1954), nor is it bound to the heme protein. These studies indicate that the coexistence of thiamine and thiamine-modifying compounds in foods such as fish limits the usefulness of present methodology for the accurate analysis of thiamine in food. Thiamine exists in tissues as the coenzyme thiamine pyrophosphate thiamine monophosphate and is also bound to protein. It is therefore unlikely that reaction between thiamine compounds and heme protein would take place in tissues. Free thiamine is formed during the analytical procedure and could react with hemin and heme protein during this process. The relationship of thiamine-modifying compounds and biological availability of thiamine needs further study.

The TMA of the various $(NH_4)_2SO_4$ fractions of beef, pork, and the light flesh of Skipjack tuna is shown in Figure 2. The TMA of the fish was spread throughout the fractions, while that of the beef and pork was more concentrated in the 70 and 80% saturation fractions. The solutions of lower $(NH_4)_2SO_4$ saturation fractions of the fish had a pink color, indicating the presence of hemoglobin-type proteins, while the equivalent fractions of the beef and pork were colorless. This would indicate that the physical properties of the Skipjack tuna heme proteins differ from the beef and pork heme proteins and are precipitated to some extent at lower $(NH_4)_2SO_4$ saturation levels. The lack of correlation between the TMA of the 70–80% $(NH_4)_2SO_4$ myoglobin precipitations and the TMA of the fish, beef, and pork suggests that TMA factors other than heme protein may be involved.

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Structure Activity Correlations of Biodegradability of DDT Analogs

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Methoxy-methiochlor [2-(*p*-methoxyphenyl) - 2-(*p*-methylthiophenyl) - 1,1,1-trichloroethane], methyl-ethoxychlor [2-(*p*-methylphenyl) - 2-(*p*-ethoxyphenyl) - 1,1,1-trichloroethane], and chloro-methylchlor [2-(*p*-chlorophenyl) - 2-(*p*-methylphenyl) - 1,1,1-trichloroethane] were studied for metabolic pathways in mice and insects and for biodegradability in a model ecosystem. Methoxy-methiochlor and methyl-ethoxychlor were good substrates for multifunction oxidases and showed biodegradability indices of 2.75 and 1.20, respectively. Chloro-methylchlor, with only a single degradophore on the aromatic ring, was also a satisfactory substrate and showed a biodegradability index of 3.43, compared to 0.015 for DDT.

Recent reports from this laboratory have described the principles of biodegradability of DDT-type molecules (Hirwe *et al.*, 1972; Kapoor *et al.*, 1970, 1972; Metcalf *et al.*, 1971b). Here we report the comparative metabolism and biodegradability of three asymmetrical biodegradable DDT molecules: methoxy-methiochlor or 2-(*p*-methoxyphenyl)-2-(*p*-methylthiophenyl) - 1,1,1-trichloroethane; methyl-ethoxychlor or 2-(*p*-methylphenyl)-2-(*p*-ethoxyphenyl) - 1,1,1-trichloroethane; and chloro-methylchlor or 2-(*p*-chlorophenyl)-2-(*p*-methylphenyl) -1,1,1-trichloroethane. The insecticidal properties of these compounds were described by Metcalf *et al.* (1971a). These data, together with comparable information on symmetrical DDT analogs (Kapoor *et al.*, 1970, 1972), permit discussion of the relationships of the chemical structure of the DDT analogs to biodegradability.

MATERIALS AND METHODS

Radiolabeled Compounds. ^{3}H-Ring-substituted methoxy-methiochlor and methyl-ethoxychlor were synthesized by the method of Hilton and O'Brien (1964) and purified by column chromatography on silica gel using 2% diethyl ether in petroleum ether (60–68°). The products had a radiopurity of 99.9+%, evaluated by thin-layer chromatography (tlc) using solvent system P.B. (Table I), with specific activities of 9.3 and 3.35 mCi/m*M*, respectively.

^{14}C-Ring-labeled chloro-methylchlor was prepared in 72.8% yield by condensation of 100 μCi (5 mCi/m*M*) of ^{14}C-ring labeled chlorobenzene diluted to 10 μl with 30 mg of *p*-methylphenyl trichloromethyl carbinol in 5 vol of H_2SO_4. The product was purified by column chromatography on silica gel with petroleum ether (60–68°) as the eluent and had a radiochemical purity of 99%, with a specific activity of 0.43 mCi/m*M*.

Radioassay. Tritium-labeled compounds were assayed by scintillation counting in 10 ml of ^{3}H cocktail (200 g of naphthalene, 10 g of PPO, and 0.25 g of POPOP in dioxane to make 1 l.). The ^{14}C-labeled compounds were similarly assayed in 10 ml of cocktail D (7 g of PPO, 100 g of naphthalene in dioxane to make 1 l.).

Chromatographic and Chromogenic Techniques. Thin-layer chromatography was performed in the usual manner using 0.25 mm silica gel with a fluorescent indicator coat-

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Table I. Properties of Methoxy-Methiochlor, Methyl-Ethoxychlor, and Chloro-Methylchlor and Their Model Metabolites

Compound	mp, °C	Thin-layer chromatography, R_f[a]							Detection (D–Z)[b]
		P.E_1	P.E_2	P.A	P.B	A.C	BDA_1	BDA_2	
I. Methoxy-methiochlor									
$CH_3OC_6H_4HCCCl_3C_6H_4SCH_3$	92–93	0.44	0.67			0.73			Bluish black
$CH_3OC_6H_4CCCl_2C_6H_4SCH_3$	88	0.52	0.67			0.73			Pink
$CH_3OC_6H_4HCCCl_3C_6H_4SOCH_3$	142–143		0			0.50			Grey
$CH_3OC_6H_4HCCCl_3C_6H_4SO_2CH_3$	92		0.12			0.73			Grey
$CH_3OC_6H_4CCCl_2C_6H_4SO_2CH_3$	80		0.19			0.73			Pink
II. Methyl-ethoxychlor									
$CH_3C_6H_4HCCCl_3C_6H_4OC_2H_5$	96			0.70	0.37		0.67		Black
$CH_3C_6H_4CCCl_2C_6H_4OC_2H_5$	51			0.77	0.43		0.67		Pink
$CH_3C_6H_4HCCCl_3C_6H_4OH$	107–109			0.30	0		0.67		Black
$CH_3C_6H_4CCCl_2C_6H_4OH$	116–117			0.38	0		0.67		Pink
$HOOCC_6H_4HCCCl_3C_6H_4OC_2H_5$	160			0	0		0.48		Grey
III. Chloro-methylchlor									
$ClC_6H_4HCCCl_3C_6H_4CH_3$	82		0.79		0.53			0.73	Dark brown
$ClC_6H_4CCCl_2C_6H_4CH_3$	67–68		0.79		0.63			0.73	Pink
$ClC_6H_4HCCCl_3C_6H_4CHO$	85		0.69		0			0.73	Bluish red
$ClC_6H_4HCCCl_3C_6H_4COOH$	94–96		0		0			0.33	
$ClC_6H_4COC_6H_4CH_3$	121–122		0.56		0			0.73	None

[a] Tlc development systems: P.E_1 = petroleum ether (60–68°)-9 to diethyl ether-1. P.B = petroleum ether (60–68°)-8 to benzene-2. P.A = petroleum ether (60–68°)-8 to acetone-2. A.C = acetone-1 to cyclohexane-1. BDA_1 = benzene-90 to dioxane-30 to acetic acid-1. BDA_2 = benzene-100 to dioxane-3 to acetic acid-1.5. P.E_2 = petroleum ether-1 (60–68°) to diethyl ether-1. [b] Color detection: D–Z = spraying with 0.5% diphenylamine + zinc chloride (in acetone), heating at 110° for 10 min, and exposure to uv light for 5 min.

ed on glass plates (E. Merck GF-254). Solvent systems and chromogenic techniques are given in Table I.

Model Metabolites. Since the three asymmetrical compounds were expected to serve as substrates for multifunction oxidase enzyme systems, the following model metabolites were prepared.

Methoxy-Methiochlor or 2-(*p*-methoxyphenyl)-2-(*p*-methylthiophenyl) - 1,1,1-trichloroethane (I), mp 92–93°, was described by Metcalf *et al.* (1971a).

2-(*p*-Methoxyphenyl)-2-(*p*-methylthiophenyl) - 1,1-dichloroethylene (II), mp 88°, was obtained from I by alkaline dehydrochlorination in ethanol.

2-(*p*-Methoxyphenyl)-2 - (*p*-methylsulfinylphenyl)-1,1,1-trichloroethane (III) was prepared from I by oxidation with H_2O_2 in glacial acetic acid. The product was crystallized from ethanol to mp 142–143°. Nmr spectrometry showed CH_3O protons at δ 3.78, CH_3SO at δ 2.72, and α-H at δ 5.07. Infrared spectrometry showed SO absorption at 1040 cm^{-1}.

2-(*p*-Methoxyphenyl) - 2-(*p*-methylsulfonylphenyl)-1,1,1-trichloroethane (IV) was synthesized by oxidation of I with performic acid in glacial acetic acid and crystallization from ethanol, mp 92°. Nmr spectrometry showed CH_3O protons at δ 3.73, CH_3SO_2 at δ 3.00, and α-H at δ 5.06. CH_3SO_2 absorption in infrared spectrometry was at 1140 and 1300 cm^{-1}.

2-(*p*-Methoxyphenyl) - 2-(*p*-methylsulfonylphenyl)-1,1-dichloroethylene (V), mp 80°, was obtained from IV by alkaline dehydrochlorination in ethanol.

Methyl-ethoxychlor or 2-(*p*-methylphenyl)-2-(*p*-ethoxyphenyl)-1,1,1-trichloroethane (VI), mp 96°, was described by Metcalf *et al.* (1971a).

2-(*p*-Methylphenyl)-2-(*p*-ethoxyphenyl) - 1,1-dichloroethylene (VII), mp 51°, was prepared from VI by dehydrochlorination in ethanolic KOH.

2-(*p*-Methylphenyl)-2-(*p*-hydroxyphenyl) - 1,1,1-trichloroethane (VIII), mp 107–109°, was synthesized by condensation of equimolar quantities of phenol, *p*-methylphenyl trichloromethyl carbinol, and anhydrous $AlCl_3$ in chloroform. Nmr spectrometry showed CH_3 protons at δ 2.28, OH at δ 4.68 (confirmed by its disappearance in D_2O), and α-H at δ 4.90. Infrared spectrometry also confirmed the presence of phenolic OH at 3410 cm^{-1}.

2-(*p*-Methylphenyl)-2-(*p*-hydroxyphenyl) - 1,1-dichloroethylene (IX), mp 116–117°, was obtained from VIII by alkaline dehydrochlorination in ethanol.

2-(*p*-Ethoxyphenyl)-2-(*p*-carboxyphenyl) - 1,1,1-trichloroethane (X), mp 160°, was obtained from VI by oxidation with CrO_3 in acetone. Ir spectrophotometry showed C=O at 1670 cm^{-1} and nmr identification showed $-CH_3$ at δ 1.23 and $-OCH_2$ at δ 3.67 (carboxylic proton was along with the aromatic ones).

Chloro-methylchlor or 2-(*p*-chlorophenyl)-2-(*p*-methylphenyl)-1,1,1-trichloroethane (XI), mp 82°, was described by Chattaway and Muir (1934), who gave mp 81°.

2-(*p*-Chlorophenyl)-2-(*p*-methylphenyl) - 1,1-dichloroethylene (XII), mp 67–68°, was obtained from XI by dehydrochlorination in ethanolic KOH.

2-(*p*-Chlorophenyl)-2-(*p*-formylphenyl) - 1,1,1-trichloroethane (XIII), mp 85°, was obtained from XI by oxidation as described by Perron and Barre (1952). Infrared spectrometry showed C=O peak at 1695 cm^{-1}. Nmr identification showed α-H at δ 5.15 and aldehyde proton at δ 9.02.

2-(*p*-Chlorophenyl)-2-(*p*-carboxylphenyl) - 1,1,1-trichloroethane (XIV), mp 94–96°, was obtained from XI by oxidation with $K_2Cr_2O_7$, as described by Haskelberg and Lavie (1949). Nmr spectrometry confirmed the presence of α-H at δ 5.57; carboxylic proton was, along with the aromatic ones, confirmed by its disappearance in the presence of D_2O. Infrared absorption for C=O was at 1695 cm^{-1}.

p-Chlorophenyl-*p*-methylphenyl ketone (XV), mp 121–122°, was described by Skerrett and Woodcock (1950, mp 126–127°).

Partition Coefficients. The octanol-water partition coefficients of the various labeled compounds were determined essentially as described by Hansch and Fujita (1964) by shaking a trace of the radiolabeled compound in a mixture of 10 ml of water and 10 ml of octanol on a mechanical shaker for 30 min, centrifuging for 1 hr at 2500 rpm, and counting aliquots of each layer by liquid scintillation.

RESULTS AND DISCUSSION

Metabolism of Asymmetrical Analogs by Salt Marsh Caterpillar. The salt marsh caterpillar larvae were fed 0.3-mg portions of radiolabeled methoxy-methiochlor,

Table II. Metabolism of Methoxy-Methiochlor, Methyl-Ethoxychlor, and Chloro-Methylchlor in Salt Marsh Caterpillar (*Estigmene acrea*)

	Percent total radioactivity	
	Homogenate	Excreta
I. Methoxy-methiochlor treatment (98.3% excreta, 1.7% homogenate)		
$CH_3OC_6H_4HCCCl_3C_6H_4SCH_3$	60.9	
$CH_3OC_6H_4HCCCl_3C_6H_4SOCH_3$		93.8
Unknown (R_f 0.27)[a]	14.3	
Base (conjugates)	19.6	2.8
II. Methyl-ethoxychlor treatment (98.0% excreta, 2.0% homogenate)		
$CH_3C_6H_4HCCCl_3C_6H_4OC_2H_5$	92.0	98.0
$CH_3C_6H_4CCCl_2C_6H_4OC_2H_5$	4.4	
Base (conjugates)	1.3	1.2
III. Chloro-methylchlor treatment (94.0% excreta, 6.0% homogenate)		
$CH_3C_6H_4HCCCl_3C_6H_4Cl$	22.6	79.7
$CH_3C_6H_4CCCl_2C_6H_4Cl$	63.3	
$HOOCC_6H_4HCCCl_3C_6H_4Cl$		11.4
Base (conjugates)	14.1	6.4

[a] Solvent system petroleum ether (60–68°)-8 to benzene-2.

methyl-ethoxychlor, and chloro-methylchlor impregnated into 1.0-g blocks of synthetic diet (Kapoor *et al.*, 1970), with the results shown in Table II. Distribution patterns of the extractable radioactivity in feces and total body homogenate of the caterpillars were nearly identical for methoxy-methiochlor and methyl-ethoxychlor, while with chloro-methylchlor the amount of radioactivity in the homogenate was appreciably higher. Tlc data on the specific metabolites showed that methoxy-methiochlor was almost totally detoxified by conversion of CH_3S- to CH_3SO, with the absence of any parent material in the feces indicating the ease with which the insect carries out microsomal sulfoxidation. Methyl-ethoxychlor is much more stable in the insect and undergoes dehydrochlorination only to a limited extent. Despite the availability of handles for O-dealkylation and side-chain oxidation, this compound is excreted nearly intact by the insect indicating the relatively low efficiency of these mechanisms. Chloro-methylchlor has only one degradable handle, the aryl-CH_3 group which is substantially degraded to -COOH. Because of the lesser availability of electrons at the α-carbon from the inductive effect of the *p*-Cl, this compound is also dehydrochlorinated to a large extent (Metcalf and Fukuto, 1968).

Metabolism in the Mouse. Male Swiss white mice were given the radiolabeled asymmetrical analogs orally at 50 mg/kg in olive oil. The metabolic fate is shown in Table III. The distribution patterns in urine and feces were virtually identical for all three compounds. Methoxy-methiochlor was almost completely detoxified by oxidation to

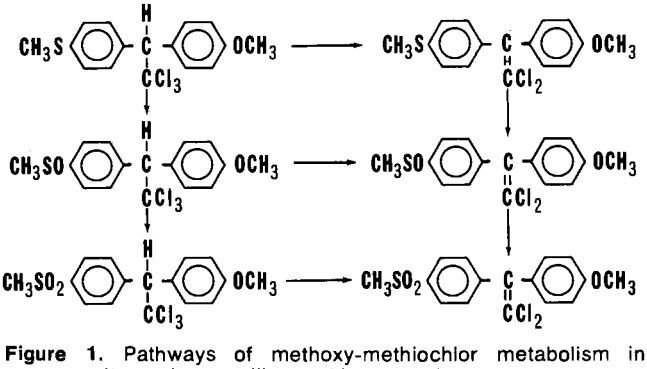


Figure 1. Pathways of methoxy-methiochlor metabolism in mouse, salt marsh caterpillar, and in a model ecosystem.

Table III. Metabolism of Methoxy-Methiochlor, Methyl-Ethoxychlor, and Chloro-Methylchlor in Mouse

	Percent total radioactivity			
	Urine		Feces	
	Hexane	Polar	Hexane	Polar
	Methoxy-methiochlor treatment (feces 73.7%; urine 26.3%)			
	14.6	85.4	23.2	76.8
$CH_3OC_6H_4HCCCl_3C_6H_4SCH_3$			8.7	
$CH_3OC_6H_4HCCCl_3C_6H_4SO_2CH_3$	6.1	2.3	5.3	4.8
$CH_3OC_6H_4CCCl_2C_6H_4SO_2CH_3$	10.3	3.6	18.0	16.2
$CH_3OC_6H_4HCCCl_3C_6H_4SOCH_3$	36.6	44.6	13.2	24.0
Conjugates	35.6	39.7	39.4	41.8
	Methyl-ethoxychlor treatment (feces 76.3%; urine 23.7%)			
	6.7	93.3	29.1	70.9
$CH_3C_6H_4HCCCl_3C_6H_4OC_2H_5$	12.0		29.4	
$CH_3C_6H_4CCCl_2C_6H_4OC_2H_5$	8.7		6.8	
Unknown I (R_f 0.55)[a]	26.2		30.3	
$CH_3C_6H_4HCCCl_3C_6H_4OH$	11.0		12.5	3.0
$CH_3C_6H_4CCCl_2C_6H_4OH$			2.9	
$HOOCC_6H_4HCCCl_3C_6H_4OC_2H_5$	33.2	17.3	7.0	27.3
Unknown II (R_f 0.41)[b]		52.8		46.5
Unknown III (R_f 0.33)[c]		15.5		8.7
	Chloro-methylchlor treatment (feces 74%; urine 26%)			
	12.7	87.3	28.9	71.1
$ClC_6H_4HCCCl_3C_6H_4CH_3$	78.6		9.8	
Unknown I (R_f 0.47)[c]			61.0	
Unknown II (R_f 0.42)[c]			15.9	
$ClC_6H_4HCCCl_3C_6H_4COOH$			5.0	57.8
Unknown III (R_f 0.18)[d]	21.4	Trace	Trace	Trace
Unknown IV (R_f 0.10)[d]		54.9		16.1
Conjugates		35.3		17.6

Solvent systems = [a] Petroleum ether (60–68°)-8 to acetone-2. [b] Benzene-90 to dioxane-30 to acetic acid-1. [c] Petroleum ether (60–68°)-8 to benzene-2. [d] Benzene-100 to dioxane-3 to acetic acid 1.5.

sulfoxide and sulfone analogs, with some dehydrochlorination of the relatively unstable sulfone (Metcalf and Fukuto, 1968). The polar products which remained at the origin of the tlc plates may be conjugates formed as a result of S-dealkylation.

With methyl-ethoxychlor, the microsomal processes of O-dealkylation and side-chain oxidation were both significant steps in metabolism, in contrast to their inactivity in the salt marsh caterpillar, and the major identified metabolites were the phenol, 2-(*p*-methylphenyl)-2-(*p*-hydroxyphenyl) - 1,1,1-trichloroethane, and the carboxylic acid, 2-(*p*-ethoxyphenyl)-2-(*p*-carboxyphenyl) - 1,1,1-trichloroethane. Unknown I (R_f 0.55) is probably 2-(*p*-methylphenyl)-2-(*p*-ethoxyphenyl) - 1,1-dichloroethane and unknown II (R_f 0.41) may be the O-dealkylation product of the carboxylic acid, 2-(*p*-hydroxyphenyl)-2-(*p*-carboxyphenyl) - 1,1,1-trichloroethane.

Chloro-methylchlor is oxidized in the mouse to give large amounts of 2-(*p*-chlorophenyl)-2-(*p*-carboxyphenyl) - 1,1,1-trichloroethane and other more polar metabolites. This molecule differs from DDT in having one ring-Cl replaced by the biodegradable aryl-CH_3, and this alteration obviously makes chloro-methylchlor a substrate for the mixed function oxidase (MFO) enzymes. Thus, a single biodegradable handle on the aryl ring of the DDT-type molecule provides for its ready conversion to water-soluble metabolites and consequent excretion, rather than for lipid storage as DDE and DDD in the case of DDT (Kapoor *et al.*, 1972; Metcalf *et al.*, 1971b).

Table IV. Distribution of [^{3}H]Methoxy-Methiochlor, [^{3}H]Methyl-Ethoxychlor, and [^{14}C]Chloro-Methylchlor and Their Metabolites in a Model Ecosystem

		Concentration, ppm				
		H_2O	*Oedogonium*, (algae)	*Physa*, (snail)	*Culex*, (mosquito)	*Gambusia*, (fish)
I. Methoxy-methiochlor treatment						
Total ^{3}H		0.016	0.074	3.61	1.19	0.15
Unknown I (R_f 0.90)[a]	(Np[f])		0.002		0.042	0.035
$CH_3OC_6H_4HCCCl_3C_6H_4SCH_3$	(Np)	0.00001		0.034		0.003
$CH_3OC_6H_4CCCl_2C_6H_4SCH_3$	(Np)	0.00002				0.002
$CH_3OC_6H_4HCCCl_3C_6H_4SOCH_3$	(P[g])	0.0041	0.049	0.950	0.734	0.005
$CH_3OC_6H_4HCCCl_3C_6H_4SO_2CH_3$	(P)	0.0034	0.011	1.775		0.003
$CH_3OC_6H_4CCCl_2C_6H_4SO_2CH_3$	(P)	0.00099	0.005	0.626	0.033	0.002
Unknown II (R_f 0.63)[a]	(P)	0.0033				0.019
Unknown III (R_f 0.56)[a]	(P)	0.0006	0.002			
Unknown IV (R_f 0.43)[a]	(P)	0.0006				
Unknown V (R_f 0.36)[a]	(P)	0.0015		0.133		0.004
Base (conjugates)	(P)	0.00104	0.005	0.009		0.075
II. Methyl-ethoxychlor treatment						
Total ^{3}H		0.01845	38.84	10.83	0.74	0.24
$CH_3C_6H_4HCCCl_3C_6H_4OC_2H_5$	(Np[f])	0.00012	27.89	5.03	0.11	0.04
$CH_3C_6H_4CCCl_2C_6H_4OC_2H_5$	(Np)	0.00020		2.99		0.03
Unknown I (R_f 0.63)[b]	(Np)		1.24	0.63	0.05	0.04
Unknown II (R_f 0.49)[b]	(P[g])			0.62	0.05	0.02
$CH_3C_6H_4HCCCl_3C_6H_4OH$	(P)	0.00039	3.42			0.02
$CH_3C_6H_4CCCl_2C_6H_4OH$	(P)	0.00015	1.13		0.11	0.02
$HOOCC_6H_4HCCCl_3C_6H_4OC_2H_5$	(P)	0.00271			0.06	0.03
Unknown II (R_f 0.35)[c]	(P)	0.00119			0.09	
Base (conjugates)	(P)	0.00149	1.63	0.21	0.09	0.04
Polar	(P)	0.01130				
III. Chloro-methylchlor treatment						
Total ^{14}C		0.0697	3.38	31.80	22.94	2.88
$ClC_6H_4HCCCl_3C_6H_4CH_3$	(Np[f])	0.00045	1.63	9.46	9.96	0.63
$ClC_6H_4CCCl_2C_6H_4CH_3$	(Np)		0.13	1.08	3.02	0.02
Unknown I (R_f 0.10)[d]	(P[g])		0.88	17.64		
$ClC_6H_4HCCCl_3C_6H_4COOH$	(P)	0.04955	0.23			1.79
Unknown II (R_f 0.21)[e]	(P)	0.00112				
Unknown III (R_f 0.19)[e]	(P)	0.00300				
Base (conjugates)	(P)	0.00603	0.51	3.60	9.96	0.44
Polar metabolites	(P)	0.0095				

Solvent systems = [a] Acetone-1 to cyclohexane-1. [b] Petroleum ether (60–68°)-8 to acetone-2. [c] Benzene-90 to dioxane-30 to acetic acid-1. [d] Petroleum ether (60–68°)-8 to benzene-2. [e] Benzene-100 to dioxane-3 to acetic acid-1.5. [f] Nonpolar compound. [g] Polar compound.

An essential feature of the insecticidal activity of the biodegradable DDT compounds is the much lower rates of MFO oxidation in insects than in vertebrates. This is demonstrated by comparisons of metabolism in the salt marsh caterpillar (Table II) and the mouse (Table III). Methyl-ethoxychlor, for example, is excreted almost unchanged by the caterpillar but is very substantially metabolized by the mouse. Chloro-methylchlor, which is converted only to a small extent to the carboxylic acid metabolite in the salt marsh caterpillar, is predominately oxidized to both carboxylic acid and much more polar compounds in the mouse.

Ecological Fate in a Model Ecosystem. The use of a model ecosystem to demonstrate biodegradability has been described by Metcalf *et al.* (1971b). The comparable fates of [^{3}H]methoxy-methiochlor and [^{3}H]methyl-ethoxychlor, and of [^{14}C]chloro-methylchlor are presented in Table IV. All three compounds are obviously highly degradable, with methoxy-methiochlor being the most fugitive. This compound is readily oxidized and dehydrochlorinated and the parent compound was ecologically magnified from the 0.00001 ppm in the water only 300-fold to the 0.003 ppm in the fish *Gambusia*. The parent compound in the fish represented only 2% of the total ^{3}H activity, with the remainder being largely polar conjugates (R_f 0.0 in acetone–cyclohexane solvent). Methoxy-methiochlor consequently has a biodegradability index (ppm polar compounds/ppm nonpolar compounds) of 2.75 in *Gambusia*. In the snail, the accumulated radioactivity was only 0.034 ppm of parent compound, or about 1% of the total ^{3}H and the sulfoxide and sulfone oxidation products, 2-(*p*-methoxyphenyl)-2-(*p*-methylsulfinylphenyl) - 1,1,1-trichloroethane and 2-(*p*-methoxyphenyl)-2-(*p*-methylsulfonylphenyl) - 1,1,1-trichloroethane, and the ethylene of the latter represented the bulk of the total activity. The overall pathways of metabolism are shown in Figure 1.

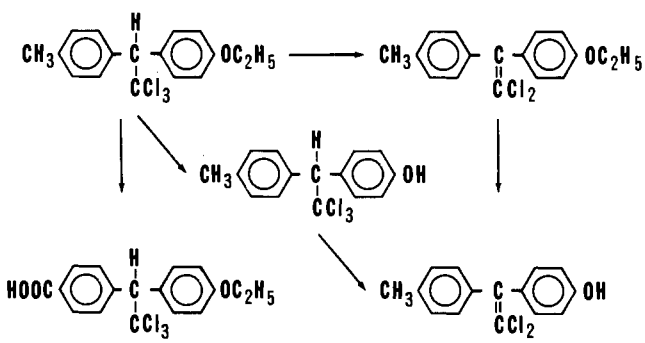
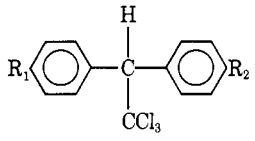


Figure 2. Pathways of methyl-ethoxychlor metabolism in mouse, salt marsh caterpillar, and in a model ecosystem.

Table V. Ecological Magnification and Biodegradability Constants for DDT Analogs



R_1	R_2	Ecological magnification[a] Fish	Ecological magnification[a] Snail	Biodegradability index[b] Fish	Biodegradability index[b] Snail	Σ sigma	Partition coefficient	H_2O solubility, ppm	Σ, P_i[c]
Cl	Cl	84,500	34,500	0.015	0.045	0.454	9.49×10^3	0.002	1.17 (1.40)
CH_3O	CH_3O	1,545	120,000	0.94	0.13	−0.536	2.05×10^3	0.62	0.50 (−0.08)
C_2H_5O	C_2H_5O	1,536	97,645	2.69	0.39	−0.480	1.18×10^3	0.163	0.26 (0.92)
CH_3	CH_3	140	120,270	7.14	0.08	−0.340	3.74×10^3	2.21	0.76 (1.04)
CH_3S	CH_3S	5.5	300	47	0.77	0.0	7.08×10^3	0.57	1.04 (1.24)
CH_3O	CH_3S	310	3,400	2.75	105	−0.268	7.84×10^2	0.189	0.08
CH_3	C_2H_5O	400	42,000	1.20	0.25	−0.410	9.19×10^3	0.028	1.15
Cl	CH_3	1,400	21,000	3.43	2.0	0.057	2.93×10^4	0.10	1.66

[a] Ratio of concentration in organism/concentration in water. [b] Ratio of polar/nonpolar metabolites. [c] Partition coefficient of 2,2-diphenyl-1,1,1-trichloroethane = 6.45×10^2.

Methyl-ethoxychlor was biologically magnified only 333-fold from water to fish, and the parent compound represented only about 17% of the total radioactivity in the fish, with the remainder being predominately polar oxidation products produced by O-dealkylation and side-chain oxidation. The biodegradability index in the fish was 1.20. The snail accumulated substantial quantities of the parent compound and its dehydrochlorination product, concentrating the compound 42,000-fold. The overall pathways of metabolism are shown in Figure 2.

Chloro-methylchlor, with only a single biodegradable handle and with an aryl chlorine atom, should behave more like DDT. Chloro-methylchlor was concentrated 1400-fold in the fish and the parent compound represented about 25% of the total ^{14}C, with the remainder being almost entirely polar metabolites to give a biodegradability index in the fish of 3.43. The ecological magnification in the snail was 21,000 times that in water. The overall pathways of metabolism are shown in Figure 3.

CORRELATION OF STRUCTURE AND BIODEGRADABILITY

The studies in the present paper plus those on the symmetrical DDT analogs previously described, (Kapoor *et al.*, 1970, 1972; Metcalf *et al.*, 1971b) provide data on eight insecticidally active DDT analogs for which extensive data are available on comparative metabolism in insect, mouse, and a model ecosystem. This information provides an opportunity to assess the influence of molecular structure on biodegradability, particularly in terms of the parameters of "ecological magnification," or ratio of concentration of parent material in the organism to concentration in water, and "biodegradability index," or the ratio of polar to nonpolar metabolites in the organism.

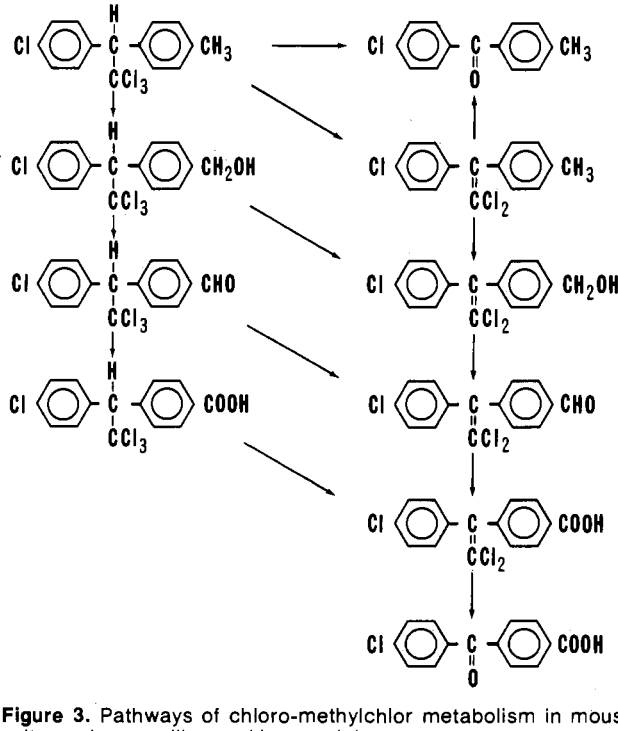


Figure 3. Pathways of chloro-methylchlor metabolism in mouse, salt marsh caterpillar, and in a model ecosystem.

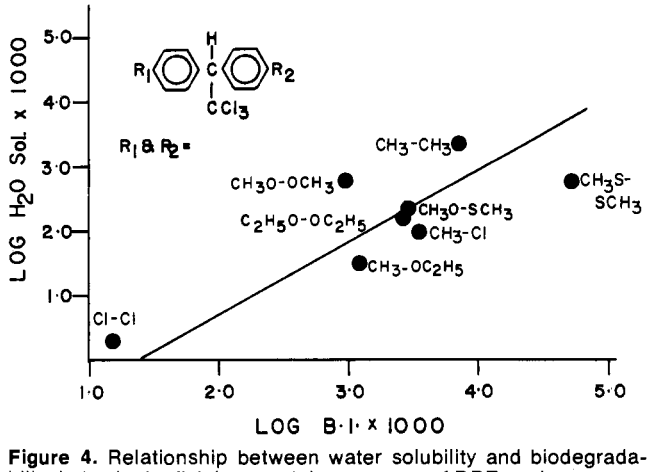


Figure 4. Relationship between water solubility and biodegradability index in the fish in a model ecosystem of DDT analogs.

Ecological magnification describes the ability of the organism to accumulate the xenobiotic, and biodegradability index describes the ability of the organism to degrade the xenobiotic. Data on these properties for the eight DDT analogs are shown in Table V, together with water solubility and the linear free energy parameters of Hammett's σ for the para substituents, and octanol–water partition coefficients.

The ecological magnification data for the snail, *Physa*, show that analogs with CH_3O, C_2H_5O, CH_3, and Cl substituents contain relatively enormous accumulations of 20,000 to 120,000-fold over the water concentrations. The two analogs with CH_3S substituents contained much lower accumulations, 300- to 3400-fold. This, together with inspection of the chemical nature of the metabolites found, indicates that the snail is able to carry out oxidation of the CH_3S group to CH_3SO- and CH_3SO_2-, but cannot readily oxidize $-CH_3$ groups to -COOH or O-dealkylate CH_3O- or C_2H_5O- to HO-. The deficiency of microsomal oxidase enzymes in *Physa* is confirmed by unpublished biochemical data (Hansen *et al.*, 1972), showing this organism to be deficient in cytochrome P_{450}. Because of the deficient oxidative pathways in the snail, the dehydrochlorination pathways are emphasized as with DDT, and relatively large amounts of the ethylenes were stored with ethoxychlor, methoxy-methiochlor (as sulfoxide), methyl-ethoxychlor, and chloro-methylchlor.

The fish *Gambusia* responds otherwise and rapidly degraded all of the compounds except DDT, so that ecological magnification values ranged from 5 to 1500, as compared with 84,500 for DDT. The accumulation of the asymmetrical analogs was intermediate between the extremes of their symmetrical parents; *i.e.*, methoxychlor 1500, methoxy-methiochlor 300, and methiochlor 5.5; ethoxychlor 1500, methyl-ethoxychlor 400, and methylchlor 140; and DDT 84,500 chloro-methylchlor 1400, and methylchlor 140. Data for chloro-methylchlor demonstrate that a single biodegradable handle is sufficient to impart a substantial rate of biodegradability to the compound (Table V).

It is tempting to relate biodegradability to a single molecular parameter so that quantitative extrapolations might be feasible. The bioaccumulation of organic compounds seems to be related to the results of a series of partitionings from water to lipids, and the concentration factors for highly lipid-soluble compounds should be inversely proportional to their water solubilities (Hamelink *et al.*, 1971). Hansch and Fujita (1964) have suggested the octanol–water partition constant as a measure of the lipophilicity of various organic compounds, and their linear free energy substituent constant, $P_i = \log P_x - \log P_H$, has been widely studied in relating chemical structure and biological activity. Partition constants for such water-insoluble compounds as DDT and its analogs are very difficult to determine, but can be approximated by radiotracer investigations. Our radiotracer results for the determinations of water solubility, octanol–H_2O partitioning, and for P_i of the DDT analogs are presented in Table V. These P_i values for DDT analogs as obtained experimentally agree reasonably well with those tabulated by Hansch *et al.* (1963), except for the CH_3O group, which is much higher experimentally than tabulated. A plot of log values of biodegradability index for fish *vs.* log partition coefficient showed very poor correlation and was insignificant at the 5% level in regression analysis. However, as shown in Figure 4, a plot of log BI *vs.* log H_2O solubility clearly indicates a relationship, and regression analysis produced an equation log BI = 1.41 + 0.87 log H_2O solubility, with a correlation coefficient $r = 0.82$, which explains 67% of the variation. Considering the difficulties of measurement of the parameters involved, the agreement is excellent. Table V shows that the values for water solubility of the highly lipid-partitioning compounds vary over a 1000-fold range, while the partition coefficients vary over only about a 30-fold range. Therefore, for such very slightly water-soluble compounds, partitioning from water to tissues and consequent storage away from sites of detoxication seems to be controlled by water solubility, as suggested by Hamelink *et al.* (1971).

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