Table IV. Inhibition of Development of *Anopheles quadrimaculatus*[a]

compd	lethal concn[b] LC_{50}, ppm	LC_{90}, ppm
1b	0.02	0.04
1c	0.12	0.22
6	0.035	0.15
9c	0.10	0.35
9d	0.03	0.04
9i	0.02	0.03
10d	0.02	0.04
10e	0.01	0.02

[a] Late third-fourth larval stage in water containing ground hog supplement for larval food. [b] Data based on the percentages of treated insects that fail to complete development to the free-flying adult stage.

and, as previously observed with 5 and other 2,6-di-*tert*-butylphenols (Pridantseva and Volod'kin, 1974), are mosquito juvenile hormone mimics. The inhibitory activity of some representative housefly sterilants on the development of the mosquito (*Anopheles quadrimaculatus*) are shown in Table IV (Dame and Jurd, 1978).

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Supplementary Material Available: The preparation and physical properties of new compounds listed in Table I are given (6 pages). Ordering information is given on any current masthead page.

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Physical-Chemical and Biological Degradation Studies on DDT Analogues with Altered Aliphatic Moieties

Joel R. Coats,[1] Robert L. Metcalf,* Inder P. Kapoor, Li-Chun Chio, and Patricia A. Boyle

A series of diethoxy 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) analogues with eight different aliphatic moieties were subjected to photodegradation studies, utilizing a sterile soil, an aqueous solution, and a film on glass. The 2-chloroisobutane analogue, a tertiary chloride, was quite photolabile in all trials. Photolability followed the order of the ease of formation of free radicals by loss of chlorine from the aliphatic moiety: tertiary > secondary > primary ≈ no chlorine. Several series of analogues were evaluated for biological degradation rates and products in the following types of studies: housefly synergistic ratios, housefly penetration–metabolism–excretion, salt marsh caterpillar metabolism, mouse metabolism, and in vitro metabolism by mouse liver microsomes. Both aliphatic and aromatic substituents influence biological degradation of the DDT analogues. In model ecosystem assessments of environmental fate, the two most important factors affecting bioaccumulation in fish were the type of alkyl chloride and water solubility of the molecule.

Biodegradable analogues of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) have been of interest since it was shown by Metcalf et al. (1971a) that replacement of the aromatic chlorine atoms by more degradable groups (alkyl, alkoxy) resulted in molecules that retained insecticidal potency but did not biomagnify or persist in the environment to the extent that DDT did. The possibility of finding analogues that possess the advantages of DDT such as high mammalian LD_{50}'s, low cost, and residual activity, but minimize environmental hazards, has inspired

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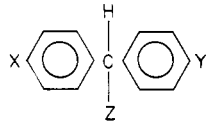
Table I. Physical Properties and Specific Activities of ^{3}H-Labeled Biodegradable DDT Analogues



analogue no.	X	Y	Z	sp act., mCi/mM	octanol/water partition coeff.	water solubility, ppm
I	C_2H_5O	C_2H_5O	CCl_3	3.7	1.27×10^3 [a]	0.16[a]
II	C_2H_5O	C_2H_5O	CCl_2CH_3	10	4.34×10^3	0.050
III	C_2H_5O	C_2H_5O	$CCl(CH_3)_2$	7.5	1.77×10^3	0.19
IV	C_2H_5O	C_2H_5O	$C(CH_3)_3$	3.1	1.46×10^3	0.14
V	C_2H_5O	C_2H_5O	$CHCl_2$	13	4.45×10^3	0.026
VI	C_2H_5O	C_2H_5O	$CHClCH_3$	9.8	3.81×10^3	0.88
VII	C_2H_5O	C_2H_5O	$CH(CH_3)_2$	9.3	4.04×10^3	0.043
VIII	C_2H_5O	C_2H_5O	$CHNO_2CH_3$	9.0	2.82×10^3	0.36
IX	CH_3O	CH_3O	CCl_3	3.8	1.12×10^3 [a]	0.62[a]
X	CH_3O	CH_3O	$CHClCH_3$	9.2	3.39×10^3	1.7
XI	CH_3O	CH_3O	$C(CH_3)_3$	0.60	3.69×10^3	0.69
XII	CH_3O	CH_3O	$CHNO_2CH_3$	4.1	1.45×10^3	8.6
XIII	CH_3	C_2H_5O	CCl_3	11	2.71×10^3 [a]	0.028[a]
XIV	CH_3	C_2H_5O	$CHClCH_3$	0.44	1.55×10^3	1.6
XV	CH_3	C_2H_5O	$CH(CH_3)_2$	14	3.53×10^3	0.18
XVI	CH_3	C_2H_5O	$CHNO_2CH_3$	0.72	2.86×10^2	2.3
DDT	Cl	Cl	CCl_3	0.77[b]	9.49×10^3 [a]	0.002[a]

[a] Data from Kapoor et al. (1973). [b] ^{14}C (ring-UL).

recent synthetic efforts in this direction (Metcalf et al., 1971a; Holan, 1969, 1971; Hirwe et al., 1972; Coats et al., 1977, Lee et al. 1977). Commercial interest in biodegradable DDT analogues is evident as agricultural chemicals divisions of several companies recently have devoted developmental efforts to such compounds as Abbott Laboratories' 1,1-bis(*p*-ethoxyphenyl)isobutane (Coats et al., 1977), Mobil Oil's 1-*p*-ethoxyphenyl-1-(*p*-*tert*-butylphenyl)-2-nitrobutane, and Mitsubishi's 1-*p*-tolyl-1-(*p*-ethoxyphenyl)-2,2,2-trichloroethane.

Certain DDT analogues, with the aromatic chlorines replaced by oxidizable substituents, have been demonstrated to be biodegradable in detailed insect and mouse metabolism studies and in a model ecosystem (Metcalf et al., 1971b; Kapoor et al., 1970, 1972, 1973; Reinbold et al., 1971). Three analogues with altered aliphatic moieties have been investigated: a benzylaniline (Hirwe et al., 1972), a neopentane (Coats et al., 1974), and the 2-nitropropane Prolan (Hirwe et al., 1975). Each was shown to be considerably more biodegradable than DDT.

It was the purpose of the present study to systematically assess the influence of the aliphatic portion of the DDT-type molecule on degradation. Eight bis(*p*-ethoxyphenyl) analogues with altered aliphatic moieties were compared directly in a battery of degradability trials, involving exposure to both physical–chemical and biological agents. Smaller series of four bis(*p*-methoxyphenyl) and four (*p*-methylphenyl)-(*p*-ethoxyphenyl) analogues confirmed the importance of the aliphatic moiety in determining physical, chemical, and biological properties of the molecule and provided additional comparisons of aromatic substituents.

MATERIALS AND METHODS

Radiolabeled Compounds. These investigations utilized ^{3}H-ring-labeled biodegradable DDT analogues synthesized from ^{3}H-ring-labeled anisole or phenetole, prepared by the method of Hilton and O'Brien (1964). Synthetic methods have been reported earlier for the alkane and chlorinated alkane analogues (Coats et al., 1977) as well as the nitroalkane analogues (Lee et al., 1977). Abbott Laboratories, North Chicago, IL, provided a sample of ^{14}C-(bridge C)-labeled 1,1-bis(*p*-ethoxyphenyl)isobutane, or A-47171, with a specific activity of 8.7 mCi/mM. ^{14}C-ring-labeled DDT was supplied by the World Health Organization, Geneva, Switzerland. All radiolabeled chemicals were purified by silica gel chromatographic methods, either by column or 1.0-mm TLC plates, to a radiochemical purity of 99+%. Specific activities of the synthesized compounds are listed in Table I.

Model Metabolites. Previous metabolism studies on DDT, methoxychlor, ethoxychlor, and other trichloroethane analogues (Kapoor et al., 1970, 1972, 1973) and one neopentane analogue (Coats et al., 1974) provided insights as to the anticipated major degradative pathways.

For each of the 12 dialkoxy analogues in Table I, the monophenolic and bisphenolic model metabolites were synthesized via mixed Baeyer condensations using phenol, an alkoxy benzene, and the appropriate aldehyde. For the four methylethoxy analogues in Table I, the O-deethylation product was synthesized by a Friedel–Crafts alkylation, using $AlCl_3$, phenol, and the appropriate substituted tolyl carbinol. These reactions are similar to the ones used to make the parent compounds and are discussed in more detail in Coats et al. (1977).

The dialkoxybenzophenones were synthesized by alkylation of commercial (Aldrich Chemical Co.) *p,p'*-dihydroxybenzophenone, using sodium methoxide and methyl iodide in methanol. The *p*-methyl-*p'*-ethoxybenzophenone was synthesized from *p*-methylbenzoyl chloride and phenetole by the method of Skerrett and Woodcock (1950).

Refluxing chlorinated analogues in alcoholic KOH yielded the corresponding ethylene, propene, and isobutene products via dehydrochlorinations. Propenes were also obtained for the nitropropane analogues since Hirwe et al. (1975) showed Prolan to undergo elimination, with loss of HNO_2, to form 1,1-bis(*p*-chlorophenyl)-1-propene.

The α-hydroxyl model metabolite was made for the dialkoxyphenyl alkanes by reacting 2 mol of the Grignard reagent *p*-alkoxyphenylmagnesium bromide with the ethyl ester of pivalic or isobutyric acid (Rogers et al., 1953).

The β-hydroxyl derivative was prepared for 1,1-bis(*p*-ethoxyphenyl)isobutane by the method of Skerrett and Woodcock (1950), utilizing the Grignard methylmagnesium iodide and the ethyl ester of 1,1-bis(*p*-ethoxyphenyl)acetic acid or ethoxy-DDA. The ethoxy-DDA was synthesized by a sulfuric acid condensation of phenetole with the morpholine salt of α,α-dimorpholinoacetic acid (prepared from morpholine and dichloroacetic acid) according to Brault and Kerfanto (1964).

All model metabolites were purified by silica gel column chromatography and/or recrystallization. All structures were confirmed by NMR spectroscopy, using tetramethylsilane as an internal standard. D_2O shakes were done for all hydroxyl mode metabolites. Infrared spectrometry was also used to confirm the presence of an OH group.

Chromatography and Radioassay. Thin-layer silica gel plates (0.25 mm), with fluorescent indicator, were used for cochromatography of model metabolites with radioactive extracts. Two TLC plates were used for all cochromatographic assays, each being developed in a system of different solvents (e.g., hexane/acetone, 9:1; and benzene/diethyl ether, 8:2). Sections were scraped into scintillation vials containing cocktail consisting of 200 g of naphthalene, 10 g of PPO, and 0.25 g of POPOP in dioxane to make 1 L. Quench correction was by the channels ratio method. The Schöniger oxygen flask technique (Kelly et al., 1961) was used for combustion of samples of mouse feces (dried at 30 °C and powdered) and urine (in methanol, and spotted onto combustion paper).

Physical Properties. Water solubilities were determined by placing 5 mL of 1 mg/mL acetone solution of the ^{3}H-labeled analogue in a 3.8-L amber glass bottle and evaporating the solvent under dry nitrogen. After 3 L of distilled water was added, a Teflon-coated magnetic stirring bar was added, and the bottle was placed in the dark and stirred. After 24 h the water was filtered through Whatman no. 2 filter paper, twice, and two aliquots of 1 L each were extracted three times with diethyl ether. Volumes were reduced to 50 mL and two 10-mL aliquots were transferred to scintillation vials and evaporated. Cocktail was added, and the vials were counted.

Partition coefficients were determined using the 1-octanol/water system of Hansch and Fujita (1964) and were carried out in duplicate by the method of Kapoor et al. (1973), with equal volumes of water and octanol, assaying each layer by liquid scintillation.

To insure accuracy, all ^{3}H-labeled analogues were repurified by preparative TLC immediately prior to water solubility and partition coefficient determinations, as small amounts of polar impurities in samples of these very lipophilic compounds could have altered results significantly.

Degradation. Drummer soil, a postmature poorly drained Central Illinois Brunizem clay loam (25% sand, 45% silt, 30% clay), had a pH of 6.5 and contained 6.3% organic matter. It was autoclaved at 120 °C for 30 min, air-dried for 1 week, and sifted through a 1-mm mesh. Twenty-five grams of soil was placed in each glass petri dish, treated with 2.5 μg of ^{3}H-labeled analogue in 4 mL of reagent grade acetone, and was allowed to dry. Final concentration on the soil was 0.1 ppm. The petri dish was left open and was put in an environmental plant growth chamber at 27 °C with a 12-h diurnal cycle of 5000 ft candles. After 6 weeks of exposure, the soil was extracted with acetone by stirring at room temperature for 1 h. The extract was dried with Na_2SO_4 (anhydrous) and its volume reduced for spotting on TLC.

For evaluation of stability of the analogues in water, solutions were prepared in distilled water at 0.02 ppm, below solubility limits, for all analogues; for DDT the solution was prepared at its solubility limit of 0.002 ppm. The analogues, in reagent grade acetone, were pipetted into 125-mL Erlenmeyer flasks, and the solvent was evaported under dry nitrogen. The water (125 mL) was added and the solution was stirred for 24 h with a magnetic stirring bar, and a ground glass stopper was used to close the flask tightly. After 24 h, the stirring bar was removed, and the flask was stoppered and placed into the growth chamber conditions that were described above. Six months later the water was analyzed by diethyl ether extraction, and the extract was prepared for spotting on TLC.

Each analogue was also applied as a thin film to an open petri dish. One milligram of compound was added in 1 mL of acetone which was evaporated off by a stream of air; the petri dish was gently shaken while evaporation was occurring to insure reasonably even distribution of the chemical on the glass surface. The resulting film was 1 mg/64 cm^2 or 16 μg/cm^2. The petri dish was left open and placed in the growth chamber for 8 weeks; dishes were then rinsed out with acetone, and the extract was spotted on TLC. All soil, water, and glass experiments were run in duplicate and the results were averaged.

Metabolism. Fifth instar salt marsh caterpillars, *Estigmene acrea*, ingested the ^{3}H-labeled analogues, as 1 mg was applied in acetone to a 5-g block of artificial medium (Vail et al., 1967). After 24 h the bodies and feces were extracted with acetonitrile and analyzed for degradation products.

Metabolism and distribution studies in the housefly followed the methods of Kapoor et al. (1970), utilizing topical application of a dose of 1 μg/female, a 24-h holding period, body rinse with acetone, body extraction with acetonitrile, feces extraction with methanol, and hydrolyzed body and feces extraction with 1 N HCl/methanol (1:1). Trials were run in triplicate.

Synergistic ratios were determined from topical application by the method of Brattsten and Metcalf (1970).

Mouse metabolism studies were conducted with female Swiss white mice, dosed orally with 50 mg/kg of ^{3}H analogue in olive oil. Two mice were kept in each metabolism cage. Urine and feces were collected daily and sampled to determine rate of excretion. The remaining urine and feces from the first 48 h after dosing were each pooled and extracted with diethyl ether, acidified to pH 2 with HCl, refluxed, reextracted, and prepared for analysis on TLC.

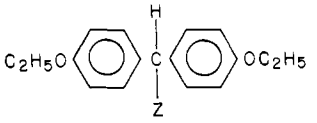
The mouse liver microsomes were prepared by a method derived from that of Hansen and Hodgson (1971) utilizing a generating system of NADP, G-6-P, G-6-P dehydrogenase, and $MgCl_2$. The procedure was the same as previously described (Coats et al., 1974) with these exceptions: homogenization was in a 0.1 M Tris buffer of pH 7.4 (0.15 M KCl), resuspension was in a 0.05 M Tris buffer of pH 8.0, substrate was added at 10^{-5} M in 50 μL of methyl Cellosolve, incubation was for 30 min, and the reaction was stopped by addition of 1 mL of acetone prior to extraction with diethyl ether.

Model ecosystem methodology has been described in detail by Metcalf et al. (1971b).

RESULTS AND DISCUSSION

Physical Properties. Two properties that have been shown to influence biological effects and fate of organic chemicals are the water solubility (Metcalf and Sanborn, 1975; Kapoor et al., 1973) and the partition coefficient (Lu et al., 1977; Coats et al., 1976). The water solubilities of

Table II. Relative Degradability of Eight Diethoxy DDT Analogues on Sterile Soil, in Distilled Water, and as a Film on Glass



Z	percent parent remaining		
	soil (6 wk)	water (6 mo)	glass (8 wk)
no chlorine			
- $CH(CH_3)_2$	79	87	74
-$C(CH_3)_3$	48	98	72
- $CHNO_2CH_3$	81	87	91
1° chlorine			
- CCl_3	32	80	95
- $CHCl_2$	92	80	78
2° chlorine			
- CCl_2CH_3	7.5	3.9	64
- $CHClCH_3$	6.9	0.8	67
3° chlorine			
- $CCl(CH_3)_2$	1.2	1.0	7.1
DDT	78	95	95

biodegradable DDT analogues are quite low, but one to three orders of magnitude higher than DDT (0.002 ppm).

A comparison of the aromatic substituents listed in Table I indicates that the diethoxy analogues are generally less soluble than the other biodegradable analogues. For a given aliphatic moiety, the dimethoxy analogue provides the highest water solubility value. This is consistent with observations that the dimethoxy analogues have lower R_f values than the other analogues on silica gel TLC, using nonpolar solvent systems.

Among the various aliphatic groups, the 2-nitropropane contributes the greatest degree of polarity to a DDT analogue, followed by the 2-chloropropane. The 2-chloropropanes are 10–30 times as soluble as either the dichloroethane or isobutane isosteres; although both chloro and methyl groups typically impart a lipophilic character to a compound, one chloro and one methyl substituted on the β carbon apparently create a substantial dipole within that aliphatic moiety, leading to larger water solubility values than expected.

The partition coefficients, given in Table I, are not very different for the 16 biodegradable analogues and DDT. Such high lipophilicity is necessary in contact insecticides to allow rapid penetration of the insect cuticle. DDT has the highest partition coefficient among the compounds in Table I, and analogue XVI, the asymmetric 2-nitropropane, the lowest.

Physical–Chemical Degradation. A series of eight diethoxy analogues were compared for degradability, using DDT as a standard, in the presence of air and light (a) on sterile Drummer soil, (b) dissolved in distilled water, and (c) as a film on glass. Under each of the three sets of conditions the same trend was observed: the 2-chloroisobutane degraded very rapidly, the 2-chloropropane and the 2,2-dichloropropane were moderately degradable, and the other five compounds were generally quite stable. As Table II shows, the tertiary chloride was most rapidly degraded, the secondary chlorides were of intermediate susceptibility to degradation, and the five most stable chemicals were the primary chlorides and analogues containing no chlorine.

Early studies on DDT isosteres showed that 1,1-bis-(*p*-chlorophenyl)-2-chloroisobutane possessed very poor insecticidal activity, which could not be explained on the basis of structure–activity relationships (Skerrett and Woodcock, 1950). In a more recent structure–activity study, six analogues with the 2-chloroisobutane aliphatic moiety all exhibited lower potency than expected relative to other DDT isosteres (Coats et al., 1977). During our syntheses we observed this aliphatic moiety to be so unstable, rapidly dehydrochlorinating, that purification attempts by silica gel chromatography (column or 1 mm TLC) or recrystallization usually were not profitable. When performed in the dark, purifications yielded somewhat larger percentages of pure parent compound. Light was suspected of causing degradation via the formation of the Cl· free radical since DDT and methoxychlor have both been shown to photodegrade by that process (Mosier et al., 1969; Zepp et al., 1976).

The energy required for radical formation is quite low for a 2-chloroisobutane since the heat of formation (ΔH_f) of a tertiary radical is less than that of a secondary radical, which is less than that of a primary radical (Franklin, 1968). This ease of formation and resultant stability of the tertiary free radical as an intermediate contributes directly to the instability of tertiary chlorides.

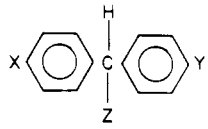
Elimination reactions are common among alkyl halides but are especially favored for aromatic, tertiary, and secondary alkyl halides due to the carbonium ion stabilizing (and free radical stabilizing) properties of these types of molecules (O'Ferrall, 1973). The elimination of HCl from DDT to form DDE proceeds readily in alkaline solution by an E2 mechanism as proposed by Cristol (1945). The concerted loss of H and Cl occurs, however, through a carbanion-like transition state giving the reaction some E1cB character, with the e^- withdrawing aromatic and aliphatic chlorines enhancing the carbanionic character (England and McLennan, 1966). Replacement of the aromatic chlorines by e^- donating groups (lower σ values) slows the rate of elimination considerably and in direct proportion to the e^- donating capacity of the group utilized (Metcalf and Fukuto, 1968). The effect is quite different, however, when the aliphatic chlorines are replaced by methyl groups (slightly e^- donating). As one chlorine is replaced with a methyl, a secondary chloride is produced, i.e., a 2,2-dichloropropane. The replacement of two chlorines by methyls results in a tertiary chloride, i.e., a 2-chloroisobutane. While such stepwise replacement decreases carbanion character of the transition state for E2 elimination, it simultaneously increases the stability and ease of formation of the free radicals which can disproportionate readily to the olefins. These types of radicals have also been shown to undergo oxidation (Plimmer et al., 1970) as well as dimerization (Zepp et al., 1976).

As a result, the 2-chloroisobutane analogues eliminate HCl in hydroxide solutions like many other DDT-type chemicals, but can also eliminate HCl at neutral and acidic pH's, probably at rates dependent on light, temperatures, solvents, and the presence of free radical initiators or traps. Thus their poor insecticidal activity can be explained by the inherent instability of the molecule which can eliminate HCl through a free radical intermediate or by the action of the enzyme DDT'ase, forming inactive isobutenes.

The secondary chlorides investigated, i.e., the 2-chloropropanes and 2,2-dichloropropanes, are quite active insecticides (Coats et al., 1977), but in the current study did show a tendency to degrade somewhat more rapidly than the primary chlorides and the chlorine-free analogues.

Rates of O-Dealkylation in the Housefly. Metabolism data have shown that dimethoxy DDT analogues are degraded by the housefly principally via O-dealkylation (Kapoor et al., 1970; Coats et al., 1974). The same pathway

Table III. Rates of O-Dealkylation in NAIDM Housefly



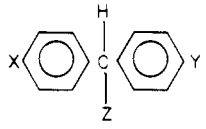
no.	Z	synergistic ratio (SR) (LD_{50} alone/LD_{50} with piperonyl butoxide) X = Y = EtO	X = EtO, Y = MeO	X = Y = MeO
1	CCl_3	4.0	4.3	13
2	CCl_2CH_3	2.5	7.9	8.1
3	$CCl(CH_3)_2$	9.7	13	46
4	$C(CH_3)_3$	4.1	2.7	5.0
5	$CHCl_2$	4.1	4.5	85
6	$CHClCH_3$	4.8	10	267
7	$CHNO_2CH_3$	4.0	17	327
8	$CHNO_2C_2H_5$	10	260	450
9	$CH(CH_3)_2$	6.1	5.4	13

also has been demonstrated to be the most important one in the metabolism of two diethoxy DDT analogues, 1,1-bis(*p*-ethoxyphenyl)isobutane (this paper) and ethoxychlor (Kapoor et al., 1972; Hansen et al., 1974). Using housefly toxicity data (Coats et al., 1977; Lee et al., 1977), in particular synergistic ratios (SR, the ratio of the LD_{50} of a compound alone to the LD_{50} of the compound when applied with a synergist), deductions can be made comparing the relative ease of removal of methyl and ethyl groups by housefly microsomal enzyme systems. For each of nine different aliphatic moieties, three analogues were compared: diethoxy, ethoxymethoxy, and dimethoxy.

Examination of the SR values for the 27 analogues in Table III reveals two definite trends. (1) As the two ethoxy groups were replaced, one at a time, by methoxy groups, the SR increased, quite dramatically in some cases. This indicates more rapid dealkylation of methoxy groups than ethoxy groups. This may be the case for other mixed function oxidase systems as well since dimethoxy DDT analogues have been shown to be much less toxic than diethoxy ones to fish (Metcalf et al., 1974) and to mice (Coats et al., 1977). It was demonstrated by Hansen et al. (1974), both in vitro and in vivo, that O-demethylation is more rapid than O-deethylation for the trichloromethyl analogues methoxychlor and ethoxychlor in houseflies, flesh flies, and mice. (2) The highest SR's observed (267–450) are quite unusual for DDT analogues, synergism attained to a degree usually expected only with certain pyrethroid, carbamate, or organophosphorus insecticides. In each case of a high SR, the aliphatic moiety is one responsible for rendering an analogue relatively water soluble, i.e., $CHNO_2CH_3$, $CHNO_2C_2H_5$, and $CHClCH_3$ (see Table I). This is entirely reasonable since one would expect the more water-soluble compounds to be more available for enzymatic attack in an aqueous biological system. It is further noted that the analogues with pure alkyl (no chloro, no nitro) Z groups (series 4 and 9) did not produce high SR's, even when the aromatic substituents were both methoxy. Such unsubstituted hydrocarbons had the greatest number of available sites of attack by mixed function oxidases, yet yielded relatively low SR's, indicating slower rates of detoxication. The low water solubilities of these alkyl types of analogues seem to best explain the paradox.

Penetration, Metabolism, and Excretion by the Housefly. The distribution of DDT, methoxychlor, and three methoxychlor analogues was examined 24 h after topical application to female houseflies. The radioactivity extracted was analyzed for determination of the amounts of intact parent compound and major metabolites. A budget of the remaining parent compound is presented in Table IV and provides explanations for the very wide range of toxicities exhibited by these five insecticides. DDT, the most toxic, showed the largest amount of intact parent compound in the body. Methoxychlor and dianisylneopentane, both of moderate toxicity, showed lesser amount of parent compound in the body. The chloropropane and the nitropropane analogues, the least toxic compounds, showed very small amounts of parent in the body. These latter two analogues have been shown previously to be only slightly toxic alone but extremely toxic when applied with the synergist piperonyl butoxide (Coats et al., 1977). Such a synergist allows more parent to accumulate in the body and reach the site of action, by reducing the rate of detoxication. The budgets for these two unusual compounds clearly show that little of the chloropropane remained intact anywhere while the nitropropane did not penetrate well and was rapidly excreted.

Table IV. Distribution of Parent Compound 24 h after Treatment of Female Houseflies, DDT, and Four Methoxy Analogues



X = Y	Z	LD_{50} μg/g	% of total recov. radiolabel external rinse	body	feces	total
Cl	CCl_3	14	5.5	33	2.0	41
OCH_3	CCl_3	45	11	16	11	38
OCH_3	$C(CH_3)_3$	95	38	21	2.6	62
OCH_3	$CHClCH_3$	935	13	2.4	1.7	17
OCH_3	$CHNO_2CH_3$	900	38	4.7	24	67

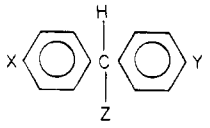
Table V. Metabolism of DDT and Four Dimethoxy Analogues by the Salt Marsh Caterpillar, *Estigmene acrea*

compound	% recov. radioactivity parent	non-polar metab.	polar metab.
DDT	95	5	
methoxychlor	96		4
dianisylneopentane	94		6
dianisyl-2-chloropropane	91	2	7
dianisyl-2-nitropropane	85		15

The primary metabolites found in the body and the feces of the housefly, for all four methoxy analogues were the mono- and bisphenols. These findings agree with earlier work which determined O-demethylation to be the primary route of detoxication for methoxychlor (Kapoor et al., 1970).

Metabolism by Salt Marsh Caterpillar. A comparison of metabolism of DDT and four dimethoxy analogues by *Estigmene acrea* larvae was measured as a total from body and feces extracts (Table V). All analogues were more degradable than DDT which degraded only to DDE. The more water-soluble analogues clearly were attacked more readily by the oxidative enzyme systems of this species. Analysis of the metabolic products revealed that, in contrast to the other species examined, the salt marsh caterpillar did not O-demethylate the di-

Table VI. Summary of Mouse Metabolism Studies for Five Biodegradable DDT Analogues (48 h after Oral Dosing at 50 mg/kg, in Olive Oil)



X	Y	Z	percent of excreted radiolabel: parent	mono-phenol[a]	bis-phenol[b]
CH_3O	CH_3O	CCl_3	7.0	30	23
CH_3O	CH_3O	$C(CH_3)_3$	11	51	17
CH_3O	CH_3O	$CHClCH_3$	10	21	6.0
CH_3O	CH_3O	$CHNO_2CH_3$	1.0	23	17
C_2H_5O	C_2H_5O	$CH(CH_3)_2$	14	25	22

[a] Metabolite resulting from one O-dealkylation. [b] Metabolite resulting from two O-dealkylations.

methoxy analogues, with the exception of the nitropropane compound.

In Vivo Mouse Metabolism. A comparison of four dimethoxy analogues and one diethoxy analogue demonstrates, in Table VI, that all are degraded substantially as they pass through the mouse. The dimethoxy 2-nitropropane analogue was almost completely degraded (1% remaining). It is the most water soluble of the analogues, and 70% of the excreted radioactivity was found in the urine and 30% in the feces. For the other four analogues, the distribution of radiolabel was reversed with 10–20% in the urine and 80–90% in the feces. Also, the other four compounds were all somewhat less degradable, with the diethoxy isobutane analogue yielding the greatest percentage of intact parent (14%). This is consistent with the mouse liver microsome studies (below) which demonstrated that diethoxy analogues were not as rapidly metabolized as dimethoxy analogues. The diethoxy isobutane analogue also is the least water-soluble of the five chemicals studied in vivo in the mouse. The percent of the administered dose that was excreted during the first 48 h varied from 56 to 99%. Kapoor et al. (1972) found that for DDT less than 20% of the administered radiolabel was excreted during the first 48 h. Of that radioactivity which was excreted, 15% was DDT (parent), 6% was DDE, and 26% was DDD, for a total of 47% nonpolar products. Clearly the mouse can excrete the biodegradable analogues more rapidly and can degrade them more easily to polar metabolites, principally the phenolic products mentioned in Table VI. Several minor metabolites (benzophenones, α- and β-hydroxylation products) were also detected by cochromatography, and one major unknown degradation product of the diethoxy isobutane analogue was determined by mass spectrometry to be the γ-hydroxylated metabolite.

In Vitro Degradation by Mouse Liver Microsomes. A comparison of DDT, eight diethoxy-, four dimethoxy-, and four methylethoxy analogues revealed that all analogues were degraded more readily than DDT. The four dimethoxy analogues were oxidized most rapidly, with an average of 9.8% intact parent compound remaining at the end of 30 min of incubation with the microsomal preparation. The diethoxy and methylethoxy series of compounds showed considerably more resistance to oxidative degradation in this system. The eight diethoxy analogues averaged 41% intact parent, and the four methylethoxy analogues averaged 52% intact parent, while 95% of the DDT remained intact. The principal oxidation products

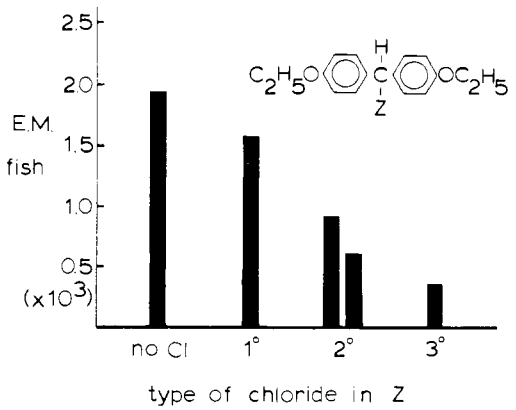


Figure 1. Ecological magnification (E.M. = concentration in fish/concentration in water) in a model ecosystem for diethoxy DDT analogues, with the aliphatic moiety Z = $CH(CH_3)_2$ (no Cl); CCl_3 (primary Cl); CCl_2CH_3 and $CHClCH_3$ (secondary Cl); $CCl(CH_3)_2$ (tertiary Cl).

identified were the mono- and bisphenols which resulted from O-dealkylation. However, within the group of eight diethoxy compounds it was noticeable that the analogues with the more highly chlorinated aliphatic moieties were somewhat less degradable than those with few or no chlorines. Thus side-chain oxidations of the aliphatic moiety also appear to be a factor in the metabolism of DDT analogues by mouse liver microsomes.

Model Ecosystem. Nine analogues were studied in terrestrial–aquatic model ecosystems to determine the influence of the aliphatic moiety on (1) the overall environmental degradability of a DDT-type molecule and (2) potential of the analogues to bioaccumulate through food chains. A biodegradability index (B.I. = polar metabolites/nonpolar metabolites and parent) was calculated for each organism as by Kapoor et al. (1973). The potential for bioaccumulation was measured by determining the ecological magnification (E. M. = concentration of parent compound in the organism/concentration of parent compound in the water) for each species, as calculated by Kapoor. Special attention was paid to those values obtained for the mosquito fish, *Gambusia affinis*, the top predator in the model ecosystem.

A series of five diethoxy analogues were compared: the trichloroethane, isobutane, 2-chloropropane, 2,2-dichloropropane, and the 2-chloroisobutane. The E.M. values, as shown in Figure 1, are related to the arrangement of chlorines on the aliphatic moiety. The phenomenon of bioaccumulation occurs only when a chemical meets all the following criteria: a low water solubility, a high lipid/water partition coefficient, and resistance to degradation. Model ecosystem E.M.'s have been correlated previously with the water solubilities (Kapoor et al., 1973) and partition coefficients (Lu and Metcalf, 1975) of chemicals evaluated. In the present study, the susceptibility to degradation, especially photodegradation, was the most important single factor governing the potential for bioaccumulation as measured by the fish E.M. values for five diethoxy analogues in the model ecosystem. The fish were added to the model ecosystem 30 days after the chemical was added; a photolabile chemical in this system tends to be more thoroughly degraded before the fish, or their major prey, mosquito larvae (added on the 26th day), are exposed to it. In this instance the 3° chloride or 2-chloroisobutane accumulated the least, followed by the 2° chlorides, a 1° chloride, and a chlorine-free alkane; these findings are consistent with the pattern observed in the physical–chemical degradation experiments. It should be noted that the five diethoxy analogues compared have very similar

partition coefficients and water solubilities, which permitted the variation in their photolability to emerge as an important parameter.

A series of four dimethoxy analogues, the trichloroethane, the neopentane, the 2-chloropropane, and the 2-nitropropane, also was evaluated in model ecosystems, and they yielded fish E.M.'s of 1545, 1636, 1340, and 26, respectively. The dimethoxynitropropane is by far the most water soluble (8.6 ppm) of the 16 DDT analogues discussed here and can be excreted more easily. Thus it accumulated less than the others in this series, as predicted from earlier studies (Kapoor et al., 1973).

From the two series it is evident that (1) water solubility is definitely important in influencing bioaccumulation and (2) that when factors such as water solubility and partition coefficient are equal, photodegradability plays an important role in determining degree of bioaccumulation. For the nine "biodegradable" DDT analogues considered in this study, model ecosystem E.M. values ranged from 26 to 1920, considerably lower than those for DDT (84 000) and most other chlorinated hydrocarbon insecticides.

CONCLUSIONS

DDT analogues with altered aromatic and aliphatic substituents were much more degradable than DDT, by both physical–chemical and biological agents. The rate of chemical decomposition in the presence of light and air was primarily dependent on the arrangement of chlorine atoms in the aliphatic moiety, due to differential susceptibility to free radical formation. Biological degradation was influenced strongly by the aromatic substituents as they were major points of enzymatic attack for analogues with *p*-alkoxy or *p*-alkyl substituted rings. Aliphatic moieties were important in biological degradation to the extent that they greatly influenced water solubilities, and hence availability to enzyme systems, as well as penetration and excretion rates. Overall environmental stability was a function of physical–chemical and biological degradation. Water solubility and persistence both affected degree of bioaccumulation in a model ecosystem for nine highly lipophilic analogues. The aliphatic moiety of a DDT-type molecule proved to be extremely important to the environmental fate of the chemical because of its influence on the water solubility and photostability of that compound.

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