to chlorocatechols (Spokes and Walker, 1974). Beall (1976) found that although most of the hexachlorobenzene applied to soil is

lost via volatilization after 19 months, the remaining chemical is not metabolized.

Bacterial nitroreductases, using $NADPH_2$ or $NADH_2$, are involved in reduction reactions of aromatic nitro compounds. Nitrobenzene is first reduced to aniline, then further transformed to catechol (Bachofer et al., 1975), which enters the pathway described earlier. Parathion is metabolized by this mechanism also. Ketones (e.g., methyl ethyl ketone) can be reduced to alcohols by ketone reductases and NADPH (Sipes and Gandolfi, 1986).

In addition to biotransformation of endogenous compounds, conjugation reactions are important pathways in xenobiotic metabolism. Conjugation reactions carried out by microorganisms include methylation and acetylation (Bollag, 1974).

From the large data set on the degradation of pesticides and other organic compounds in soil, including early work by Alexander and Aleem (1961), Alexander and Lustigman (1966), and Bartha et al. (1967), generalizations regarding effects of chemical structure on biodegradability have emerged. Studies of xenobiotic and drug metabolism in eukaryotes also contributed to structure-activity type relationships that allow qualitative estimations of chemical persistence. For example, highly branched, short chain compounds are more resistant to biodegradation than long chain, unbranched compounds. In addition, unsaturated aliphatic compounds are more readily degraded than saturated aliphatic compounds. Finally, the type, number, and position of substituent(s) play a large role in determining

degradability. The existence of these qualitative relationships provide an impetus to explore the use of quantitative structure-activity relationships in biodegradation research.

2.2 QSAR in Microbial Degradation Studies

Since biodegradation is one of the most important processes in determining the persistence of a chemical in surface soils, screening-level predictors of biodegradation rates are very desirable (Howard et al., 1987). Biodegradation rates can theoretically be predicted by (1) parameters describing the rates at which compounds cross bacterial membranes (e.g., octanol-water partition coefficient) or (2) parameters describing the rates at which compounds are enzymatically transformed (e.g., electronic or steric parameters) (Parsons et al., 1987). The use of quantitative structure-activity relationships (QSARs) to predict degradability of organic pollutants in aquatic systems has been documented for alcohols and ketones (Vaishnav et al., 1987); chloro- and nitro-phenols (Beltrame et al., 1984; 1988); substituted phenols and anilines (Pitter, 1985); various chloro-derivatives of phenol, anisol, and resorcinol (Banerjee et al., 1984); aromatic and aliphatic aldehydes, phenols, carboxylic acids, halogenated hydrocarbons, amino acids, and aromatic and aliphatic amines (Dearden and Nicholson, 1986); phthalate esters (Urushigawa and Yonezawa, 1979); and aliphatic alcohols (Yonezawa and Urushigawa, 1979). However, their use in soil environments has been

rare (Cantier et al., 1986; Bossert and Bartha, 1986) and of limited success.

Early work by Wolfe and co-workers (1980) demonstrated the possible usefulness of QSARs in predicting microbial degradation rates. The second-order degradation rate constants of phthalate esters in natural water samples were shown to correlate well ($r^2 = 0.93$) with second order alkaline hydrolysis rate constants. In addition, better correlations ($r^2 = 0.97$) were observed for the degradation in natural waters, of malathion [O,O-dimethyl-S-1,2di(carboethoxy) ethyl phosphorodithioate], methyl benzoate, methyl anisate, chlorpropham (isopropyl mchlorocarbanilate), and an ester of 2,4-dichlorophenoxy acetic acid.

Beltrame et al. (1984; 1988) found that the increased inhibitory action of chloro- and nitro-phenols to phenol degradation in activated sludge was correlated with increased octanol-water partition coefficients (K_{ow}) and Hammett constants. In the 1984 study, a structure-activity relationship (SAR) was found for the entire set of chemicals (N = 25), however, the 2,6-disubstituted phenols (N = 7) gave the best correlation (r = 0.94). These findings are consistent with their observations that increased values of $\log_{10} K_{ow}$ and Hammett constants enhance the recalcitrance of phenols to microbial decomposition. A similar SAR was observed by Vaishnav and Lopas (1985) for primary alcohols. They found that log K_{ow} could be used to estimate the concentration of an alcohol that would cause a

50% inhibition in maximum degradation rate (EC50) from the following relationship:

$$\log_{10} EC50 = -0.88(\log_{10} K_{ow}) + 0.07(\log_{10} K_{ow})^2 - 0.23 \qquad (1)$$

Banerjee and co-workers (1984) have developed a kinetic model for predicting biodegradation based on lipophilicity. The model showed that degradation rates for chlorophenols and related compounds in both pure and mixed bacterial cultures of natural water samples that increased with decreasing values of K_{ow}. This relationship suggests that chemical penetration of the lipid bacterial membrane is rate-limiting, a phenomenon also observed with alkyl esters of p-aminobenzoic acid (Parsons et al., 1987). However, Parsons and co-workers found that first-order biodegradation rate constants increased with increasing lipophilicity (K_{ow}) for these compounds.

Studies by Vaishnav et al. (1987) on the degradation of 17 alcohols and 11 ketones also showed correlations between $\log_{10} K_{ow}$ and percent theoretical oxygen demand (%ThOD), which is often used as a non-specific indicator of biodegradation. They observed that the degradation of very hydrophilic alcohols and ketones (which are believed to enter bacterial cells through membrane pores) was unrelated to K_{ow}. However, the degradability of hydrophobic alcohols and ketones (which diffuse directly through the bacterial membrane) may be positively or negatively correlated with K_{ow} depending on their degree of hydrophobicity. At high K_{ow} values, degradation rates were lower,

whereas at lower K_{ow} values, degradation rates were higher. A possible explanation for this phenomenon is that the compounds with extremely high octanol-water partition coefficients readily crossed the bacterial membranes and accumulated at concentrations that were toxic to the bacteria, thus preventing degradation. Although these results are not encouraging for those seeking simple methods to predict chemical persistence, they do help illustrate situations in which multiple mechanisms of action are operating and therefore a multiple QSAR is needed to predict this behavior.

Pitter (1985) used substituent constants of 35 substituted phenols and anilines as a predictor of biodegradation by mixed bacterial cultures and concluded that the electrophilic character of the aromatic ring determined the degradation rates of these compounds. Substituents that lowered the electron density of the aromatic ring (i.e., strong electron-withdrawing groups like Cl and NO_2) lowered the degradation rates. Pitter concluded that the electronic effects of the substituents governed the initial attack of the aromatic ring, either making it more susceptible (strong electron-repulsing substituents) or less susceptible (strong electron-withdrawing substituents) to hydroxylation.

A number of biological and chemical properties of compounds have been correlated with molecular connectivity, which is a numerical representation of the topology of a molecule, taking into account the size, shape, branching, and nature of the atom-atom connections (Kier and Hall, 1976). This parameter has been used to predict microbial

degradation of 2,4-D alkyl esters, N-3-chlorophenylcarbamates, dialkyl ethers, phthalate esters (Boethling, 1986) and a wide variety of other compounds (Niemi et al., 1987) with promising success. These findings implicate enzyme binding as an important factor in determining biodegradation (Parsons et al., 1987). In these cases, the compound to be degraded must associate with the active site on an enzyme, a process dictated by the shape of both the substrate and the receptor site.

While the QSAR approach has met with limited success for microbial degradation in soil systems, studies show that QSARs can be developed for homologous series of chemicals under experimental conditions that control or account for major loss processes such as sorption and volatilization (Walton and Anderson, in press). Bossert and Bartha (1986) found that the water solubilities of 10 polycyclic aromatic hydrocarbons were positively correlated with their biodegradation in soil samples. However abiotic losses, namely volatilization, may have obscured this SAR. Cantier and co-workers (1986) observed correlations between degradation rate constants of propyzamide analogues in soil and their Hammett and hydrophobic constants. Abiotic loss mechanisms (chemical degradation) may have interfered with this SAR also. These studies indicate the possible application of QSARs to the biological degradation of organic compounds in soil. In addition, they illustrate two fundamental concepts for the effectiveness of QSARs in soil systems: (1) designing experiments to minimize or account for competing loss mechanisms and (2) testing for QSARs using a homologous series of chemical compounds (Walton and Anderson, in press). The

implementation of these concepts will enable researchers to develop multiple QSARs to predict persistence in soil for large numbers of compounds of diverse type and structure.

CHAPTER III

MATERIALS AND METHODS

3.1 Soils

Two soils, low in carbon content, from the Southeastern United States were used in the degradation rate studies. The first soil was a Captina silt loam (Typic Fragiudult) collected from the 0800 area at Oak Ridge National Laboratory (ORNL), Roane County, Tennessee. The second soil, a McLaurin sandy loam (Typic Paleudluts) from Stone County, Mississippi, was provided by Dr. Gary McGinnis, Mississippi State University. Collection and characterization of the soils is described by Walton et al. (in press a.). Both soils were coarse sieved (6.3 mm) and air dried before being stored in the dark at 4°C until needed. Fine sieving (2 mm) and adjustment of the soils to 80% moisture content with distilled, deionized water (9.5 mL/50 g soil for the Captina silt loam and 4.6 mL/50 g soil for the McLaurin sandy loam) took place prior to use in the degradation experiments. The physical and chemical properties of the study soils are shown in Table 1. No nutrient amendments were made to the soils due to the land application context of the study where site management practices may be less than ideal. Thus, the measurements made can be viewed as conservative estimates of biodegradation potential.

Table 1. Selected physical and chemical properties of the study soils.

Parameter	Soil	Properties[a]
Type, texture	Captina silt loam	McLaurin sandy loam
Collection site	Roane County, TN	Stone County, MS
pH (distilled water)	5.33 ± 0.03	4.92 ± 0.08
pH (calcium chloride)	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]

[a]All numeric values are reported as the mean ± standard deviation for three samples unless indicated otherwise.
[b]Number reported is based on one sample.

(Adapted from Walton et al., in press a.)

3.2 Soil Sterilization

In order to distinguish losses due to biodegradation from abiotic losses, the microbial activity of the test soils must be absent or attenuated (Rozycki and Bartha, 1981). Several common methods were tested to inactivate microorganisms, including gamma irradiation, autoclaving, and the use of mercuric chloride and sodium azide. Sterilization was verified by measuring carbon dioxide efflux (microbial respiration) on an infrared gas analyzer (Model 300, Mine Safety Appliances Company, Pittsburgh, PA) from 50-g soil samples for 7 days at 24-hour intervals (Edwards, 1982) and checking for viable microorganisms on soil-inoculated agar plates.

A 1.35 Mrad dose of gamma irradiation [supplied by the High Flux Isotope Reactor (HFIR) at ORNL] depressed microbial respiration, however, CO_2 efflux in the Captina silt loam and McLaurin sandy loam had returned to 15% and 16% of control values, respectively, after 5 days. Higher radiation doses were deemed impractical and were not used due to the time and expense involved. In addition, the ambient temperatures associated with this method of sterilization permit the retention of heat-labile, nonviable substances in soil that can ultimately accelerate the degradation of certain organic compounds (Getzin and Rosefield, 1968).

The use of metabolic inhibitors (sodium azide and mercuric chloride) was effective but only at relatively high concentrations, which undoubtedly changed the chemical and physical properties of the soils. The combining of mercuric chloride and sodium azide forms

mercuric azide, a percussion detonator. Although this combination may be effective as a soil sterilant, its explosiveness (inadvertently confirmed in the laboratory) precludes routine usage.

The procedure of autoclaving soil samples for 1 hour on three consecutive days prior to experimental use proved to be effective for sterilization of soil samples. No microorganisms grew on nutrient agar (Difco Laboratories, Detroit Mich.) plates inoculated with autoclaved soils and only trace microbial respiration occurred over a seven-day period relative to control samples. Autoclaving can alter soil and microbial properties by increasing extractable N, P, and S (Eno and Popenoe, 1964), and Mn, Cu, B, Ca, Mg and Na (Salonius et al., 1967), which can result in increased microbial growth. Steam sterilization has also been associated with slight changes in soil pH (Skipper and Westermann, 1973). However, the ease and economy of this procedure supported its use in these studies.

3.3 Chemicals

The chemicals included in the study (See Table 2) were a group of volatile and semi-volatile organic compounds from Appendix VIII (U.S. Environmental Protection Agency, 1980) of the Resource Conservation and Recovery Act (RCRA) and are commonly found in hazardous waste mixtures. Microoganisms and their enzymes must operate in aqueous environments. As a group, the compounds were relatively insoluble in water and this presented a difficult problem. Although the structure of some organic compounds would permit them to be rapidly degraded,

Table 2. Chemical Abstract Numbers (CAS No.) for the organic compounds used in studies of microbial degradation in soils.

Chemical	CAS No.
Methyl ethyl ketone	78-93-3
Tetrahydrofuran	109-99-9
Chlorobenzene	108-90-7
Benzene	71-43-2
Chloroform	67-66-3
Carbon tetrachloride	56-23-5
p-Xylene	106-42-3
1,2-Dichlorobenzene	95-50-1
cis-1,4-Dichloro-2-butene	764-41-0
1,2,3-Trichloropropane	96-18-4
2-Chloronaphthalen	91-58-7
Ethylene dibromide	106-93-4
Hexachlorobenzene	118-74-1
1,2,4,5-Tetrachlorobenzene	98-95-3
Nitrobenzene	98-95-3
Toluene	108-88-3

their limited water solubility can hinder their decomposition (Huddleston et al., 1986).

The study compounds, purchased from the vendors indicated, and their respective purities are as follows: 1,2-dibromoethane (ethylene dibromide) (99+%); 1,2,3-trichloropropane (99+%); p-xylene (99+%); 1,2,4,5-tetrachlorobenzene (98%); hexachlorobenzene (97%); cis-1,4-dichloro-2-butene (95%) were obtained from Aldrich Chemical Co., Inc., Milwaukee, WI. In addition, Fisher Scientific, Pittsburgh, PA, provided reagent grade carbon tetrachloride, tetrahydrofuran, methyl ethyl ketone, and chlorobenzene. Nitrobenzene (reagent grade), 2-chloronaphthalene, and 1,2-dichlorobenzene (99%) were supplied by Eastman Kodak Co., Rochester, NY. Toluene and benzene (both analytical reagent grade) were obtained from Mallinkrodt Co., Paris, KY. EM Science, Cherry Hill, NJ, supplied chloroform (99%) and Sigma Chemical Co., St. Louis, MO, provided ^{14}C-Toluene (99+%).

Selected physicochemical properties of the chemicals are listed in Table 3.

3.4 Chemical Toxicity

High concentrations of potentially degradable compounds that are toxic to soil microorganisms may persist in soils simply because they depress or inhibit microbial activity. Successful microbial degradation studies require that the chemicals of interest be placed on the soil at a concentration that does not surpass the assimilative capacities of the soil. Studies undertaken in our laboratory to

Table 3. Selected physicochemical properties of the organic compounds used in the studies of microbial degradation in soils.

Chemical	MW[a]	$\log_{10}K_{ow}$[b]	VP[c]	H_2O Solubility (M/liter)	$^1\chi$[d]
Methyl ethyl ketone	72	0.28	74	353,000	
Tetrahydrofuran	72	0.46	0.173	300,000	
Chlorobenzene	113	2.83	8.8	500	3.394
Benzene	78	1.95	76	1,780	3.000
Chloroform	119	1.97	160	8,000	
Carbon tetrachloride	154	2.64	90.5	800	
p-Xylene	106	3.15	6.5	175	3.788
1,2-Dichlorobenzene	147	3.38	1.08	100	3.805
cis-1,4-Dichloro-2-butene	125	----	----	----	
1,2,3-Trichloropropane	147	2.01	2	16	
2-Chloronaphthalene	163	----	0.017	6.74	
Ethylene dibromide	188	1.76	11	4,310	
Hexachlorobenzene	285	6.35	0.0000109	0.29	5.464
1,2,4,5-tetrachlorobenzene	216	4.93	44	0.692	4.609
Nitrobenzene	123	1.87	0.15	1,900	
Toluene	92	2.50	22	515	3.394

[a]Molecular weight.
[b]National Library of Medicine. 1986. Toxicology Data Bank.
[c]From vapor pressure(mm Hg).
[d]Molecular connectivity ($^1\chi$) as reported in Sabljic, 1986.

establish safe loading rates for each individual chemical (Walton et al., in press b.) aided in this requirement, however, additional data on a safe concentration for a mixture of all 16 compounds was needed.

Carbon dioxide evolution, which is occasionally used as a non-specific test for biodegradation (Lyman et al, 1982), can be quite useful for assessing the toxicity of chemicals to soil microorganisms simply by comparing the respiration rates of treatment soils relative to controls. An infrared gas analyzer (Model 300, Mine Safety Appliances Co., Pittsburgh, PA) was used to measure CO_2 efflux over a 7-day period from incubated (20°C) 50-g soil samples according to the methods of Edwards (1982). A mixture of all 16 compounds was introduced into the soils gravimetrically (μg chemical/g soil dry weight) however, soils were not mixed so that losses due to volatilization would be minimized. Every 24 hours a flow of moist, CO_2-free air (See Figure 1) was passed through the 8 x 5-cm incubation jars to measure CO_2 efflux and also to maintain aerobic conditions. Toxicity determinations were based on whether microbial respiration in the treatment soils had returned to control levels by the end of the 7-day experiment. From the results of this study, a loading rate of 100 μg chemical/g soil dry weight was selected. In other words, all 16 compounds were added from a stock solution to the 50-g soil samples such that their final concentration was 100 parts per million (ppm).

3.5 Degradation Measurements

Degradation experiments were carried out using equipment and conditions similar to those described previously for toxicity

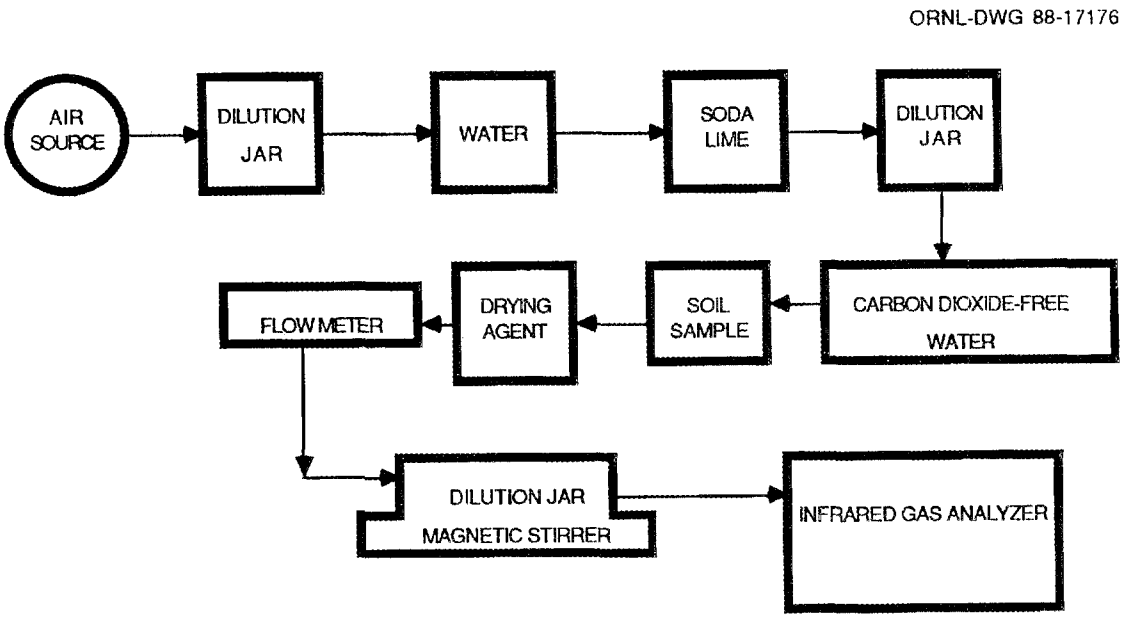

Figure 1. Flow diagram (adapted from Edwards, 1982) for determining CO_2 evolution from incubated soil samples using an infrared gas analyzer. The inlet air was bubbled through water, then passed through soda lime to remove CO_2. The CO_2-free water (boiled and flushed with N_2) was used to moisten the air passing through the sample jar. The CO_2 evolved from the soil samples, was dried, and then diluted by a known volume of air (250 mL) before entering the infrared gas analyzer.

determinations (Walton et al., in press a.). In addition, the incubation jars were fitted with charcoal traps (459004, Mine Safety Appliances Co., Pittsburgh, PA) to account for headspace vapors so that mass balances could be calculated. Because cost prohibited the use of radiolabelled chemicals for determining degradation rates, the loss of individual, non-radiolabelled chemicals from the soil was measured over time. Soil samples (50 g) were incubated in the dark at 20°C in 8 x 5-cm glass jars fitted with teflon-lined stoppers and charcoal traps (See Figure 3). Soils were adjusted to 80% moisture content with sterile, distilled, deionized water. Sterile controls were obtained by autoclaving as described above (Section 3.2). The test chemicals were introduced in a mixture at 100 μg chemical/g soil dry weight by pipetting a known volume of standard solution of liquids after weighed amounts of the solid chemicals (1,2,4,5-tetrachlorobenzene, hexachlorobenzene, 2-chloronaphthalene) were added to the soils. The incubation jars were immediately stoppered and incubated in the dark at 20°C. Every 24 hours, the jars were removed from the incubator and flushed with a stream of air (Figure 2) that had passed through a sterile cotton plug and a charcoal trap. The exit air passed through the charcoal tubes fastened to the incubation jars. The sample jars were disassembled and the soil extracted in toto by the procedure described below (Section 3.7) on days zero, two, three, six, and seven of the experiment.

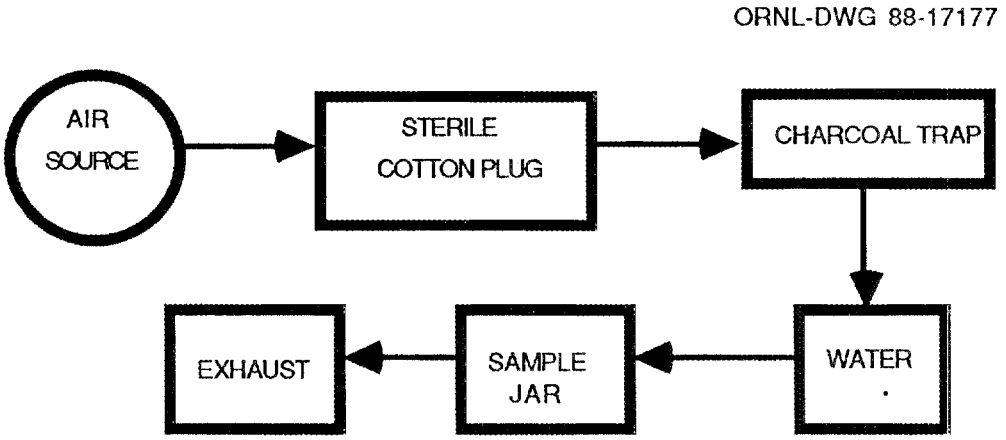

Fig. 2. Flow diagram for the method of aerating soil samples used in the study of microbial degradation. Air was passed through a sterile cotton plug to remove air-borne microorganisms and a charcoal trap to filter out any organic contaminants in the air source. Water contained in 250 mL erlenmeyer flasks helped moisten the air to prevent drying of the soil samples. After passage through the sample jar, the air was vented into a fume hood.

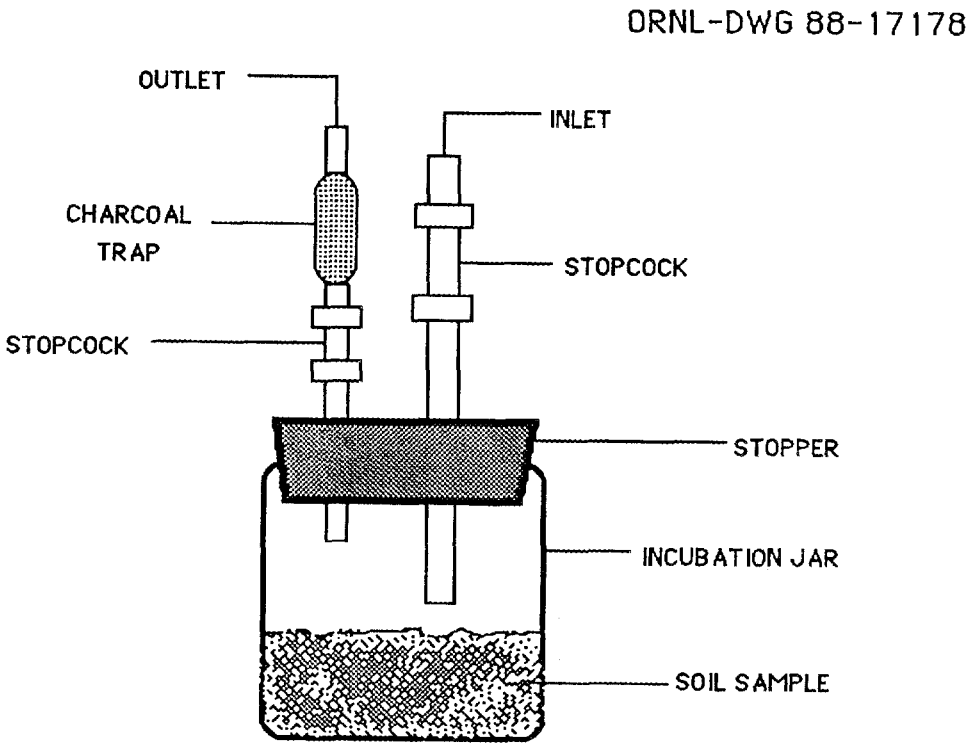

Fig. 3. Incubation jar (not to scale) used in the study of microbial degradation in soil. Glass sample jars (8 x 5 cm) were equipped with teflon-lined rubber stoppers. The organic compounds were applied in a mixture to the 50-g soil samples. Sterile, moist air was passed over the soil, via the inlet stopcock, every 24 hours to maintain aerobic conditions and trap headspace vapors in the charcoal traps.

3.6 Sorption and Degradation of ^{14}C-Toluene

Experiments designed to trace the fate of a representative chemical (i.e., ^{14}C-toluene) were conducted under conditions identical to those used for the degradation experiments. The purpose of these experiments was to determine possible mechanisms (e.g., irreversible sorption to the soil) contributing to unaccountable losses of compounds. A standard mixture of all 16 chemicals (^{14}C toluene replaced toluene) was placed on sterile Captina silt loam soil such that the final concentration of each individual chemical was 100 μg chemical/g soil dry weight. Samples were incubated at 20°C in the dark and were flushed with air (see description above) every 24 hours. On days 0 and 7, triplicate samples were extracted with methanol for subsequent analysis. To obtain a mass balance, ^{14}C remaining in the soil was determined (described in Section 3.8).

3.7 Organic Chemical Extraction From Soil

Extracting volatile organic chemicals from soil samples produces several complications. The most common solvent used to extract organics from soils, methylene chloride, could not be used in this study because of interference during gas chromatographic separation and analysis of the analytes. In addition, sonication and soxhlet extraction, two common methods often employed to extract chemicals from soil, could not be used because of the temperatures involved. The heat generated by the probe during sonication provides a potential loss mechanism for volatile compounds. Soxhlet extraction was inappropriate for this study because it depends on a continuous flux

of solvent cycling through the soil sample by volatilization and condensation. The high vapor pressures of the compounds in this study would prevent their concentration in the collection flask. Also, the volume of extracting solvent is critical in that it must be sufficient to reach all the microsites in the soil yet must not dilute the analytes below detectable limits. Based on these difficulties, method development was needed to achieve acceptable recovery of the analytes from the soil without losses due to volatilization, and without excessive dilution by the extraction solvent.

Preliminary experiments designed to determine an appropriate solvent and solvent volume based on detection and percent recoveries of the chemicals showed methanol to be acceptable. Triplicate extractions with methanol (30 mL and 45 mL total volume for 50-g samples of the McLaurin sandy loam and Captina silt loam, respectively) gave reproducible recoveries of the analytes (See Tables 4 and 5). The extraction procedure was as follows: Methanol was introduced by pipeting a known volume (15 mL and 10 mL for 50-g samples of the Captina silt loam and McLaurin sandy loam, respectively) into the 8 x 5-cm glass incubation jars. The jars were mechanically agitated with a stirring rod for approximately 2 minutes then vortexed (Scientific Products Model K-550-G) for 1 minute. Samples were centrifuged (Beckman Model J-21) at 1500 revolutions per minute (rpm) for 15 minutes. The liquid extract was transferred into 15-mL glass centrifuge tubes and spun on a table-top centrifuge

Table 4. Average percent recoveries for the test compounds in the McLaurin sandy loam based on 3 determinations. Soil samples were extracted with methanol by mechanical agitation. After two centrifugation steps, the volume of extract was measured and decanted into a marked, 20-mL, glass storage vial. The above procedure was repeated 2 more times for a total of 3 extractions, and these were pooled for the percent recovery determinations.

Chemical	Mean % recovery	S.D.[1]
Methyl ethyl ketone	74	6.6
Tetrahydrofuran	67	6.1
Chlorobenzene	104	0.7
Benzene	69	7.9
Chloroform	71	9.0
Carbon tetrachloride	58	8.1
p-Xylene	103	7.1
1,2-Dichlorobenzene	76	3.5
cis-1,4-Dichloro-2-butene	123	19.1
1,2,3-Trichloropropane	115	6.4
2-Chloronaphthalene	78	1.5
Ethylene dibromide	98	5.7
Hexachlorobenzene	40	1.5
1,2,4,5-Tetrachlorobenzene	40	13.2
Nitrobenzene	88	7.2
Toluene	93	8.6

[1]One standard deviation of the mean based on triplicate experiments.

Table 5. Average percent recoveries for the test compounds in the Captina silt loam based on 3 determinations. Soil samples were extracted by adding methanol followed by mechanical agitation. After two centrifugation steps, the volume of extract was measured and decanted into a marked, 20-mL, glass storage vial. The above procedure was repeated 2 more times for a total of 3 extractions. These 3 extracts were pooled to determine percent recovery.

Chemical	Mean % recoveries	S.D.[1]
Methyl ethyl ketone	72	2.1
Tetrahydrofuran	70	4.4
Chlorobenzene	114	19.1
Benzene	64	4.5
Chloroform	73	11.4
Carbon tetrachloride	65	20.0
p-Xylene	97	0.0
1,2-Dichlorobenzene	68	5.9
cis-1,4-Dichloro-2-butene	114	5.7
1,2,3-Trichloropropane	112	0.0
2-Chloronaphthalene	72	6.1
Ethylene dibromide	90	1.4
Hexachlorobenzene	28	4.0
1,2,4,5-Tetrachlorobenzene	28	12.5
Nitrobenzene	83	6.1
Toluene	93	12.1

[1]One standard deviation of the mean based on triplicate experiments.

(International Clinical Centrifuge) for 5 minutes. The volume of liquid was measured and decanted into a marked, 20-mL glass storage vial. Aliquots (1 mL) used for analysis were placed into 1-mL amber glass vials and sealed with crimp caps equipped with teflon-lined septa. The remaining volume was archived. The entire procedure was repeated two more times for a total of three extractions. All chemicals were stored at 4°C until analysis (no longer than 14 days).

3.8 Chemical Analyses

Chemical analyses of methanol extracts and charcoal traps used to quantify the rate of disappearance of parent chemicals from soil samples were conducted by the Analytical Chemistry Division at Oak Ridge National Laboratory under the direction of Dr. Wayne Griest.

The volatile organics (tetrahydrofuran, chloroform, carbon tetrachloride, benzene, methyl ethyl ketone, ethylene dibromide, chlorobenzene, p-xylene, cis-1,4-dichloro-2-butene, toluene, and 1,2,3-trichloropropane) were analyzed by packed column gas chromatography according to chromatographic conditions specified in U.S. EPA Method 624 (1984). A Hewlett-Packard Model 5840 gas chromatograph equipped with a flame ionization detector (FID), a 2.4 m x 3.2 mm OD stainless steel column packed with 1% SP-1000 on 60/80 mesh Carbopak B, and a model 7671A sampler were used. The conditions were as follows: Injector temperature, 200°C; FID temperature, 250°C; column temperature program, 55°C for 12 minutes to

220°C at 8°C/minute and hold at 220°C for 20 minutes; injection volume was approximately 1.5 μL.

After calibration of the instruments, the methanol extracts were injected automatically and the concentration of each organic compound was determined by the method of external standards. The results were reported in μg/mL for the soil extracts and mg/mL for the standards used to treat the soils. Methanol blanks were also analyzed with each set of samples to check the background of the instrument.

The semi-volatile organics (2-chloronaphthalene, 1,2,4,5-tetrachlorobenzene, hexachlorobenzene, nitrobenzene, and 1,2-dichlorobenzene) were analyzed by reverse phase liquid chromatography. A Hewlett-Packard Model 1090 liquid chromatograph equipped with a UV absorbance detector, a 0.1 m x 4.6 mm ID reverse phase C18 column, and an autosampler were used for these determinations. The operating conditions for the high performance liquid chromatography (HPLC) procedure were as follows: Solvent A, water; solvent B, methanol; flow rate, 2.0 mL/minute; solvent program, isocratic 30/70 (A/B) for 20 minutes, then 100% B for 10 minutes; run time, 30 minutes; detection, UV absorbance at 254 nm; injection volume, 25 μL. The concentration of each component was determined by peak areas and reported in the units described above.

The organic compounds collected on the charcoal tubes were analyzed using the procedure P & CAM 127, "Organic Solvents in Air" described by the Occupational Safety and Health Agency (1977) with modifications to improve resolution and sensitivity. The charcoal

tubes were unpacked into 2.0 mL vials and extracted for 0.5 hr with 1 mL of carbon disulfide. Separate extractions and analyses were made of the front and backup portions of the traps. HPLC was used to analyze the least volatile compounds by the procedures described above. The compounds with volatilities less than and including nitrobenzene were separated using capillary column gas chromatography. A Hewlett-Packard model 5880 gas chromatograph equipped with a flame ionization detector, a level IV data system , and a 60 m x 0.32 mm ID fused silica column with 5-μm bonded film of RSL160. The column was temperature programmed from 40°C (12 minute isothermal hold) to 175°C at 8°C/minute, with a 22 minute final isothermal hold at 175°C. The carrier gas was hydrogen with a head pressure of 57 kPa. One μL of the diluted extract was quantified by the method of external standards and was corrected for a charcoal tube/CS_2 blank.

Analysis of the ^{14}C-toluene methanol extracts was done in our laboratory by liquid scintillation spectroscopy (Packard Model 2000CA). Methanol extracts (1 mL) from the soil samples were added to 10 mL Aqueous Counting Scintillant (ACS) (Amersham, Arlington Heights, IL) and analyzed for radioactivity. Subsamples of soil (10 g) were extracted twice with 35 mL of 0.5 M NaOH at 30°C to remove the humic and fulvic acids (Parsons, 1988; Graveel et al., 1985). Aliquots (200 μL) of the NaOH extract were added directly to 10 mL ACS (Graveel et al., 1985) for subsequent radioactivity determination. The charcoal traps were extracted four times with 5 mL of CS_2 for variable lengths of time. Separate extractions were made of the inlet and

outlet halves of the charcoal traps. Aliquots (200 μL) of the CS_2 extracts were placed in 10 mL ACS and analyzed for radioactivity. The total amount of ^{14}C remaining was then calculated for use in determining total percent recovery of the radiolabel.

3.9 Degradation Kinetics

Data obtained from the degradation experiments described previously were used to calculate the half-life and degradation rate constant for each of the test compounds. Kinetic expressions to model the loss of a compound over time have been generally described by two rate laws: the hyperbolic rate law and the power rate law (Scow, 1982; Morrill et al., 1982; Larson, 1980). Although one rate law may not adequately model the loss of a compound over the entire degradation curve, it is usually assumed to do so.

The hyperbolic rate law, which is commonly used to describe microbial population growth on a single substrate and many enzyme reactions (Larson, 1980), is based on Monod kinetics where the reaction rate is a hyperbolic function of substrate concentration:

$$\text{rate} = (\text{Umax})[S] \;/\; \text{Km} + [S] \qquad (2)$$

where Umax is the maximum microbial growth rate, [S] is the concentration of substrate, and Km is the substrate concentration at Umax/2. Monod kinetics can be used to express substrate disappearance

by the addition of a yield coefficient (Yd = d[B]/d[S]) (Baughman et al., 1980; Scow, 1982), where [B] is the microbial population concentration and [S] is the substrate concentration. The rate of disappearance then becomes:

$$\text{rate} = (\text{Umax})[B][S] \;/\; (\text{Yd})(\text{Km} + [S]) \qquad (3)$$

If one assumes that Km is much greater than [S] in the denominator, then [S] can be ignored. In addition, Umax / (Yd)(Km) is equivalent to a second order rate constant k_{el} (Paris et al., 1975). The simplified rate expression then becomes a function of the microorganism and substrate concentrations:

$$\text{rate} = k_{el}[B][S] \qquad (4)$$

Finally, if one assumes that the microbial population concentration, [B], remains constant during the degradation experiment, the kinetic expression simplifies further to the first-order expression:

$$\text{rate} = k_{el}[S] \qquad (5)$$

First-order reaction kinetics, which are applicable to pesticides (Morrill et al., 1982), are a common assumption at low pollutant concentrations or if the kinetic relationship is not well

understood (Scow, 1982). It also allows the simple calculation of half-life ($t_{1/2}$) by the following equation:

$$t_{1/2} = 0.693/k_{el}. \quad (6)$$

Based on these features, first-order reaction kinetics were selected for the degradation rate data in this study.

Plots of the $\log_{10}$ total % remaining (soil + trap) vs. time were made for each chemical using Cricket Graph software on an Apple Macintosh Plus personal computer. The program derived a least squares linear regression equation from the data, and the slope of this equation was used to calculate the first-order degradation rate constant for each chemical from the following relationship (Klaassen, 1986) :

$$k_{el} = -\text{slope} \times 2.303 \quad (7)$$

Once the rate constants had been obtained, the half-lives of each compound were calculated from the relationship described in equation (6).

3.10 Analysis for QSAR

QSAR analyses were done using linear regression techniques on Cricket Graph software. The purpose was to check for a relationship between half-lives or degradation rate constants and selected

physicochemical properties of the compounds in the study. Simple linear regression, rather than multiple regression, was used because the parameters are not orthogonal.

3.11 Incorporation of Data into a Prebiological Screen

The environmental impact of the organic compounds in the study was evaluated by criteria outlined in Gillett (1983) where the exposure hazard of a chemical is a function of its environmental concentration. The predicted environmental concentration (PEC) of a chemical is dependent on its partitioning between media and its lifetime in the medium of concern. Therefore, parameters that can be used to predict partitioning (K_{ow} and Henry's law constants) and data on half-lives of chemicals in soil or water can be used to predict potential environmental impacts based on the general criteria described by Gillett as follows:

- Chemicals with $t_{1/2} < 0.1$ days are unlikely to cause environmental problems.
- Chemicals with $t_{1/2} < 4$ days are degraded within a fraction of a plant's growing season or a fraction of the lifetime of vertebrates and invertebrates, therefore, are of little ecotoxicologic concern.
- Chemicals with $\log K_{ow} < 2.5$ are not bioaccumulated in terrestrial animals.
- Chemicals with $t_{1/2} > 14$ days and $\log K_{ow} > 3.5$ are of concern regarding chronic exposure and bioaccumulation.

The Prebiological Screen (PBS) is an IBM-compatible program written in Microsoft BASIC. The program was loaded from a floppy disc and run on an IBM-AT personal computer. The three critical parameters for each chemical; $\log_{10}$ half-life (experimentally determined), $\log_{10}$ octanol-water partition coefficient (Table 3), and $\log_{10}$ Henry's Law constant (calculated by the computer program), were inputted from the keyboard. Although the program is capable of providing testing recommendations, data comparisons, and data storage, the output of interest consisted of a three-letter code termed the Comprehensive Index. This index was used to assess the potential environmental impact of the organic compounds in this study by the criteria illustrated in Gillett (1983), Figures 1-3.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Biological Degradation

Experimentally measured half-lives and (see Table 6) were relatively short for the 16 organic compounds as a group. The first-order degradation rate constants from which the half-lives were calculated are given in Table 7. Because half-life is a more common measure of persistence, the discussion is restricted to the latter parameter. The relationship between half-life and first-order degradation rate constant is described in Section 3.9. Shorter half-lives were observed in the McLaurin sandy loam for the entire set of compounds. Nitrobenzene ($t_{1/2}$ = 23.1 d) and 2-chloronaphthalene ($t_{1/2}$ = 21.7 d) were the most recalcitrant chemicals in the Captina silt loam while hexachlorobenzene ($t_{1/2}$ = 10.8 d) and 2-chloronaphthalene ($t_{1/2}$ = 7.9 d) remained the longest in the sandy loam. Chlorobenzene, p-xylene, ethylene dibromide, chloroform, and cis-1,4-dichloro-2-butene were among the least persistent chemicals in both soils.

Half-lives and degradation rate constants found in the literature for some of these organic compounds are shown in Table 8. Although hexachlorobenzene and nitrobenzene were two of the most persistent chemicals in this study, their half-lives were considerably shorter than those observed by Beck and Hansen (1974), and Freitag et al., (1985), especially for hexachlorobenzene. However, the half-life

Table 6. Degradation half-lives for test chemicals in the Captina silt loam and McLaurin sandy loam in the dark at 20°C. Half-lives were obtained for both sterile (autoclaved) and non-sterile samples within each soil type and were calculated from the first-order kinetic expression, $t_{1/2} = 0.693/k_{el}$.

Chemical	Half-life (d)			
	Captina silt loam		McLaurin sandy loam	
	Non-sterile	Sterile	Non-sterile	Sterile
Methyl ethyl ketone	6.5	8.2	4.1	4.1
Tetrahydrofuran	7.4	10.8	4.8	4.8
Chlorobenzene	2.7	2.0	1.8	1.8
Benzene	3.1	3.3	2.4	2.4
Chloroform	2.8	3.3	2.1	2.2
Carbon tetrachloride	3.2	3.5	2.5	2.4
p-Xylene	2.8	1.9	1.6	1.9
1,2-Dichlorobenzene	5.3	6.5	3.4	7.0
cis-1,4-Dichloro-2-butene	2.5	1.9	1.9	1.8
1,2,3-Trichloropropane	4.0	2.4	2.3	2.8
2-Chloronaphthalene	12.6	16.9	7.9	9.1
Ethylene dibromide	3.1	2.4	1.9	2.0
Hexachlorobenzene	13.6	10.8	10.8	11.6
1,2,4,5-Tetrachlorobenzene	12.6	5.0	5.5	6.2
Nitrobenzene	23.1	11.2	5.8	5.8
Toluene	2.6	2.5	2.0	2.2

Table 7. First-order degradation rate constants for the test chemicals in the Captina silt loam and McLaurin sandy loam in the dark at 20°C. Rate constants were obtained for both sterile (autoclaved) and non-sterile samples within each soil type by fitting a linear plot to the data and multiplying the corresponding slope by 2.303 (ln 10).

Chemical	Degradation rate constants			
	Captina silt loam		McLaurin sandy loam	
	Non-sterile	Sterile	Non-sterile	Sterile
Methyl ethyl ketone	0.106	0.085	0.168	0.168
Tetrahydrofuran	0.094	0.064	0.145	0.145
Chlorobenzene	0.260	0.341	0.389	0.378
Benzene	0.223	0.207	0.292	0.290
Chloroform	0.251	0.210	0.329	0.320
Carbon tetrachloride	0.216	0.196	0.272	0.290
p-Xylene	0.246	0.362	0.432	0.366
1,2-Dichlorobenzene	0.131	0.106	0.205	0.099
cis-1,4-Dichloro-2-butene	0.281	0.359	0.373	0.375
1,2,3-Trichloropropane	0.173	0.286	0.302	0.244
2-Chloronaphthalene	0.055	0.041	0.088	0.076
Ethylene dibromide	0.226	0.288	0.364	0.350
Hexachlorobenzene	0.051	0.064	0.064	0.060
1,2,4,5-Tetrachlorobenzene	0.055	0.138	0.125	0.111
Nitrobenzene	0.030	0.062	0.120	0.120
Toluene	0.266	0.276	0.345	0.313

Table 8. Degradation half-lives of some organic compounds found in the literature.

Chemical	Half-life (days)	System	Source
Benzene	3.1 to 23	NA	Wakeham et al., 1983
Benzene	5.7	sediment and light	Freitag et al., 1985
Carbon tetrachloride	29.1	sediment and light	Freitag et al., 1985
Chlorobenzene	75	sediment	Lee and Ryan, 1979
Chlorobenzene	4.6 to 21	NA	Wakeham et al., 1983
Chlorobenzene	5	sediment and light	Freitag et al., 1985
Hexachlorobenzene	1386	soil	Beck and Hansen, 1974
Hexachlorobenzene	156.3	sediment and light	Freitag et al., 1985
Nitrobenzene	35.2	sediment and light	Freitag et al., 1985
Toluene	.7 to 1.7	soil	Overcash et al., 1982
Toluene	7	soil	Berkowitz et al., 1981
Toluene	7.2	sediment and light	Freitag et al., 1985

for nitrobenzene in the non-sterile Captina silt loam (23.1 days) is similar to the half-life (35.2 days) reported by Freitag and co-workers (1985).

Wakeham and co-workers (1983) found half-lives of chlorobenzene and benzene ranged from 4.6 to 21 days and 3.1 to 23 days, respectively, depending on the season. The observed half-lives for chlorobenzene (1.8 to 2.7 days) and benzene (2.4 to 3.3 days) in this study, conducted at 20°C, were similar but slightly less than the lower values of the 1983 study. In addition, Freitag and co-workers (1985) found half-lives of 5 and 5.7 days for chlorobenzene and benzene, respectively, also similar to results observed in our laboratory. However, Lee and Ryan (1979) observed a longer half-life (75 days) for chlorobenzene in sediment samples.

Half-lives for toluene degradation have ranged from .7-1.7 days (Overcash et al., 1982), to 7 days (Berkowitz et al., 1981), to 7.2 days (Freitag et al., 1985). These values are somewhat similar to the half-life of toluene observed in this study (2.0 to 2.6 days). Carbon tetrachloride was readily degraded in this study, however, it was more persistent in sediment samples (29.1 days) as observed by Freitag et al., (1985).

Disappearance curves for the 16 compounds can be found in Walton et al., in press b). Visual inspection of the graphs showed that differences in rate constants and half-lives between sterile and non-sterile soil samples were indistinguishable, similar to results found by Cantier et al., (1986) and Getzin (1968). Determination of

microbial colonies on nutrient agar plates revealed that samples used in the present study were devoid of microbial activity, possibly indicating that during the experimental time frame (7 days), non-biological factors were a more important loss mechanism than microbial degradation. Similar conclusions were drawn by Getzin (1968) during studies on the microbial degradation of diazinon (diethyl-2-isopropyl-4-methyl- 6-pyrimidinyl phosphorothionate) in soil. He found that degradation rates for diazinon were similar in autoclaved and non-autoclaved Sultan silt loam soil samples and concluded that nonbiological degradative mechanisms were primarily responsible for the breakdown of diazinon in this soil type. Cantier and co-workers (1986) also found disappearance of organic compounds (propyzamide analogues) in sterile soil samples. Another possible explanation for the nonrecoverability of compounds could be the pre-analysis storage conditions used. Data from Dr. Wayne Griest (personal communication) of the Analytical Chemistry Division at Oak Ridge National Laboratory indicate a continuous, unexplained loss of some volatile compounds including chloroform, carbon tetrachloride, benzene, toluene, and chlorobenzene from the McLaurin sandy loam soil at -20°C over a 28 day period. Since the EPA protocol for handling volatile organics calls for holding samples within 14 days when stored at a higher temperature (4°C), the possible loss of compounds via this mechanism must be considered likely.

In addition, most of the graphs are bi-phasic showing an initial fast phase followed by a slower phase, similar to drug elimination

curves in a two compartment model where there is a fast distribution phase preceded by a slower elimination phase. This would seem to indicate that distribution of the chemicals into the soil organic fraction occurs initially, and that irreversible partitioning may account for their non-recoverability. Although exhaustive extractions with methanol and methylene chloride did not recover additional analytes, the possibility of irreversible sorption could not be eliminated. It is known that lipophilic organic chemicals added to soil or sediment become increasingly difficult to desorb with time (Wu and Gschwend, 1986; Di Toro and Horzempa, 1982) and can possibly become physically entrapped in soil micropores (Sawhney et al., 1987). Although in instances where organic compounds sorb tightly to soil organic matter it is difficult to make inferences about possible microbial degradation of these compounds (adsorption by organic matter can either prevent or promote degradation), it is likely that these compounds have been immobilized by the soil and are unlikely to migrate into the hydrosphere (i.e., groundwater) except perhaps during severe thunderstorms. Pulses of dissolved organic carbon (DOC) can move through soil pores during large rainstorm events (P.M. Jardine, ORNL, personal communication). Liu (1980) noted that 77% of petroleum hydrocarbon degradation in silt loam soil occurred within the top 1 cm, which suggests the ability of this soil to effect degradation as well as retain organic compounds. Both of these features are important considerations in selecting a land treatment site. A high degree of adsorption can also decrease the volatilization of organic

compounds from soil (Thomas, 1982), thus reducing the exposure hazard of organic vapors.

4.2 ^{14}C-Toluene Degradation

The degradation experiment with ^{14}C-toluene was undertaken in an attempt to determine whether irreversible binding of the test chemicals to the soil was a probable reason for non-recoverability of compounds in the sterile soil samples. Radioactivity determinations of the methanol, NaOH, and CS_2 extracts indicated that on day 7 most (> 65%) of the recoverable radioactivity from the sterile Captina silt loam was associated with humic and fulvic acids in the soil. Only about 4% was recovered in the methanol extracts and 20% in the charcoal traps. In addition, the charcoal trap data raise an interesting point. The procedure used by the ORNL Analytical Chemistry Division in this study to remove the organic compounds from the charcoal traps (described in section 3.8) calls for one extraction with CS_2 for 0.5 hr. However, four CS_2 extractions, over 0.5, 1, 24, and 48 hours, showed that increasing radioactivity could be recovered with increasing extraction time. Less than 6% of the total radioactivity recovered from the charcoal traps was desorbed during the 0.5 hour extraction. Although the Analytical Chemistry Division determined the efficiency of the CS_2 extraction procedure with a short equilibration spike-recovery experiment, aging over the 7-day experiment plus time before analysis may have reduced the extraction efficiency. Since hydrophobic organic compounds in soil become

increasingly difficult to desorb with time (Wu and Gschwend, 1986; Di Toro and Horzempa, 1982) it would seem logical that organic compounds sorbed on charcoal traps could follow this same scenario. Whether this potential loss mechanism could account for all of the non-recoverable radioactivity is highly unlikely. A combination of this mechanism, chemical degradation, and irreversible sorption all appeared to contribute to the lack of total recovery of the test chemicals from sterile soils.

4.3 QSAR Analysis

Although attempts to correlate degradation rate constants and half-lives with physicochemical properties for all the test chemicals (N = 16) yielded poor results (See Tables 9 and 10), similar analysis for a subset of benzene derivatives (N = 7) produced good correlations in both soils (See Tables 11 and 12). A similar SAR using $\log_{10} K_{ow}$ values was also found to describe microbial toxicity (Walton, et al., in press a.). These correlations help to illustrate the applicability of QSARs in soil systems provided the researcher does not overextend the extrapolation boundaries of this technique. The QSAR approach works well under situations were one process is dominant (e.g., partitioning in soil sorption) or when standard experimental procedures are employed to minimize competing processes. Thus, if soil biodegradation experiments are carried out under defined procedures that minimize or account for competing loss processes, SARs for biodegradation in soils can be developed despite the complexity of

Table 9. Least squares linear regression coefficients (r) for physicochemical properties of all the test compounds (N=16) when correlated with degradation half-lives.

Parameter	Captina silt loam		McLaurin sandy loam	
	Sterile	Non-sterile	Sterile	Non-sterile
$\log_{10} K_{ow}$	0.03	0.26	0.61	0.55
Vapor pressure	0.18	0.30	0.33	0.25
Molecular weight	0.20	0.37	0.63	0.61
$\log_{10}$ M.W.	0.13	0.33	0.54	0.58
$\log_{10}$ H_2O solubility	0.11	0.29	0.55	0.48

Table 10. Least squares linear regression coefficients (r) for physicochemical properties of the test compounds (N=16) when correlated with first-order degradation rate constants.

Parameter	Captina silt loam		McLaurin sandy loam	
	Sterile	Non-sterile	Sterile	Non-sterile
$\log_{10} K_{ow}$	0.03	0.22	0.30	0.24
Vapor Pressure	0.09	0.28	0.23	0.09
Molecular weight	0.21	0.39	0.40	0.39
$\log_{10}$ M.W.	0.12	0.31	0.32	0.29
$\log_{10}$ H_2O solubility	0.02	0.28	0.34	0.26

Table 11. Least squares linear regression coefficients (r) for physicochemical properties of 7 benzene derivatives[1] when correlated with degradation half-lives.

Parameter	McLaurin sandy loam		Captina silt loam	
	Sterile	Non-sterile	Sterile	Non-sterile
$\log_{10} K_{ow}$	0.83	0.94	0.89	0.93
Vapor pressure	0.30	0.09	0.33	0.22
Molecular weight	0.88	0.96	0.93	0.95
$\log_{10}$ M.W.	0.84	0.94	0.90	0.89
$\log_{10}$ H_2O solubility	0.77	0.98	0.85	0.88
Molecular connectivity	0.83	0.93	0.89	0.93

[1]Benzene, chlorobenzene, 1,2-dichlorobenzene, hexachlorobenzene, 1,2,4,5-tetrachlorobenzene, toluene, and p-Xylene.

Table 12. Least squares linear regression coefficients (r) for physicochemical properties of 7 benzene derivatives[1] when correlated with first-order degradation rate constants.

Parameter	McLaurin sand loam		Captina silt loam	
	Sterile	Non-sterile	Sterile	Non-sterile
$\log_{10} K_{ow}$	0.65	0.86	0.76	0.80
Vapor pressure	0.02	0.09	0.13	0.01
Molecular weight (MW)	0.74	0.91	0.82	0.87
$\log_{10}$ MW	0.71	0.92	0.83	0.85
$\log_{10}$ H_2O solubility	0.68	0.91	0.79	0.84
Molecular connectivity	0.65	0.86	0.76	0.79

[1]Benzene, chlorobenzene, 1,2-dichlorobenzene, hexachlorobenzene, 1,2,4,5-tetrachlorobenzene, toluene, and p-Xylene.

the matrix (Walton and Anderson, in press). In situations where more than one loss process appears to be in operation, multiple QSARs may be needed to describe this behavior.

4.4 Prebiological Screen

The results of the prebiological screen are summarized in Table 13. From the exposure hazard criteria outlined in Figure 1 (chronicity and bioaccumulation) of Gillett (1983) and the PBS program, the following inferences were made for the organic compounds in the Captina silt loam soil. Benzene, chloroform, and ethylene dibromide should cause no ecotoxicologic concerns regarding chronic exposure or bioaccumulation. Chlorobenzene, carbon tetrachloride, p-xylene, 1,2-dichlorobenzene, methyl ethyl ketone, tetrahydrofuran, toluene, and 1,2,3-trichloropropane have the potential for bioaccumulation and chronic effects. Those chemicals having a high potential for persistence and bioaccumulation would be nitrobenzene, hexachlorobenzene, 1,2,4,5-tetrachlorobenzene, and 2-chloronaphthalene.

In the McLaurin sandy loam soil, benzene, toluene, chloroform, ethylene dibromide, and 1,2,3-trichloropropane should pose no ecotoxicologic concerns regarding chronic effects or bioaccumulation. Methyl ethyl ketone, tetrahydrofuran, nitrobenzene, chlorobenzene, p-xylene, carbon tetrachloride, and 1,2-dichlorobenzene however, are predicted to pose ecotoxicologic problems due to both bioaccumulation and persistence. Hexachlorobenzene, 2-chloronaphthalene, and

Table 13. Results of the pre-biological screen (PBS) program and exposure hazard criteria outlined in Figures 1-3 of Gillett (1983).

	Bioaccumulation Chronicity		Multi-media Mobility		Direct Exposure	
	CSL[1]	MSL[2]	CSL[1]	MSL[2]	CSL[1]	MSL[2]
Methyl ethyl ketone	**	**	**	**	*	*
Tetrahydrofuran	**	**	**	**	*	*
Chlorobenzene	**	**	*	*	*	*
Benzene	*	*	**	**	*	*
Chloroform	*	*	**	**	*	*
Carbon tetrachloride	**	**	**	**	*	*
p-Xylene	**	**	*	*	*	*
1,2-Dichlorobenzene	**	**	*	*	***	***
cis-1,4-Dichloro-2-butene	?	?	?	?	?	?
1,2,3-Trichloropropane	**	*	*	*	***	***
2-Chloronaphthalene	***	***	*	*	*	*
Ethylene dibromide	*	*	**	*	*	*
Hexachlorobenzene	***	***	*	*	*	*
1,2,4,5-tetrachlorobenzene	***	***	**	**	*	*
Nitrobenzene	***	**	*	*	***	***
Toluene	**	*	**	*	*	*

[1]Captina silt loam.
[2]McLaurin sandy loam.
* = No concern.
** = Concern.
*** = Heavy concern
? = Insufficient data to evaluate.

1,2,4,5-tetrachlorobenzene all have a high potential for bioaccumulation and chronic effects. The predicted bioaccumulation problems with hexachlorobenzene in both soil types is verified by previous research (Kenage, 1980). More evidence is needed for evaluation of cis-1,4-dichloro-2-butene in both soil types.

Criteria from Figure 2 in Gillett (1983) involves chronic effects and multimedia mobility. Since inferences on chronic effects of the compounds was mentioned previously, only multimedia mobility concerns will be discussed here. From data generated by the PBS, the following inferences were made for the organic compounds in the Captina silt loam soil. Chlorobenzene, hexachlorobenzene, p-xylene, 1,2-dichlorobenzene, 1,2,3-trichloropropane, nitrobenzene, and 2-chloronaphthalene should pose no concern regarding multimedia mobility. Tetrahydrofuran, methyl ethyl ketone, benzene, toluene, chloroform, carbon tetrachloride, ethylene dibromide, and 1,2,4,5-tetrachlorobenzene would have potential concerns about multimedia mobility. In the McLaurin sandy loam, toluene, p-xylene, chlorobenzene, ethylene dibromide, 1,2-dichlorobenzene, 1,2,3-trichloropropane, nitrobenzene, hexachlorobenzene, and 2-chloronaphthalene should have no concerns regarding multimedia mobility. Methyl ethyl ketone, tetrahydrofuran, benzene, chloroform, carbon tetrachloride, and 1,2,4,5-tetrachlorobenzene would have potential concerns regarding multimedia mobility.

The final criteria, shown in Figure 3 of Gillett (1983), involves mobility and direct exposure. Since mobility inferences were

discussed previously, only exposure inferences will be mentioned here. In the Captina silt loam and McLaurin sandy loam soils, nitrobenzene, 1,2-dichlorobenzene, and 1,2,3-trichloropropane have heavy concerns regarding direct exposure effects in the water column. In addition, 1,2-dichlorobenzene overlaps into zones where there are high concerns for inhalation toxicology. The remaining compounds are of no concern regarding direct exposure.

4.5 Conclusions

The application of organic chemicals on soil can be an effective and efficient disposal strategy. This research helps fill gaps in current land treatment technology in addition to providing technical data for maximizing the clean-up of spills. Although there is a two-fold difference in organic carbon content between the Captina silt loam and McLaurin sandy loam, the difference in degradation rates of the chemicals tested was minimal. Therefore, degradation of the organic compounds in the field is expected to be similar between these two soil types. With the subsequent collection of additional data on toxicity, sorption, bioaccumulation, and degradation for other compounds, regulators will be capable of making scientifically-informed decisions on land treatability of organic wastes.

The experimental design used in this study was implemented to distinguish between biotic and abiotic losses of chemicals from the two soils. Standard EPA and OSHA methods were used for sample

handling, storage, and analysis. In addition to minimizing the loss of compounds due to volatilization during the chemical extraction procedure, charcoal traps were used to account for volatilization of compounds during the experiment. Nonetheless, mass-balances for the compounds were not obtained. Simple studies with a radiolabeled tracer (^{14}Ctoluene) showed that for at least one of the test compounds, solvent extraction did not recover all of the chemical after a 7-day incubation, even though recovery was good based on a spike-recovery technique. In addition, the OSHA extraction method used failed to desorb all of the chemical from aged charcoal traps and standard EPA storage conditions appear to be inadequate for volatile compounds in light of the chemical degradation data described earlier.

This study indicates the usefulness of physicochemical properties as predictors of microbial degradation in complex systems such as soils and illustrates situations where multiple quantitative structure-activity relationships are needed to describe the biological behavior of chemicals influenced by more than one mode of action. Finally, the current research provides data which can and was used in a pre-biological screen to model the fate of organic compounds in ecosystems.

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