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Comparative Metabolism of DDT, Methylchlor, and Ethoxychlor in Mouse, Insects, and in a Model Ecosystem

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Ethoxychlor [2,2-bis(*p*-ethoxyphenyl)-1,1,1-trichloroethane] and methylchlor [2,2-bis(*p*-methylphenyl)-1,1,1-trichloroethane] were evaluated for metabolic pathways in mice and insects and for biodegradability in a model ecosystem. Ethoxychlor, like methoxychlor, was metabolized by O dealkylation to form 2-(*p*-hydroxyphenyl)-2-(*p*-ethoxyphenyl)-1,1,1-trichloroethane and subsequently to 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane. In the model ecosystem ethoxychlor was found in fish at the top of the food chain at 1500 times the amount in water, together with larger amounts of polar and dealkylation products. Methylchlor was metabolized by oxidation of the arylmethyl groups to form 2-(*p*-hydroxymethylphenyl)-2-(*p*-methylphenyl)-1,1,1-trichloroethane and by subsequent oxidation to 2,2-bis(*p*-carboxyphenyl)-1,1,1-trichloroethane. In the model ecosystem, methylchlor was found in fish at 1400 times the amount in water together with larger amounts of polar metabolites. These two DDT analogs are much more biodegradable than DDT, which was concentrated in fish at 85,000 times the amount in water.

In the search for biodegradable analogs of DDT, the metabolism of methoxychlor and methiochlor in mice, insects, and a model ecosystem was investigated by Kapoor *et al.* (1970). In further study of the ecological biochemistry of DDT analogs with biodegradable substituents in the para positions of the aryl rings, ethoxychlor or 2,2-bis(*p*-ethoxyphenyl)-1,1,1-trichloroethane and methylchlor or 2,2-bis(*p*-methylphenyl)-1,1,1-trichloroethane have now been investigated. Ethoxychlor was first synthesized by Fritsch and Feldman (1899). It is substantially more insecticidal than methoxychlor (Metcalf and Fukuto, 1968) but is also more toxic to mammals with an oral LD_{50} to the mouse of 300 mg/kg (Von Oettingen and Sharpless, 1946).

Methylchlor was first made by Fisher (1874). It was found to be substantially toxic to insects (Metcalf and Fukuto, 1968) and nontoxic to the mouse, oral LD_{50} > 1000 mg/kg (Von Oettingen and Sharpless, 1946). Ariens (1966) suggested, without evidence, that methylchlor should be substantially biodegradable through microsomal side chain oxidation to hydroxymethyl and benzoic acid moieties.

Peterson and Robison (1964) and Jensen *et al.* (1957) have reported the qualitative metabolism of DDT in mammals, and it is surprising that even now there is little data available on its metabolic pattern in the mouse. Due to this paucity of information, the problem has been restudied and information is presented on the metabolism of DDT, methylchlor, and ethoxychlor.

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MATERIALS AND METHODS

Radio-Labelled Compounds. ^{3}H-Ring substituted ethoxychlor was synthesized by the method of Hilton and O'Brien (1964), and purified by column chromatography on silica gel by elution with 2% diethyl ether in petroleum ether (boiling range 60–68° C). The product had a radio purity of 99.9+% evaluated by thin-layer chromatography (tlc),

Table I. Properties of Ethoxychlor and Its Model Metabolites

Compound	mp, °C	Thin-layer chromatography (R_f)[a] HDE$_1$	HDE$_2$	Detection[b] uv	D–Z
$C_2H_5OC_6H_4HCCCl_3C_6H_4OC_2H_5$	105	0.49	0.71	None	Steel gray
$C_2H_5OC_6H_4CCCl_2C_6H_4OC_2H_5$	107	0.55	0.71	None	Pink
$C_2H_5OC_6H_4HCCCl_3C_6H_4OCH_2CH_2OH$	117		0.40	None	Steel gray
$C_2H_5OC_6H_4HCCCl_3C_6H_4OH$	65–7		0.49	Light yellow	Black
$HOC_6H_4HCCCl_3C_6H_4OH$	194.0		0.27	Yellow	Black
$HOC_6H_4CCCl_2C_6H_4OH$	212.0		0.30	Yellow	Pink
$HOC_6H_4COC_6H_4OH$	213–215.0		0.18	Yellow	None

[a] The development systems: HDE$_1$ = hexane–dioxane–diethyl ether (80:20:10); HDE$_2$ = hexane–dioxane–diethyl ether (90:60:10). [b] Color detection: uv = exposure to uv light for 5–10 min; D–Z = spraying with 0.5% diphenylamine + zinc chloride (in acetone), heating at 110° C for 10 min and exposure to uv light for 5 min.

Table II. Properties of Methylchlor and Its Model Metabolites

Compound	mp, °C	Thin-layer chromatography (R_f)[a] P	BDA	Detection[b] D–Z
$CH_3C_6H_4HCCCl_3C_6H_4CH_3$	83	0.28	0.71	Black
$CH_3C_6H_4CCCl_2C_6H_4CH_3$	82	0.38	0.71	Pink
$CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$	74		0.53	Gray
$CH_3C_6H_4HCCCl_3C_6H_4COOH$	107		0.48	Gray
$HOH_2CC_6H_4HCCCl_3C_6H_4CH_2OH$	Gummy solid		0.30	None
$HOOCC_6H_4HCCCl_3C_6H_4COOH$	274–6		0.33	None

[a] Tlc development systems: P = petroleum ether (60–68° C); BDA = benzene-90 to dioxane-30 to acetic acid (glacial)-1. [b] Color detection: D–Z = spraying with 0.5% diphenylamine + zinc chloride (in acetone), heating at 110° C for 10 min and exposure to uv light for 5 min.

using solvent HDE (Table I) with a specific activity of 4.44 mCi/mmol.

^{14}C-Methyl-labelled methylchlor [2,2-bis(*p*-methylphenyl)-1,1,1-trichloroethane] was synthesized in vacuum line from 3 mg of ^{14}C-toluene, diluted to 20 mg with cold toluene, and condensed with 20 mg of chloral in 10 volumes of concentrated H_2SO_4 in 60% yield. The product was purified by column chromatography on silica gel with petroleum ether (60–80° C) as the eluent, and had a radiochemical purity of 99.9+% with a specific activity of 1.30 mCi/mmol. ^{14}C-Ring-labelled DDT was supplied by the World Health Organization, Geneva, Switzerland, with a specific activity of 5.48 mCi/mmol. It was purified by column chromatography to 99.9+% purity.

Radioassay. Tritium-labelled compounds in biological materials were assayed by oxygen flask combustion.

^{14}C-Labelled compounds likewise were also combusted by a procedure essentially identical to tritium combustion. For CO_2 absorption, 20 ml of N cocktail (135 ml of phenethylamine, 135 ml of methanol, 730 ml of toluene, 5 g of PPO, and 100 mg of POPOP) was injected into the flask after combustion. The flask was held over ice cold water for 15 min and periodically shaken. A 15-ml aliquot was then counted on a Packard 2002 liquid scintillation counter. Other radioassay methods were those of Kapoor *et al.* (1970).

Chromatographic techniques were carried out on 0.25 mm plates of silica gel with a fluorescent indicator (E. Merck) using the solvent systems given in Tables I and II. Chromogenic techniques for identification of individual metabolites are given by Kapoor *et al.* (1970).

Model Metabolites. The previous investigation of the metabolism of methoxychlor (Kapoor *et al.*, 1970) which showed biological degradation to mono- and dihydroxyphenols produced by O dealkylation suggested that ethoxychlor might be degraded through a similar metabolic pathway. Therefore, the following model metabolites were prepared.

Ethoxychlor or 2,2-bis(*p*-ethoxyphenyl)-1,1,1-trichloroethane (**I**), mp 105° C (Fritsch and Feldman, 1899; mp 105° C) was synthesized by condensation of ethoxybenzene (phenetole) with chloral in the presence of $AlCl_3$.

The 2,2-bis(*p*-ethoxyphenyl)-1,1-dichloroethylene (**II**), mp 107° C (Stephenson and Waters, 1946; mp 109° C) was obtained from **I** by alkaline dehydrochlorination in ethanol.

2-*p*-Ethoxyphenyl-2-*p*-(β-hydroxyethoxyphenyl)-1,1,1-trichloroethane (**III**) was obtained by reacting 2.0 g of **IV** with 1.0 g of 2-bromoethanol in 50 ml of dry acetone containing 2.0 g of K_2CO_3. The mixture was refluxed for 14 hr, filtered, and acetone removed under reduced pressure. The residue was taken up in ether, washed twice with 5% KOH and then water. The product was purified on a silica gel column by eluting with increasing concentrations of diethyl ether in petroleum ether (60–68° C). The product was recrystallized from cyclohexane to mp 107° C and identified by nmr spectrometry, showing α-H at τ 5.07, $-CH_3$ at τ 8.63, $-CH_2$ at τ 6.07, and $-OH$ at τ 7.88.

2-*p*-Ethoxyphenyl-2-*p*-hydroxyphenyl-1,1,1-trichloroethane (**IV**), mp 65–67° C, was prepared by the method of Kapoor *et al.* (1970) for the methoxy analog. Nmr spectrometry showed α-H at τ 5.03, $-CH_3$ at τ 8.62, and $-CH_2$ at τ 5.94.

Methylchlor or 2,2-bis(*p*-methylphenyl)-1,1,1-trichloroethane (**VIII**), mp 87–88° C (Fisher, 1874; mp 89° C) was obtained by condensation of 18.4 g of toluene with 14.8 g of chloral by the dropwise addition of 250 ml of concentrated H_2SO_4. The product was crystallized from ethanol.

2,2-Bis(*p*-methylphenyl)-1,1-dichloroethylene (**IX**), mp 82° C (Stephenson and Waters, 1946; mp 87° C) was prepared by alkaline dehydrochlorination in ethanol.

2-*p*-Methylphenyl-2-*p*-hydroxymethylphenyl-1,1,1-trichloroethane (**X**) was made from 2-*p*-methylphenyl-2-*p*-bromomethylphenyl-1,1,1-trichloroethane (from the bromination of **VIII**, identified by nmr, $-CH_2$ at τ 5.57, $-CH_3$ at τ 7.88, and α-H at τ 5.01) by base hydrolysis and purified by column chromatography on silica gel with increasing concentrations of diethyl ether in petroleum ether (60–68° C) as the eluent. The product was crystallized from petroleum ether, mp 74° C. Nmr spectrometry showed α-H at τ 4.92, $-CH_3$ protons at τ 7.65, $-CH_2$ at τ 5.28, and $-OH$ at τ 8.32, confirmed by its disappearance in the presence of D_2O.

2,2-Bis(*p*-hydroxymethylphenyl)-1,1,1-trichloroethane (**XI**)

was prepared from 2,2-bis(*p*-bromomethylphenyl)-1,1,1-trichloroethane (Stephenson and Waters, 1946) by alkaline hydrolysis. The resultant product was a gum, even after purification by column chromatography. The product gave a single spot on tlc. Nmr spectrum confirmed the structure by presence of $-OH$ at τ 7.4 which disappeared in the presence of D_2O.

2-*p*-Methylphenyl-2-*p*-carboxyphenyl-1,1,1-trichloroethane (**XII**) was made by oxidation of **VIII** with CrO_3 in glacial acetic acid and crystallized from dilute ethanol to mp 107° C and identified by infrared spectrometry (C=O at 1680 cm^{-1}) and nmr data which showed α-H at τ 4.88, $-CH_3$ at τ 7.72, and $-OH$ at τ 1.34. Fisher (1874) gives 174° C, which must be an error.

2,2-Bis(*p*-carboxyphenyl)-1,1,1-trichloroethane (**XIII**), mp 274–276° C, was obtained from **VIII** (Haskelberg and Lavie, 1949; mp 271–272° C). Ir showed strong absorption at 1600 cm^{-1}.

TOXICOLOGICAL METHODS

Metabolic studies in male Swiss mice were carried out by oral administration of ^{14}C-DDT, ^{14}C-methylchlor, and ^{3}H-ethoxychlor at 50 mg/kg in olive oil. Procedures for mice, houseflies, and salt marsh caterpillars have already been described (Kapoor *et al.*, 1970).

Microsomal Oxidations. Ethoxychlor and methylchlor were incubated with mouse liver homogenate. After perfusion with 1.15% KCl, mouse liver was homogenized and suspended in 1.15% KCl (10% w/v). The homogenate was centrifuged at 15,000 × *g* for 30 min and the incubation mixture, consisting of 32 ml of supernatant, 1 ml of NADPH (0.1%), 1 ml of nicotinamide (5.2×10^{-4} *M*), 2.6 ml of phosphate buffer (0.2 *M*, pH 7.8) and 20 μl of substrate in ethanol (0.1%), was incubated for 1 hr at 37° C, acidified to pH 2.0, heated on a steam bath for 1 hr, extracted with Et_2O, and processed for identifying and determining the metabolites.

Model Ecosystem. Detailed methodology has already been reported (Kapoor *et al.*, 1970; Metcalf *et al.*, 1971b).

RESULTS AND DISCUSSION

Studies of the metabolic fate of radio-labelled ethoxychlor and methylchlor in R_{SP} female houseflies are shown in Table III. The percent recovery for ethoxychlor was poor and the data are the average of three different experiments. Analysis of the results shows that ethoxychlor is O dealkylated to mono- and bis-phenolic products, but unlike methoxychlor, dehydrochlorination does play an important role. Since O deethylation is an inefficient process compared to O demethylation (Hansen *et al.*, 1971), the organism must utilize the alternative dehydrochlorination as a survival mechanism. Methylchlor is oxidized to benzyl alcohol and benzoic acid analogs.

Metabolism by Salt Marsh Caterpillar. Substantially more radioactivity fed the organism was recovered in the feces in case of ethoxychlor (99.0%) than methylchlor (93.6%). Whereas dehydrochlorination was the major metabolic pathway for ethoxychlor metabolism, methylchlor was also oxidized to benzoic acid analogs and conjugates were formed. The qualitative and quantitative distribution of metabolites is shown in Table IV.

Rates of Excretion in the Mouse. Comparative rates of elimination of radio-labelled DDT, ethoxychlor, and methylchlor, along with data for methoxychlor and methiochlor (Kapoor *et al.*, 1970), are shown in Figure 1. The rate of elimination of DDT was determined by the more efficient O_2 flask combustion technique at 50 mg/kg dosage, equivalent to the dose used for other analogs. DDT was eliminated

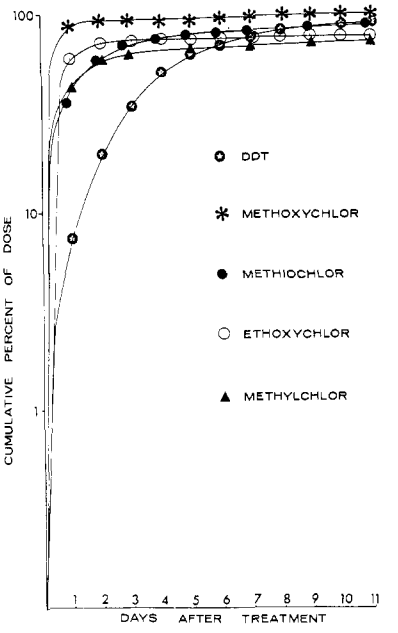

Figure 1. Accumulative rates of elimination of ^{14}C-DDT, ^{3}H-ethoxychlor, ^{14}C-methylchlor, and ^{3}H-methoxychlor and methiochlor in urine and feces of mouse following oral administration. Data for methoxychlor and methiochlor from Kapoor *et al.* (1970)

Table III. Metabolism of Ethoxychlor and Methylchlor in R_{SP} Housefly

	Percent total radioactivity	
	Homogenate	Excreta
Ethoxychlor treatment (1.8% wash, 35.8% excreta, 11.0% homogenate = 48.6% recovery)		
Ethoxychlor	53.5	14.6
$C_2H_5OC_6H_4CCCl_2C_6H_4OC_2H_5$	21.2	13.4
$C_2H_5OC_6H_4HCCCl_3C_6H_4OCH_2CH_2OH$		12.2
$C_2H_5OC_6H_4HCCCl_3C_6H_4OH$	1.3	14.0
$HOC_6H_4HCCCl_3C_6H_4OH$	4.6	18.6
$HOC_6H_4CCCl_2C_6H_4OH$	1.7	11.8
$HOC_6H_4COC_6H_4OH$		5.9
Conjugates	8.6	7.5
Methylchlor treatment (3.0% wash, 61.0% excreta, 5% homogenate = 69% recovery)		
Methylchlor	40.2	18.6
Unknown[a]		12.4
$CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$		16.5
$HOCH_2C_6H_4HCCCl_3C_6H_4CH_2OH$	41.6	12.6
$HOOCC_6H_4HCCCl_3C_6H_4COOH$		15.1
Conjugates	18.2	24.8

[a] Unknown R_f 0.64 (benzene/dioxane/acetic acid 90:30:1).

Table IV. Metabolism of Ethoxychlor and Methylchlor in Salt Marsh Caterpillar (*Estigmine acrea*)

	Percent total radioactivity	
	Homogenate	Excreta
Ethoxychlor treatment (99.0% excreta, 1.0% homogenate)		
Ethoxychlor	81.5	92.1
$C_2H_5OC_6H_4CCCl_2C_6H_4OC_2H_5$	17.9	6.9
$C_2H_5OC_6H_4HCCCl_3C_6H_4OH$	Traces	Traces
Conjugates	Traces	Traces
Methylchlor treatment (93.6% excreta, 6.4% homogenate)		
Methylchlor	84.5	95.5
$CH_3C_6H_4CCCl_2C_6H_4CH_3$	7.4	
$CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$		Traces
$HOOCC_6H_4HCCCl_3C_6H_4COOH$		1.4
Conjugates	8.1	2.1

Table V. Metabolism of Ethoxychlor and Methylchlor by Mouse Liver Homogenate

	Percent total radioactivity
Ethoxychlor treatment	
Ethoxychlor	72.5
$C_2H_5OC_6H_4HCCCl_3C_6H_4OH$	9.1
Conjugates	12.4
Methylchlor treatment	
Methylchlor	19.3
unknown I (R_f 0.64)[a]	2.1
$CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$	30.4
$CH_3C_6H_4HCCCl_3C_6H_4COOH$	5.5
$HOOCC_6H_4HCCCl_3C_6H_4COOH$	6.2
Unknown II (R_f 0.32)[a]	7.6
$HOH_2CC_6H_4HCCCl_3C_6H_4CH_2OH$	19.8
Conjugates	1.6

[a] Solvent system: benzene/dioxane/acetic acid 90:30:1.

slowly (7.4% in the first 24 hr), while methylchlor (43.7%) and ethoxychlor (99.0%) were rapidly eliminated. However, over a period of 11 days, 90.8% DDT had been eliminated compared to 73.7% for methylchlor and 77.5% for ethoxychlor, thus indicating that although initially DDT has a much slower rate of elimination, it reaches the level of the other analogs within 6 days. The degree of polarity of the excretory metabolites is indicated by the urine/feces ratio, which is 0.37 for DDT, 0.69 for ethoxychlor, and 0.12 for methylchlor. The ratio for ethoxychlor differs substantially from its methoxy analog (0.13) and may be indicative of its higher toxicity.

Metabolism by Mouse Liver Homogenate. As shown in Table V, 72.5% of ethoxychlor was recovered intact after incubation with the homogenate along with 9.1% of the mono-phenolic product 2-(*p*-hydroxyphenyl)-2-(*p*-ethoxyphenyl)-1,1,1-trichloroethane and 12.4% conjugates. In comparison, 80.7% of methylchlor was metabolized, the major components being the monoalcohol (30.4%) or 2-(*p*-methylphenyl)-2-(*p*-hydroxymethylphenyl)-1,1,1-trichloroethane and di-alcohol (19.8%) or 2,2-bis(*p*-hydroxymethylphenyl)-1,1,1-

Table VI. Metabolism of DDT, Ethoxychlor, and Methylchlor in Mouse

	Percent total radioactivity			
	Urine		Feces	
	Hexane	Polar	Hexane	Polar
Ethoxychlor treatment (feces 62.3%, urine 37.7%)				
	11.5	88.5	44.5	55.5
Ethoxychlor	53.1		15.1	1.5
$C_2H_5OC_6H_4CCCl_2C_6H_4OC_2H_5$	1.0		1.9	
$C_2H_5OC_6H_4HCCCl_3C_6H_4OCH_2CH_2OH$	1.2		46.0	18.0
$C_2H_5OC_6H_4HCCCl_3C_6H_4OH$	1.7		35.1	12.3
$HOC_6H_4HCCCl_3C_6H_4OH$	9.7	3.0		26.4
$HOC_6H_4CCCl_2C_6H_4OH$	2.1	1.4		6.4
$HOC_6H_4COC_6H_4OH$		2.8		12.1
Conjugates	31.3	92.2		23.3
Methylchlor treatment (feces 88.9%, urine 11.1%)				
	9.1	90.9	4.9	95.2
Methylchlor	74.9		72.6	
$CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$			8.2	
$CH_3C_6H_4HCCCl_3C_6H_4COOH$			9.5	5.3
Unknown I (R_f 0.44)[a]		28.0		
Unknown II (R_f 0.40)[a]		16.7		
$HOOCC_6H_4HCCCl_3C_6H_4COOH$		19.9		21.8
Unknown III (R_f 0.35)[a]				2.4
$HOH_2CC_6H_4HCCCl_3C_6H_4CH_2OH$				63.9
Unknown IV (R_f 0.18)[a]				1.2
Conjugates	25.1	35.4	9.7	4.5
DDT treatment (feces 73.02%, urine 26.98%)				
	43.3	56.7	47.8	52.2
DDE	41.5	5.3	2.1	
DDT	29.1	2.6	28.0	3.2
DDMS	1.4			
DDD	1.7	1.0	61.3	13.0
DBP	2.4			
Kelthane	1.9	0.8	Trace	Trace
Unknown I (R_f 0.64)[b]	3.3	4.5	8.7	
DDA	14.9	30.6		28.2
Unknown II (R_f 0.47)[b]		2.8		
Unknown III (R_f 0.40)[b]	1.2	3.5		
Unknown IV (R_f 0.25)[b]		15.7		10.1
Unknown V (R_f 0.11)[b]	1.3	22.7		24.0
Base (conjugates)	1.5	10.5		21.6

[a] Solvent system: benzene/dioxane/acetic acid 90:30:1. [b] Solvent system: benzene/dioxane/acetic acid 110:30:1.5.

trichloroethane along with mono- and bis-benzoic acid analogs, two unknowns and conjugates. These data suggest that the mammalian liver microsomes are more active in side chain oxidation than in O deethylation.

Recent studies in our laboratory (Hansen *et al.*, 1971) on comparative metabolism of 10^{-5} *M* methoxychlor and ethoxychlor by mouse liver microsomes have shown that methoxychlor is metabolized 2.5 times faster than ethoxychlor, explaining the relatively higher toxicity of the latter.

DDT, Ethoxychlor, and Methylchlor Metabolism in the Mouse. The metabolism of DDT has been reported previously by Jensen *et al.* (1957) and Peterson and Robison (1964), but even today no clear picture is available for its complete qualitative and quantitative metabolic pattern, which is given in Table VI and Figure 2. Over a period of 48 hr 73.0% of the recovered radioactivity was in the feces and the remaining 27.0% in urine. After the analysis it was shown that DDE (41.5%) was the major component in the nonpolar (hexane) fraction of the urine, along with DDT (29.1%) and DDA (14.9%), whereas the polar fraction comprised 30.6% DDA and a major portion of more polar unknowns. In the fecal hexane fraction, DDD (61.3%) was the major product along with DDT (28.0%), DDE (2.1%), and an unknown (8.7%), whereas DDA (28.2%), and DDD (13.0%) together with unknowns constituted the polar fraction. It is evident that DDT is biologically degradable, but it is the degradation products, especially DDE, that turn out to be environmental hazards.

As shown in Table VI, ethoxychlor like methoxychlor is O dealkylated to the phenolic products 2-*p*-(ethoxyphenyl)-2-(*p*-hydroxyphenyl)-1,1,1-trichloroethane and 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane. The other major identified component was 2-(*p*-ethoxyphenyl)-2-(*p*-hydroxyethoxyphenyl)-1,1,1-trichloroethane. Conjugates and polar material constituted almost all of the urine polar fraction and considerable amounts in the other fractions.

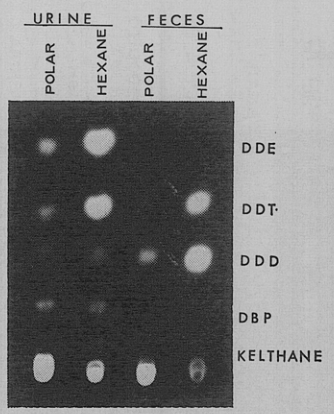

Figure 2. Radioautograph of thin-layer plate showing metabolic pattern of ^{14}C-DDT by the mouse

Methylchlor is also subject to oxidative degradation to alcoholic and carboxylic acid components. The urine fractions were comprised of parent material and 2,2-bis(*p*-carboxyphenyl)-1,1,1-trichloroethane along with unknowns and polar conjugates. The dialcoholic product 2,2-bis(*p*-hydroxymethylphenyl)-1,1,1-trichloroethane and the di-acid 2,2-bis(*p*-carboxyphenyl)-1,1,1-trichloroethane formed the major metabolic products in the feces. The results are given in Table V.

Metabolism in Model Ecosystem. The distribution of ^{3}H-ethoxychlor and ^{14}C-methylchlor and their metabolites is presented in Table VII. Ethoxychlor was concentrated

Table VII. Distribution of ^{3}H-Ethoxychlor, ^{14}C-Methylchlor and Their Metabolites in a Model Ecosystem

		Concentration (ppm)			
	H_2O	*Oedogonium* (algae)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Ethoxychlor					
Total ^{3}H	0.0904	2.014	86.16	1.138	4.806
Unknown I				0.072	
p,p'-Ethoxychlor	0.0006	1.526	58.587	0.908	0.922
p,p'-Ethoxychlor ethylene	0.0004	0.039	3.420	0.024	0.382
$C_2H_5OC_6H_4HCCCl_3C_6H_4OCH_2CH_2OH$	0.0008		0.284		0.129
$C_2H_5OC_6H_4HCCCl_3C_6H_4OH$	0.0078	0.355	21.195	0.059	1.438
$HOC_6H_4HCCCl_3C_6H_4OH$	0.0112	0.041	0.844		0.213
$HOC_6H_4CCCl_2C_6H_4OH$	0.0018		0.694		0.137
$HOC_6H_4COC_6H_4OH$	0.0108		0.163		0.099
Conjugates	0.0368	0.053	0.973	0.075	1.486
Polar metabolites	0.0197				
Methylchlor					
Total ^{14}C	0.2159	5.525	101.000	1.002	0.684
Unknown I			11.392		
p,p'-Methylchlor	0.0006	5.168	72.164	0.835	0.084
Unknown II			7.878		
Unknown III	0.0233		2.070		
$CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$		0.124	6.645	Traces	
$CH_3C_6H_4HCCCl_3C_6H_4COOH$	0.0343			Traces	
Unknown IV	0.0167				
Unknown V	0.0151				
$HOOCC_6H_4HCCCl_3C_6H_4COOH$	0.0181				
Conjugates	0.0032	0.233	0.851	0.167	0.600
Polar metabolites	0.1149				

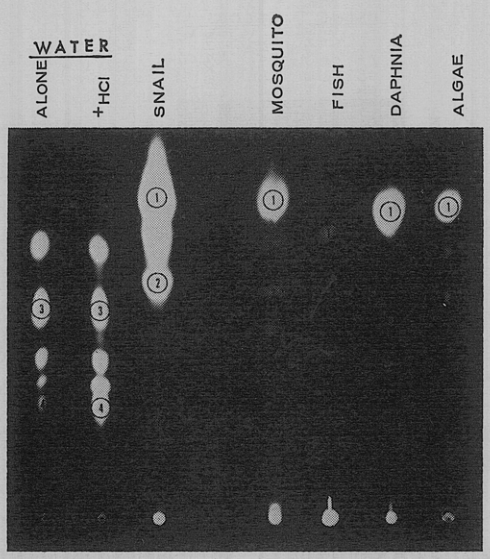

Figure 3. Radioautograph of thin-layer plate showing metabolic pattern of degradation of ^{14}C-methylchlor by elements of a model ecosystem. 1. $CH_3C_6H_4HCCCl_3C_6H_4CH_3$; 2. $CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$; 3. $CH_3C_6H_4HCCCl_3C_6H_4COOH$; 4. $HOOCC_6H_4HCCCl_3C_6H_4COOH$

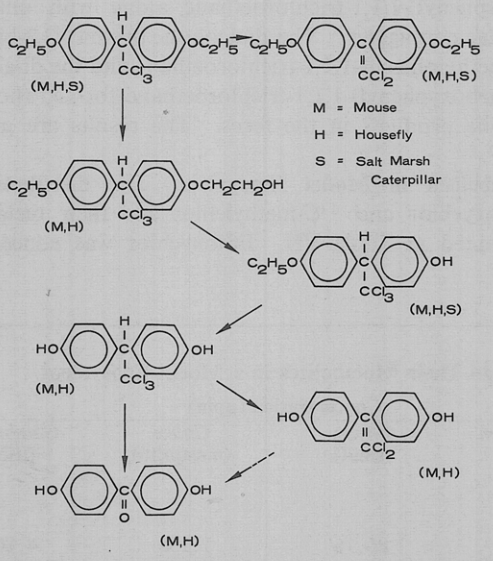

Figure 4. Pathways of ethoxychlor metabolism in mouse, housefly, and salt marsh caterpillar

in the higher positions of the food chain, *e.g.*, in fish, to a level of 1500 times that in the water. This represented only 19.2% of the total activity in the organism, the major constituent being the monophenolic product 2-(*p*-ethoxyphenyl)-2 - (*p* - hydroxyphenyl) - 1,1,1 - trichloroethane. Snails concentrate large quantities of ethoxychlor (98,000 fold) over that present in water, which is at a much higher level than DDT (35,000 fold) but less than the concentration of methoxychlor of 120,000 fold (Kapoor *et al.*, 1970).

With methylchlor, the parent material was stored in the fish at 1400 times, comparable with other biodegradable analogs investigated. Snails once again concentrated even this compound 120,000 times, indicating a metabolic deficiency of microsomal O dealkylation and side chain oxidation. Mono- and bis-alcohols and carboxylic acids were present as metabolites. Radioautographs showing the qualitative metabolic pattern of environmental degradation of methylchlor in the model ecosystem are presented in Figure 3.

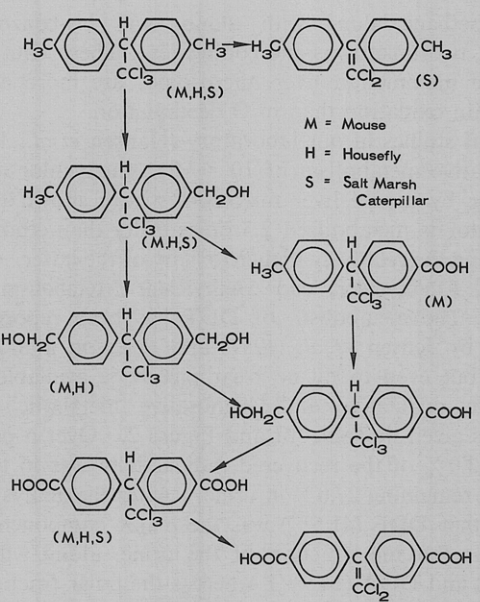

Figure 5. Pathways of methylchlor metabolism in mouse, housefly, and salt marsh caterpillar

SUMMARY AND CONCLUSIONS

The pathways of metabolism for ethoxychlor and methylchlor in insects, mice, and in the model ecosystem (Figures 4 and 5) demonstrate that these compounds, like methoxychlor and methiochlor previously investigated (Kapoor *et al.*, 1970) are persistent, biodegradable analogs of DDT. It is evident that the CH_3–, CH_3O–, C_2H_5O–, and CH_3S– moieties can serve as degradative handles in the DDT type molecule and that the incorporation of any one of these can markedly accelerate the biodegradability of the molecule. This information has been used to synthesize a number of new asymmetrical DDT analogs, highly toxic to insects and of greatly reduced toxicity to the mouse. Ethoxychlor is more toxic than DDT to some insects and both ethoxychlor and methylchlor are much effective than DDT to the moderately DDT-resistant housefly (Metcalf *et al.*, 1971a). This can be attributed to a lower rate of dehydrochlorination because of the electron donating C_2H_5O– and CH_3– groups (Metcalf *et al.*, 1968).

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