Effect of Chemical Structure on the Biodegradability of 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT)

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Thirty-seven analogues of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its metabolites were tested for their susceptibility to biodegradation by means of the biological oxygen demand technique. Of the diphenylmethanes, benzhydrols, benzophenones, and phenylacetates tested, the unsubstituted chemicals and those with a single hydroxyl, amino, or methoxy group in the para position were readily metabolized by soil microorganisms. Chemicals with hydroxyl, chlorine, or methoxy groups in both para positions were resistant to biodegradation.

Several hypotheses have been advanced to explain the resistance of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and other persistent molecules to biodegradation (Alexander, 1973). Considerable evidence indicates that particular substituents are important in conferring resistance to microbial attack. Focht and Alexander (1970, 1971) reported that the presence of a chlorine in the para position and substituents on the methylene carbon governed the resistance of DDT and related compounds to aerobic attack. However, nonchlorinated analogues of DDT metabolites are readily attacked by microorganisms (Subba-Rao and Alexander, unpublished data). Studies of the biodegradability of such molecules are needed to establish the reasons for the resistance of these types of chemicals and to help in the design of biodegradable analogues of DDT that still have insecticidal activity.

The present investigation was designed to ascertain the effect of chemical structure on the biodegradability of a large number of analogues of DDT.

MATERIALS AND METHODS

The relative biodegradability of DDT analogues was measured by the depletion of dissolved O_2 in an O_2-saturated solution in biological oxygen demand (BOD) bottles (American Public Health Association, 1971). The inorganic salts solution of Hammond and Alexander (1972) was used for preparing O_2-saturated water and for the isolation of bacteria.

Bis(*p*-nitrophenyl)methane, bis(*p*-chlorophenyl)-methane, and benzhydrol were obtained from Eastman Organic Chemicals, Rochester, N.Y. *p,p'*-Dimethoxybenzophenone, 1,1-diphenylethane, and *p*-methoxy-, *p*-methyl-, *p*-amino-, and *p*-nitrophenylacetic acids were from K&K Laboratories, Plainview, N.Y. 1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)ethane was provided by E. I. DuPont De Nemours and Co., Wilmington, Del. Tetraphenyldimethyl ethers were synthesized by the method of Pratt and Draper (1949). All other analogues of DDT were obtained from Aldrich Chemical Co., Milwaukee, Wis.

The chemicals were introduced into the BOD bottles as sole carbon sources at a concentration of 2 mg of carbon per bottle. The compounds were added in acetone solutions, and the acetone was evaporated prior to the addition of O_2-saturated water. Each bottle received 5 mg of Hudson Collamer silt loam as a source of the microbial inoculum. The bottles were filled with the air-saturated salts solution and closed with glass stoppers. Bottles containing O_2-saturated water inoculated with soil but no carbon source were also included in the study to account for the O_2 depletion resulting from microbial oxidation of organic matter and ammonium. Each compound was also tested in combination with glucose (both at a concentration of 2 mg of carbon per bottle) to test whether the possible lack of biodegradation was a result of toxicity of the test chemical. The bottles were incubated in the dark at 25 °C.

Dissolved O_2 in the bottles was measured at regular intervals using a Yellow Spring Instrument Co. (Yellow Springs, Ohio) oxygen analyzer, Model 53. The instrument was calibrated with the salts solution, the O_2 content of which was determined by the Alsterberg modification of the Winkler method (American Public Health Association, 1971). At regular intervals, the dissolved O_2 in the samples was measured after calibrating the instrument with a BOD bottle containing inoculated O_2-saturated water supplemented with 0.1% KCN. The solutions in bottles showing O_2 depletion were used to obtain microorganisms capable of utilizing the substrate. The microorganisms were isolated by the enrichment culture technique with 0.1% of the test chemical as sole source of carbon in the medium. Nitrite and nitrate were determined by the methods of Montgomery and Dymock (1961, 1962).

Table I. Biodegradability of Test Chemicals

Test chemical	O_2 consumed, μg/ml			
	5 days	10 days	20 days	30 days
Benzhydrol[a]	0.0	1.1	0.8	2.1
p,p'-Dimethoxybenzhydrol	0.1	0.1	0.2	0.3
p-Chlorobenzhydrol	0.0	0.0	0.3	0.5
p,p'-Dichlorobenzhydrol	0.0	0.0	0.2	0.5
1,1,1-Trichloro-2,2-bis-(*p*-methoxyphenyl)ethane	0.7	0.5	0.4	1.4
1,1-Diphenylethane[a]	0.2	0.2	0.3	0.3
1,1-Diphenylethylene[a]	0.2	0.3	0.4	0.4
Diphenylacetaldehyde[a]	0.5	0.9	1.0	1.5
p-Chlorodiphenylmethane[a]	0.0	0.1	0.2	0.4

[a] Organisms were isolated from enrichment cultures containing these compounds as carbon sources.

RESULTS

Nitrite and nitrate were not evident in the BOD bottles until day 40, at which time 0.1 μg of nitrite N and 0.6 μg of nitrate N were present. These figures rose to 1.5 and 4.6 μg on day 60. In flasks containing glucose and the test chemicals, most of the O_2 was consumed in 2 days; hence, none of the substances was appreciably toxic.

The biodegradability of diphenylmethane and substituted diphenylmethanes is presented in Figure 1. *p*-Hydroxydiphenylmethane and diphenylmethane were degraded extensively in 20 and 40 days, respectively. It

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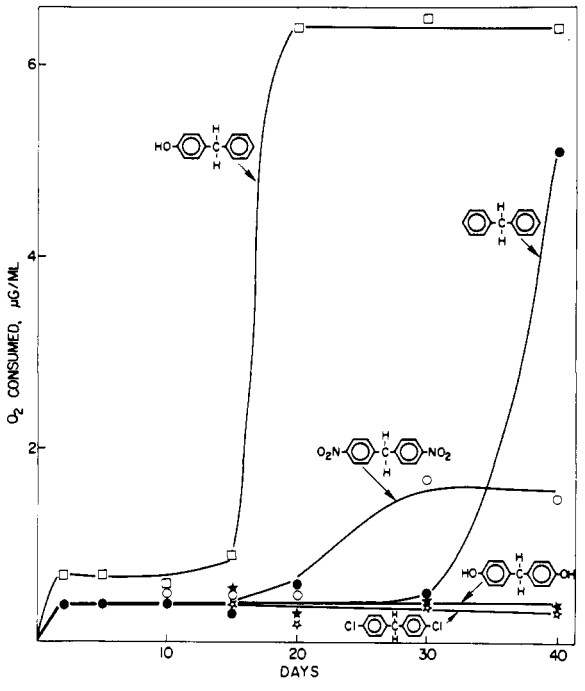

Figure 1. Biodegradation of diphenylmethanes in soil suspensions.

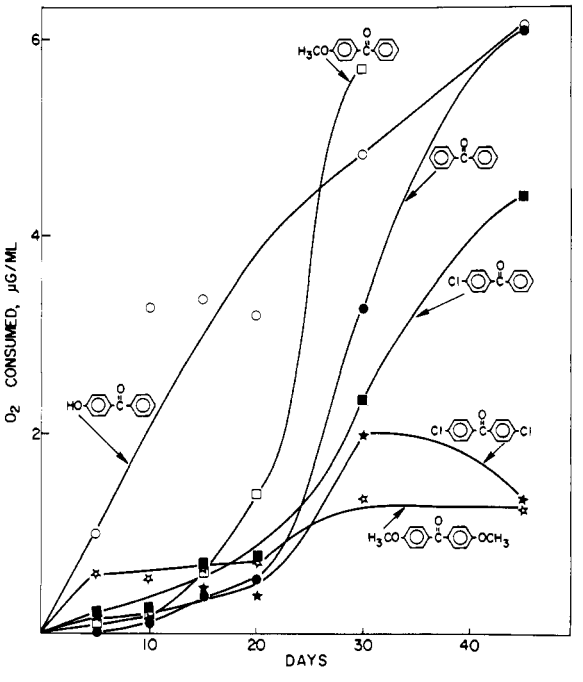

Figure 2. Biodegradation of benzophenones in soil suspensions.

is surprising that these compounds were degraded only after a prolonged lag period. The *p,p'*-dinitro compound was only attacked slowly and incompletely, whereas the most resistant compounds were bis(*p*-hydroxyphenyl)-methane and bis(*p*-chlorophenyl)methane.

Among the benzhydrols tested, only benzhydrol appeared to be biodegradable, and the substituted benzhydrols were refractory (Table I). Although O_2 consumption by the microflora exposed to benzhydrol was only 2.1 μg/ml, the chemical was considered to be biodegradable because of the isolation of many bacteria from enrichments containing this compound as sole source of carbon.

Benzophenone and *p*-hydroxy-, *p*-methoxy-, and *p*-chloro-substituted benzophenones were degraded, although appreciable disappearance of O_2 sometimes required more than 20 days (Figure 2). The most resistant molecules of this group were *p,p'*-dimethoxybenzophenone and *p,p'*-dichlorobenzophenone.

Less than 1.0 μg of O_2/ml was consumed in 30 days with the following chemicals: diphenylacetic acid, bis(*p*-chlorophenyl)acetic acid, 2,2-diphenylpropane, 2,2-diphenyl-1,1,1-trichloroethane, *p,p'*-dichlorodiphenylmethane, DDT, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane, 1,-1,1',1'-tetraphenyldimethyl ether, and 1,1,1',1'-tetra(*p*-chlorophenyl)dimethyl ether. Moreover, no isolates were obtained from enrichment cultures with these chemicals as carbon sources. Diphenylacetaldehyde and 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane (methoxychlor) showed modest O_2 depletion, but neither 1,1-diphenylethane or 1,1-diphenylethylene gave rise to significant O_2 utilization (Table I).

Biodegradability studies were also conducted with phenylacetic acid analogues. Unsubstituted phenylacetic acids and *p*-hydroxy-, *p*-methoxy-, and *p*-aminophenylacetic acids were biodegradable, and appreciable destruction was evident after only a few days (Figure 3). On the other hand, *p*-methyl-, *p*-nitro-, and *p*-chlorophenylacetic acids all appeared to be resistant under the test conditions.

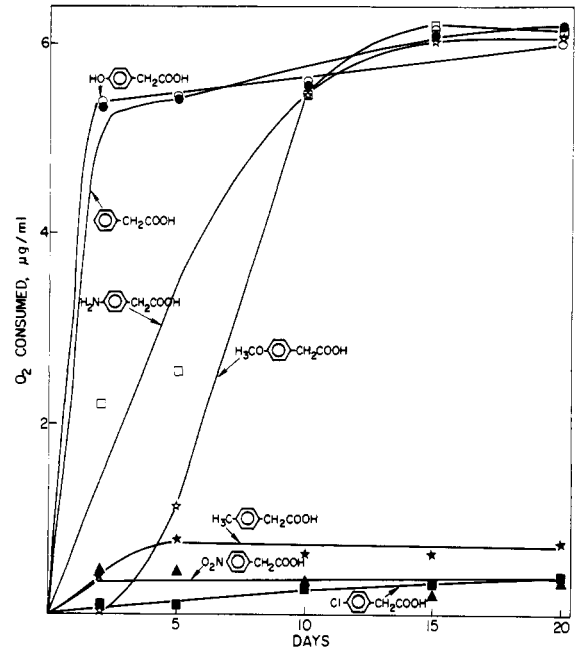

Figure 3. Biodegradation of phenylacetic acids in soil suspensions.

Enrichments set up during the biodegradation tests confirmed that the compounds showing the greatest O_2 depletion in the BOD bottles were also able to support growth of microorganisms. Organisms were isolated with diphenylmethane, *p*-hydroxydiphenylmethane, benzhydrol, benzophenone, *p*-hydroxybenzophenone, *p*-methoxybenzophenone, 1,1-diphenylethane, 1,1-diphenylethylene, 1,1-diphenylacetaldehyde, *p*-chlorodiphenylmethane, phenylacetic acid, *p*-methoxyphenylacetic acid, and *p*-aminophenylacetic acid as sole carbon sources in the enrichments. Although the microbial inoculum for the BOD bottles containing diphenylethane, diphenylethylene, and *p*-chlorodiphenylmethane consumed little O_2, the enrichments maintained for these compounds showed microbial growth, and organisms were isolated in pure cultures. Hence, these chemicals are also biodegradable.

DISCUSSION

DDT persists in soils for long periods of time, and its half-life has been estimated to be between 3 and 20 years (Kiigemagi and Terriere, 1972; Menzie, 1972). A possible way to reduce the longevity of this insecticide is to introduce or change substituents on the molecule to favor microbial utilization. Thus, Alexander and Lustigman (1966) reported that the presence of chloro, sulfonate, and nitro groups retarded the rate of biodegradation of monosubstituted benzenes, but carboxyl and hydroxyl groups favored the decomposition of the chemicals. Comparable information for DDT and related compounds might lead to the development of suitable and short-lived replacements. Although many studies have indeed been conducted of the insecticidal activity of molecules of this class with different substituents or substituents in different positions, published information on the biodegradation of many of these compounds is lacking.

In the present investigations, the para,para'-substituted dichloro, dimethoxy, dihydroxy, and dinitro compounds tested were utilized poorly if at all. Conversely, the unsubstituted chemicals or the para-substituted monohydroxy and monomethoxy chemicals were attacked readily. 1,1,1',1'-Tetraphenyldimethyl ether, a product synthesized microbiologically from diphenylmethane (Subba-Rao and Alexander, unpublished data), and its chlorinated analogue 1,1,1',1'-tetra(*p*-chlorophenyl)dimethyl ether were resistant to biodegradation in soil suspensions. These two molecules were included in the studies as the chlorinated ether had been suspected to be formed from chlorinated diphenylmethane in soil or water.

Biodegradability of DDT analogues has been expressed by Kapoor et al. (1973) in terms of the amount of polar metabolites generated during the metabolic transformation. However, the finding of polar products does not necessarily mean extensive metabolism or even total mineralization of the parent molecule, inasmuch as diphenylacetic acid, bis(*p*-chlorophenyl)acetic acid, and *p*-chlorophenylacetic acid, though water soluble, were not found to be decomposed appreciably in BOD bottles. Additional evidence for the resistance of these compounds can be found in the absence of microbial growth in enrichments containing such chemicals as sole sources of carbon.

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Crystal and Molecular Structure of Carbamate Insecticides. 1. Mesurol

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The crystal and molecular structure of Mesurol (4-methylthio-3,5-dimethylphenyl *N*-methylcarbamate, $C_{11}H_{15}NO_2S$) has been determined by three-dimensional x-ray analysis. The crystals are triclinic with space group $P\bar{1}$ and with unit cell dimensions a = 11.786 (2), b = 12.149 (5), and c = 8.896 (3) Å, α = 101.84 (3), β = 90.88 (2), and γ = 74.95 (2) °, and Z = 4. The structure was refined to a final conventional discrepancy factor of 0.062 for 2301 observed reflections ($|F_o| > 3\sigma_{F_o}$). The molecule consists of three approximately planar sections; the methylthio and carbamate groups twist by about 80 and 60°, respectively, out of the plane of the 3,4-dimethylphenyl group. In the carbamate group, the C–O single bond distance in both molecules (average 1.370 (4) Å) indicates a possible lengthening ($\sim 3\sigma$) relative to those usually found in esters.

In recent years the production and use of both carbamate and organophosphorus insecticides have greatly increased as they replace the much less biodegradable and carcinogenic chlorinated hydrocarbon insecticides. The mode of action of the carbamate and organophosphorus insecticides is accepted to be via the inhibition of the enzyme acetylcholinesterase. The enzyme appears to be phosphorylated or carbamoylated at a serine hydroxyl with release of a leaving group. The enzyme then loses the phosphoryl or carbamoyl group by a very slow reaction with water, and during this period the enzyme cannot react with acetylcholine (Krupka, 1964). We feel that it is important to obtain as much information as possible concerning the details of these interactions at the molecular level and, hence, have initiated a program of crystal-structure determinations of selected organophosphorus and carbamate insecticides to yield firm structural data for use by investigators in this area. We have previously reported the details of crystal structures of three organophosphorus insecticides, ronnel (Baughman and Jacobson, 1975), Coroxon (Gifkins and Jacobson, 1976), and azinphosmethyl (Rohrbaugh et al., 1976). We

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