PESTICIDE BIOCHEMISTRY AND PHYSIOLOGY 4, 201–211

Metabolism of the Methoxychlor Isostere, Dianisylneopentane, in Mouse, Insects, and a Model Ecosystem

JOEL R. COATS, ROBERT L. METCALF,[1] AND INDER P. KAPOOR[2]

Department of Entomology, University of Illinois, Urbana, Illinois 61801

Received August 27, 1973; accepted November 28, 1973

Dianisylneopentane or 1,1-bis(*p*-methoxyphenyl)-2,2-dimethylpropane was metabolized largely by *O*-demethylation to form mono- and diphenol derivatives. Only a small percentage was degraded by α-hydroxylation and rearrangement. In the model ecosystem, dianisylneopentane reacted very similarly to methoxychlor, accumulating in fish to about the same extent and yielding a slightly higher ratio of polar to nonpolar metabolites. The neopentyl group proved to be approximately as stable in biological systems as the isosterically equivalent trichloromethyl group.

INTRODUCTION

Recent pressure for the replacement of DDT by more selective and biodegradable substitutes has resulted in extensive studies of DDT analogues. Variation of the aromatic substituents has indicated the potential usefulness of symmetric and asymmetric analogues (1). Changes in bridging structures have demonstrated the activity of benzylaniline, benzylphenyl ether, and benzylphenyl thioether analogues (2). Analogues with modified aliphatic moieties such as dimethylpropanes, nitroalkanes, and dimethyloxetanes have been extensively investigated by Holan (3).

In light of methoxychlor's increased usage over the past decade as a persistent yet biodegradable insecticide and its steric similarity to the neopentane analogue, it was decided to examine more closely the comparative insecticidal activity, selectivity, and biodegradative pathways of the chlorine-free isostere 1,1-bis(*p*-methoxyphenyl)-2,2-dimethylpropane or dianisylneopentane. This compound was first investigated by Brown and Rogers (4), and it became the cornerstone of their trihedralization theory of DDT mode of action (5). Subsequently the neopentane analogues were described by Stringer *et al.* (6) as having negligible insecticidal activity to *Calandra* and *Dysdercus*. Holan (3) proposed a degradation pathway on the basis of observed breakdown in the presence of weak acid.

Extensive metabolism studies have been completed for DDT and several analogues (2, 7–9); the work described herein represents the first metabolism study for a neopentane analog and includes data for houseflies, salt marsh caterpillars, mice, and a model ecosystem. Direct comparisons are made between the metabolism of dianisylneopentane and methoxychlor; DDT data is also considered, as an indicator of resistance to degradation. Except for the mouse microsomal assay, the methoxychlor and DDT data are from Kapoor *et al.* (7).

[1] To whom correspondence should be addressed.

[2] Present address, American Cyanamid Co., Princeton, NJ.

MATERIALS AND METHODS

Radiolabeled compounds. The investigations were conducted with [*ring*-^{3}H]dianisylneopentane, synthesized from [*ring*-^{3}H]anisole, prepared by the method of Hilton and O'Brien (10), and the bisulfite adduct of pivaldehyde, condensing with a mixture of sulfuric and acetic acids. Purification was achieved by column chromatography on silica gel by elution with 5% diethyl ether in petroleum ether, bp 60–68°C (Skellysolve B). Thin-layer chromatography determined the radiochemical purity to be 99.7%; the specific activity was 0.83 mCi/mmole. [*ring*-^{3}H]methoxychlor was prepared in this laboratory to a radiochemical purity of 99% and specific activity of 4.66 mCi/mmole by the method of Kapoor *et al.* (7).

Model metabolites (*Table 3*). It has been shown that *O*-dealkylation is a significant detoxication mechanism for aromatic alkoxy compounds (7, 11); thus mono- and diphenolic products were synthesized as suspected primary metabolites. Holan (3) proposed α-hydroxylation as the major degradative pathway, based on small amounts of dicofol (Kelthane) detected in various DDT metabolism studies; he observed the α-hydroxylated neopentane to undergo a rearrangement under acidic conditions to 2,3-dianisyl-3-methylbut-1-ene. Thus, the propan-l-ol, the rearrangement product, and other model metabolites were synthesized.

Dianisylneopentane or 1,1-bis(*p*-methoxyphenyl)-2,2-dimethylpropane (I), mp 58°C [59–61°C (4)] was produced from the condensation of 2 moles of anisole with 1 mole of pivaldehyde bisulfite adduct by dropwise addition of a mixture of 50% concentrated sulfuric acid and 50% glacial acetic acid at 0°C; recrystallization was achieved from ethanol. Nuclear magnetic resonance spectrometry showed CH_3O– protons at δ3.65 and the α-proton at δ3.61.

The 1,1-bis(*p*-methoxyphenyl)-2,2-dimethylpropan-1-ol (II) was prepared by adding dropwise 1 mole of ethyl pivalate into an ether solution of 2 moles of *p*-methoxyphenyl magnesium bromide. The product was crystallized from Skellysolve B to mp 81–82°C. [81–83°C (4)]. Nuclear magnetic resonance spectrometry showed the hydroxyl proton at δ2.19, and infrared spectrometry revealed hydroxyl absorption at 3500 cm^{-1}.

The 2,3-di(*p*-methoxyphenyl)-3-methylbut-1-ene (III) was obtained from the rearrangement-dehydration of II in ethanolic solution of dilute HCl. The compound was a viscous liquid at room temperature. Nuclear magnetic resonance revealed the *p*-methoxy protons at δ3.58 and δ3.68 for anisyl groups on the 3-carbon and 2-carbon, respectively. The two equivalent methyl groups absorbed at δ1.38; the two ethylenic protons each absorbed as a doublet, one at δ5.03, and one at δ5.27, with coupling constants of 1.6–1.7 Hz.

The bisphenol, or 1,1-bis(*p*-hydroxyphenyl)-2,2-dimethylpropane (IV) was synthesized from phenol and pivaldehyde adduct by the method described for I. The pure product was crystallized from benzene to a mp 157–158°C [158–160°C (5)]. Nuclear magnetic resonance spectrometry indicated two hydroxyl protons at δ8.92, confirmed by their disappearance with the addition of D_2O, and the α-proton at δ3.50. Infrared revealed hydroxyl stretching absorption at 3440 cm^{-1}.

The monophenol, or 1-(*p*-methoxyphenyl)-1-(*p*-hydroxyphenyl)-2,2-dimethylpropane (IV) was prepared by treating a methanolic solution of the bisphenol (V) with 1 mole of sodium methoxide, then adding dropwise 1 mole of methyl iodide and refluxing for 1 hr. Crystallization was from a mixture of benzene and Skellysolve B to mp 136–137°C. Nuclear magnetic resonance spectrometry disclosed a hydroxyl proton at δ5.06 which disappeared with the addition of D_2O, and the α-proton at δ3.55. Infrared spectrometry showed strong hydroxyl stretching absorption at 3370 cm^{-1}.

Bis(*p*-hydroxy)-benzophenone (VII) was purchased from Aldrich Chemical Company and recrystallized from dilute ethanol to mp 213–215°C.

The bis(*p*-methoxy)-benzophenone (VI) was prepared by methylation of VII using 1.5 moles of sodium methoxide, then 2 moles of methyl iodide. Crystallization to a mp 142–143°C was achieved from ethanol.

Radioassay. All samples were assayed by liquid scintillation in 10 ml of scintillation cocktail consisting of: 200 g naphthalene, 10 g PPO, and 0.25 g POPOP, made up to 1 liter with dioxane. Water samples from the ecosystem were added directly to the cocktail; samples in organic solvents were evaporated under nitrogen before cocktail was added; samples adsorbed on silica gel were added to the cocktail in the vials and allowed to set in the dark for 12 hr prior to counting, to reduce phosphorescence.

The percent of the dose of dianisylneopentane excreted by mice in 48 hr was determined by the Schöniger oxygen flask technique (12) for combustion. Small samples of dried powdered feces (40 mg) and urine (1 ml of 25 ml methanol–urine solution) were combusted using Whatman combustion paper. After the flasks had cooled, scintillation cocktail was injected.

Chromatography was carried out on thin-layer glass plates, coated with 0.25-mm silica gel containing a fluorescent indicator (E. Merck, FG-254); the two solvent systems used are described in Table 3. The chromogenic indicator spray used (Table 3) was made from 20 g ceric ammonium nitrate in 100 ml 2 *N* nitric acid.

Toxicity determinations. These were carried out as described for houseflies and *Phormia regina* in Ref. (1) and for mosquitoes in Ref. (13). Mouse LD_{50}s were determined by oral administration of olive oil solutions to female Swiss white mice, using a micrometer-driven Hamilton syringe; 1-week mortalities were recorded. Fish LC_{50}s were carried out by adding the compounds in acetone solution to jars of water which were then aerated for 24 hr to allow the compound to dissolve and the acetone to evaporate. One liter of water was provided per gram of fish, and mortalities were recorded every 24 hr for 4 days.

Metabolic studies in the DDT-resistant housefly R_{SP} and in the salt marsh caterpillar *Estigmene acrea* (Drury) were carried out with [*ring*-^{3}H]dianisylneopentane (7), but using acetonitrile for all extractions. Female Swiss white mice were used for metabolism studies, with an oral dose of 50 mg/kg administered in olive oil to each mouse. Urine and feces were collected after 48 hr and were processed according to Ref. (7), except that polar fractions were extracted after acidifying the aqueous phase to pH 2 with HCl and refluxing for 6 hr.

Model ecosystem methodology has been described in detail by Metcalf *et al.* (14).

Mouse liver microsomes were prepared according to Hansen and Hodgson (15); each incubation contained a generating system consisting of: NADP (2 mmoles), G-6-*P* (10 mmoles), G-6-*P* dehydrogenase (2 units) and $MgCl_2$ (30 mmoles) in 2 ml distilled water. Mouse livers were homogenized in 0.05 *M* Tris buffer (with 0.15 *M* KCl) of pH 7.4, centrifuged at 10,000*g* for 20 min, filtered, and centrifuged at 105,000*g* for 1 hr. The pellet was resuspended in 0.1 *M* Tris buffer at pH 8.0, and a 2-ml volume of resuspended microsomes was used in each incubation representing microsomes from 0.13 g of mouse liver. Substrate was added in 50 μl of acetone to a final concentration of 1×10^{-5} *M*. Incubations were carried out at 37°C for 1 hr with agitation, the labeled products were removed with diethyl ether, separated by tlc, and quantitatively assayed by liquid scintillation counting.

The water solubility of dianisylneopentane was determined using 99.9% pure ^{3}H-labeled compound by placing 5 ml of 1 mg/ml acetone solution in a 1-gallon brown glass bottle and evaporating the solvent under nitrogen. Three liters of distilled water were added, and the bottle, kept in the dark, was shaken vigorously

TABLE 1

Toxicity and Synergism with Piperonyl Butoxide (PB) for DDT, Methoxychlor, and Dianisylneopentane (DANP)

	Musca domestica topical LD_{50} (μg/g)						*Phormia regina* topical LD_{50} (μg/g)			*Culex fatigans* LC_{50}		*Anopheles albimanus* LC_{50}	
	S_{NAIDM}[a]			R_{SP}[c]						adults	larvae	adults	larvae
	alone	P.B.	S.R.[b]	alone	P.B.	S.R.	alone	P.B.	S.R.	(μg/cm²)	(ppm)	(μg/cm²)	(ppm)
DDT	14.0	5.5	2.5	270	70	3.9	11.2	8.2	1.4	7.9	0.07	0.23	0.015
Methoxychlor	45.0	3.5	12.8	48	4.6	10.4	10.0	4.5	2.2	16	0.067	13	0.18
DANP	95.0	19.0	5.0	62.5	12.0	5.2	17.0	5.75	2.9	22	0.36	4.5	0.85

[a] Susceptible strain NAIDM.
[b] Synergistic ratio.
[c] Resistant (to DDT and dieldrin) strain Super-Pollard.

every 12 hr over a period of 4 days. The water was filtered twice, and two 1-liter aliquots were extracted four times with petroleum ether (bp 62–68°C). The volumes were reduced to 50 ml each, then two 10-ml aliquots were removed to scintillation vials, evaporated, and counted. The activity of the water after extraction was not significantly above background.

RESULTS AND DISCUSSION

Toxicity to Insects

Comparative toxicities for dianisylneopentane, methoxychlor, and DDT are given in Table 1 for: susceptible housefly (S_{NAIDM}), DDT-resistant housefly (R_{SP}), black blowfly *Phormia regina*, *Culex fatigans* adults and larvae (susceptible), and *Anopheles albimanus* adults and larvae (DDT-resistant). Piperonyl butoxide (PB) was preapplied by 1 hr, at 50 μg, in the fly toxicity determinations to evaluate the intrinsic toxicities of the compounds through inhibiting their detoxication by the mixed function oxidases (MFO).

When applied alone, both dianisylneopentane and methoxychlor are approximately five times as toxic as DDT to resistant flies. Synergized by PB the methoxychlor molecule is more toxic than DDT or dianisylneopentane, in both susceptible and resistant flies. Thus the *p,p'*-methoxy aromatic substituents with the trichloromethyl aliphatic moiety appear to represent an optimal steric and electronic structure for inducing toxicity at the site of action. An additional factor to be considered is that of penetration. In metabolism studies it was observed that 24 hr after topical application to R_{SP} houseflies, 90% of the methoxychlor and DDT recovered had penetrated into the bodies as compared to 62% for the dianisylneopentane. Methoxychlor may be superior to the neopentane in its rate of absorption into the lipid nerve tissues as well, further enhancing its overall toxicity to the fly. Synergistic ratios (SR) given for the three insecticides indicate

comparable titres of MFO in the two strains of houseflies; the higher SRs for methoxychlor are then the result of easier detoxication by MFO, as compared to dianisylneopentane. Very little dehydrochlorination occurs with methoxychlor [Kapoor *et al.* (7)], so the relatively rapid rate of metabolism, mostly via *O*-demethylation, must certainly be a function of MFO degradation. Although the dianisylneopentane possesses sites for side-chain oxidation as well as *O*-demethylation, it resists attack by housefly MFO considerably better than methoxychlor, as shown by the low SRs (in both strains of housefly) for dianisylneopentane. This may be a result of more strongly bonded methoxy groups due to the electron-donating methyls (σ^*) or a lower affinity for the oxidizing enzymes.

The mosquito data probably reflects the variation in uptake into lipoid tissues, with the neopentane showing generally poor toxicity, and methoxychlor varying from good activity to poor.

Toxicity to Nontarget Organisms

As shown in Table 2 both methoxy analogs are considerably less toxic than DDT to mice; they are both safer to the mosquito fish as well, although methoxychlor is a little more toxic than the neopentane.

TABLE 2

Comparative Toxicity of DDT, Methoxychlor, and Dianisylneopentane to Nontarget Organisms

	Mouse oral LD_{50} (μg/g)	Mosquito fish (*Gambusia affinis*) LC_{50} ppm
DDT	200[a]	0.30, 0.32[d]
Methoxychlor	1000[b], 1850[c]	1.0
Dianisylneopentane	1000	3.0

[a] Ref. (1).
[b] Ref. (16).
[c] Ref. (17).
[d] Ref. (18).

Metabolism and accumulation studies demonstrating why these compounds are less toxic to mice and fish are discussed later.

Metabolism in the R_{SP} Housefly

Female houseflies of the DDT-resistant strain R_{SP} were treated topically with 1 μg of insecticide and assayed 24 hr later for penetration, excretion, and degradation products in the excreta and bodies. Table 4 compares the metabolism of dianisylneopentane with that of methoxychlor. The total activity ratios between the excreta and body homogenates are comparable

TABLE 3

Chromatographic Properties of Model Metabolites of Dianisylneopentane

	Thin-Layer Chromatography R_f[a]			Detection[b]	
	mp (°C)	SA	SB	uv	Chromogenic
I. $CH_3OC_6H_4HCC(CH_3)_3C_6H_4OCH_3$	58	0.65	0.30	none	orange
II. $CH_3OC_6H_4C(OH)C(CH_3)_3C_6H_4OCH_3$	81–82	0.41	0.09	none	none
III. $CH_3OC_6H_4C(=CH_2)C(CH_3)_2C_6H_4OCH_3$	liquid	0.65	0.39	none	red-orange
IV. $CH_3OC_6H_4HCC(CH_3)_3C_6H_4OH$	136–137	0.29	—	yellow	orange-brown
V. $HOC_6H_4HCC(CH_3)_3C_6H_4OH$	157–158	0.15	—	yellow	brown
VI. $CH_3OC_6H_4COC_6H_4OCH_3$	142–143	0.33	—	none	none
VII. $HOC_6H_4COC_6H_4OH$	213–215	0.06	—	light yellow	light brown

[a] Thin-layer chromatography systems: SA, 80% petroleum ether (60–68°C)/20% acetone; SB, 50% petroleum ether (60–68°C)/50% benzene.

[b] Detection: uv, exposure to ultraviolet light for 1 hr; chromogenic, ceric ammonium nitrate spray reagent.

TABLE 4

Metabolism of Dianisylneopentane and Methoxychlor in R_{SP} Housefly[a]

	Percent total radioactivity	
	Excreta	Body homogenate
Dianisylneopentane treatment (wash 38%, excreta 16%, body 46%)		
Dianisylneopentane	16	46
$CH_3OC_6H_4C(=CH_2)C(CH_3)_2C_6H_4OCH_3$	2	2
Unknown A (R_f 0.43 in SA)[b]	—	3
$CH_3OC_6H_4C(OH)C(CH_3)_2C_6H_4OCH_3$	4	4
$CH_3OC_6H_4COC_6H_4OCH_3$	1	1
$CH_3OC_6H_4HCC(CH_3)_2C_6H_4OH$	17	19
Unknown B (R_f 0.23 in SA)[b]	14	6
$HOC_6H_4HCC(CH_3)_2C_6H_4OH$	8	5
$HOC_6H_4COC_6H_4OH$	9	6
Conjugates and acids	28	8
Methoxychlor treatment (wash 10%, excreta 26%, body 64%)		
Methoxychlor	42	25
$CH_3OC_6H_4HCCCl_3C_6H_4OH$	18	—
Conjugates	29	63

[a] Dose: 1 μg/female. Recovery: Dianisylneopentane treatment 86%, Methoxychlor treatment 94%.
[b] See Table 3 for solvent system.

though penetration through the cuticle is observed to be faster with methoxychlor. Considerably more parent compound was excreted intact in the methoxychlor treatment than in the neopentane treatment (16%). In both cases the major metabolite identified is the monophenol, the product of *O*-demethylation by the MFO.

Metabolism in Salt Marsh Caterpillar

The caterpillars ingested the [^{3}H]dianisylneopentane as they fed on Vail moth medium (19) into which powdered compound had been pressed just prior to feeding. In both methoxychlor and dianisylneopentane assays the major portion of the

TABLE 5

Metabolism of Dianisylneopentane and Methoxychlor in Salt Marsh Caterpillar

	Percent total radioactivity	
	Excreta	Body
Dianisylneopentane treatment (excreta 96%, body 4%)		
Dianisylneopentane	94	97
$CH_3OC_6H_4C(=CH_2)C(CH_3)_2C_6H_4OCH_3$	1	1
Conjugates and acids	4	1
Methoxychlor treatment (excreta 93.5%, body 6.5%)		
Methoxychlor	96	100
$CH_3OC_6H_4C(CCl_2)C_6H_4OCH_3$	trace	—
Conjugates	trace	—

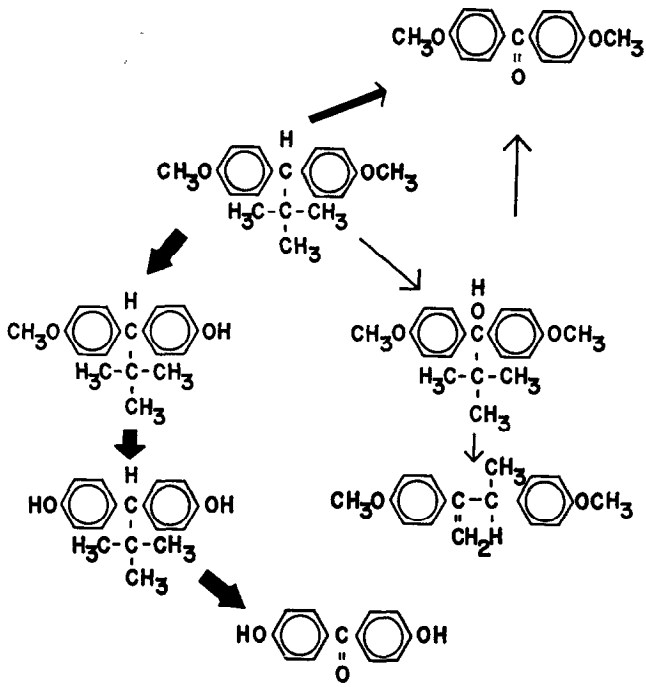

FIG. 1. *Degradative pathways for dianisylneopentane in mouse, insects, and in a laboratory model ecosystem.*

radioactivity recovered was found in the feces; as shown in Table 5 the degradation was slight for both compounds with essentially no *O*-demethylation occurring in this insect species. A little more methoxychlor was found intact than was the parent neopentane.

Metabolism in the Mouse

The 48-hr excretion accounted for 64% of the total dose of dianisylneopentane administered, and for 99% of the total dose of methoxychlor. Both compounds show 90% of the excreted activity in the feces, 10% in the urine.

Major metabolites in both cases are the mono- and diphenols (Fig. 1) resulting from *O*-demethylation by the MFO; the complete qualitative and quantitative distribution of metabolites is shown in Table 6.

The *in vitro* liver microsomal assays for the two insecticides revealed mono- and diphenols as prominent metabolites, and a significant amount of dihydroxybenzophenone resulting from the dianisylneopentane incubation. The highly efficient mixed-function oxidases of the mouse liver are evidently capable of attacking the extra available aliphatic sites in dianisylneopentane, resulting in 20% parent compound remaining for dianisylneopentane compared to 44% parent compound for methoxychlor. The results of the microsomal incubations are shown in Table 7.

Biodegradation in a Model Ecosystem

The use of the model ecosystem for demonstration of biodegradability in pesticides has been described by Metcalf *et al.* (14). Kapoor *et al.* (9) correlated structure to biodegradability for a series of DDT analogues, bearing different combinations of aromatic substituents, as to their relative stabilities in the model ecosystem and accumulations in various elements of the food chains present.

The dianisylneopentane was compared to methoxychlor to evaluate the ecological

TABLE 6

Metabolism of Dianisylneopentane and Methoxychlor in the Mouse[a]

	Percent total radioactivity			
	Urine		Feces	
	Hexane	Polar	Hexane	Polar
Dianisylneopentane treatment (urine 10%, feces 90%)	(19%)	(81%)	(56%)	(44%)
Dianisylneopentane	34	1	14	6
$CH_3OC_6H_4C(=CH_2)C(CH_3)_2C_6H_4OCH_3$	—	1	—	—
$CH_3OC_6H_4C(OH)C(CH_3)_3C_6H_4OCH_3$	1	—	—	—
$CH_3OC_6H_4COC_6H_4OCH_3$	2	—	2	1
$CH_3OC_6H_4HCC(CH_3)_3C_6H_4OH$	35	7	77	28
Unknown B (R_f 0.23 in SA)[b]	5	2	1	3
$HOC_6H_4HCC(CH_3)_3C_6H_4OH$	8	14	2	37
$HOC_6H_4COC_6H_4OH$	6	7	2	6
Conjugates and acids	7	64	1	13
Methoxychlor treatment	(36%)	(64%)	(14%)	(86%)
Methoxychlor	17	—	53	—
$CH_3OC_6H_4HCCCl_3C_6H_4OH$	62	10	42	28
$HOC_6H_4HCCCl_3C_6H_4OH$	5	22	—	28
$HOC_6H_4CCCl_2C_6H_4OH$	—	17	—	—
$HOC_6H_4COC_6H_4OH$	—	14	—	12
$HOC_6H_4CH(COOH)C_6H_4OH$	5	7	—	trace

[a] Dose: 50 mg/kg. Percent excreted in 48 hr: dianisylneopentane treatment 64%, methoxychlor treatment 99%.

[b] See Table 3 for solvent system.

effects of the neopentane structure as opposed to the chlorinated aliphatic moiety of methoxychlor. The biodegradability index (BI), a ratio of polar to nonpolar metabolites present in an organism, for the fish *Gambusia* was found to be 1.04 for dianisyl neopentane, compared to 0.94 for methoxychlor; in snails the BIs were 0.23

TABLE 7

Metabolism of Dianisylneopentane and Methoxychlor by Mouse Liver Homogenate

	Percent total radioactivity
Dianisylneopentane incubation	
Dianisylneopentane	20
$CH_3OC_6H_4C(=CH_2)C(CH_3)_2C_6H_4OCH_3$	2
$CH_3OC_6H_4HCC(CH_3)_3C_6H_4OH$	7
Unknown B (R_f 0.23 in SA)[a]	2
$HOC_6H_4HCC(CH_3)_3C_6H_4OH$	48
$HOC_6H_4COC_6H_4OH$	11
Conjugates	10
Methoxychlor incubation	
Methoxychlor	44
$CH_3OC_6H_4HCCCl_3C_6H_4OH$	19
$HOC_6H_4HCCCl_3C_6H_4OH$	29
Conjugates	8

[a] See Table 3 for solvent system.

TABLE 8

Distribution of Dianisylneopentane, Methoxychlor, and Their Metabolites in a Model Ecosystem

	Concentration (ppm)			
	H_2O	alga (*Oedogonium*)	snail (*Physa*)	fish (*Gambusia*)
Dianisylneopentane				
Total ^3H	0.065	6.0	32.0	4.0
Dianisylneopentane	0.00066	4.74	25.6	1.8
$CH_3OC_6H_4C(=CH_2)C(CH_3)_2C_6H_4OCH_3$	0.00036	0.12	0.32	0.08
Unknown A (R_f 0.42 in SA)[a]	0.00024	0.48	0.32	0.08
$CH_3OC_6H_4C(OH)C(CH_3)_3C_6H_4OCH_3$	0.00036	—	2.56	0.16
$CH_3OC_6H_4COC_6H_4OCH_3$	0.00012	—	—	0.12
$CH_3OC_6H_4HCC(CH_3)_3C_6H_4OH$	0.0013	0.24	—	1.36
Unknown B (R_f 0.23 in SA)[a]	0.0011	0.18	0.32	0.08
$HOC_4H_4HCC(CH_3)_3C_6H_4OH$	0.0022	0.06	—	0.12
$HOC_6H_4COC_6H_4OH$	0.0011	—	—	0.12
Conjugates and acids	0.0051	0.12	3.2	0.20
Unextractable	0.053			
	H_2O	mosquito (*Culex*)	snail (*Physa*)	fish (*Gambusia*)
Methoxychlor				
Total ^3H	0.0016	0.48	15.7	0.33
Methoxychlor	0.00011	—	13.2	0.18
$CH_3OC_6H_4CCCl_2C_6H_4OCH_3$	—	—	0.7	—
$CH_3OC_6H_4HCCCl_3C_6H_4OH$	0.00013	—	1.0	—
$HOC_6H_4CCCl_2C_6H_4OH$	0.00003	—	—	—
$HOC_6H_4HCCCl_3C_6H_4OH$	0.00003	—	—	—
Unknowns	0.00009	—	—	—
Polar metabolites	0.00125	—	0.8	0.16

[a] See Table 3 for solvent system.

and 0.13, respectively. The concentration factor, the ratio of parent compound in the body to parent compound in the water, was determined to be 1636 for the neopentane and 1545 for methoxychlor in the fish; for the snail the concentration factors were 23,300 for the neopentane and 120,000 for methoxychlor. Table 8 contains complete data for the ecosystem studies on the two insecticides.

Summary and Conclusions

Dianisylneopentane is metabolized by insects and by the mouse in a manner both qualitatively and quantitatively similar to its isostere methoxychlor. The principal biochemical pathway of metabolism is by *O*-demethylation of aryl methoxy groups to form mono- and bisphenols. The somewhat more rapid microsomal *O*-dimethylation of methoxychlor compared to its bioisostere dianisylneopentane, in the housefly, is probably a function of the electron withdrawing properties of the $-CCl_3$ group ($* + 2.65$) vs the $-C(CH_3)_3$ group ($* - 0.30$) (20) which decreases the electron density at the CH_3O-aryl bonds in methoxychlor and should make them more susceptible to enzymatic attack. Further studies in this area are now in progress. Only a relatively small amount of α-hydroxylated dianisylneopentane and its rearrangement product, 2,3-dianisyl-3-methylbut-1-ene, were formed *in vivo*. However, in the mouse, *in vivo* and microsomal *in vitro* metabolism produced

a higher percentage of polar metabolites for dianisylneopentane than for methoxychlor, possibly due to microsomal oxidations of aliphatic sites available on the neopentane molecule. The degradative pathways of dianisylneopentane are shown in Fig. 1. The model ecosystem evaluation showed almost equivalent environmental stability and ecological magnification between dianisylneopentane and methoxychlor. The water solubility of dianisylneopentane was determined radiometrically to be 0.69 ppm at 25°C; the value for methoxychlor is 0.62 ppm. The 11.3% higher BI of dianisylneopentane corresponds to a 10.6% increased water solubility over methoxychlor and agrees with the proposal by Kapoor *et al.* (9) that water solubility of DDT analogues is significantly correlated with biological degradation. These data indicating equivalent biological and environmental behavior for dianisylneopentane and methoxychlor are not in agreement with preconceptions that the $-CCl_3$ group is environmentally much more objectionable than the equivalent $-C(CH_3)_3$ group. They emphasize the importance of quantitative measurement of environmental parameters such as biodegradability index and ecological magnification.

ACKNOWLEDGMENTS

This research was supported in part by research grants from The Rockefeller Foundation for the "Development of Novel, Selective, and Non-persistent Pesticidal Chemicals" and by the Department of Health, Education and Welfare, Environmental Protection Agency, Grant EP 826. From a thesis submitted for the Ph.D. degree in Entomology, University of Illinois, Urbana–Champaign, by Joel R. Coats.

REFERENCES

1. R. L. Metcalf, I. P. Kapoor, and A. S. Hirwe, Biodegradable analogues of DDT, *Bull. World Health Org.* **44**, 363 (1971).
2. A. S. Hirwe, R. L. Metcalf, I. P. Kapoor, α-Trichloromethylbenzylanilines and α-trichloromethylbenzyl phenyl ethers with DDT-like insecticidal action, *J. Agr. Food Chem.* **20**, 818 (1972).
3. G. Holan, Rational design of degradable insecticides, *Nature (London)* **232**, 644 (1971).
4. H. D. Brown and E. F. Rogers, The insecticidal activity of 1,1-dianisylneopentane, *J. Amer. Chem. Soc.* **72**, 1864 (1950).
5. E. F. Rogers, H. D. Brown, I. M. Rasmussen, and R. E. Heal, The structure and toxicity of DDT insecticides, *J. Amer. Chem. Soc.* **75**, 2991 (1953).
6. A. Stringer, D. Woodcock, and E. J. Skerrett, Insecticidal activity and chemical constitution: analogues and isosteres of DDT, *Ann. Appl. Biol.* **43**, 366 (1955).
7. I. P. Kapoor, R. L. Metcalf, R. F. Nystrom, and G. K. Sangha, Comparative metabolism of methoxychlor, methiochlor, and DDT in mouse, insects, and in a model ecosystem, *J. Agr. Food Chem.* **18**, 1145 (1970).
8. I. P. Kapoor, R. L. Metcalf, A. S. Hirwe, P. Y. Lu, J. R. Coats, and R. F. Nystrom, Comparative metabolism of DDT, methylchlor, and ethoxychlor in mouse, insects, and in a model ecosystem, *J. Agr. Food Chem.* **20**, 1 (1972).
9. I. P. Kapoor, R. L. Metcalf, A. S. Hirwe, J. R. Coats, and M. S. Khalsa, Structure activity correlations of biodegradability of DDT analogues, *J. Agr. Food Chem.* **21**, 310 (1973).
10. B. D. Hilton and R. D. O'Brien, A simple technique for tritiation of aromatic insecticides, *J. Agr. Food Chem.* **12**, 236 (1964).
11. L. G. Hansen, R. L. Metcalf, and I. P. Kapoor, Biochemistry of selective toxicity and biodegradability: comparative *in vivo* and microsomal O-dealkylation by mice and flies, *Comp. Gen. Pharmacol.* (in press).
12. R. G. Kelly, E. A. Peets, S. Gordon, and D. A. Buijske, Determination of C^{14} and H^3 in biological samples by Schöniger combustion and liquid scintillation techniques, *Anal. Biochem.* **2**, 267 (1961).
13. R. L. Metcalf and T. R. Fukuto, The comparative toxicity of DDT and analogues to susceptible and resistant houseflies and mosquitoes, *Bull. World Health Org.* **38**, 633 (1968).
14. R. L. Metcalf, G. K. Sangha, and I. P. Kapoor, Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification, *Environ. Sci. Tech.* **5**, 709 (1971).
15. L. G. Hansen and E. Hodgson, Biochemical characteristics of insect microsomes: *N*- and *O*-demethylation, *Biochem. Pharmacol.* **20**, 1569 (1971).
16. W. F. Von Oettingen and N. Sharpless, The toxicity and toxic manifestations of 2,2-bis-

(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) as influenced by chemical changes in the molecule. A contribution to the relation between chemical constitution and toxicological action, *J. Pharmacol. Exp. Ther.* **88**, 400 (1946).

17. R. Domenjoz, Action biologique des quelques dérivés du DDT, *Helv. Chim. Acta* **29**, 1317 (1946).
18. R. W. Hatch, Relative sensitivity of salmonids to DDT, *Progve. Fish Cult.* **19**, 89 (1957).
19. P. V. Vail, T. J. Henneberry, and R. Pengalden, An artificial diet for rearing the salt marsh caterpillar, *Estigmene acrea* (Lepidoptera: Arctiidae), with notes on the biology of the species, *Ann. Entomol. Soc. Amer.* **60**, 134 (1967).
20. R. W. Taft, Separation of polar, steric, and resonance effects in reactivity, *in* "Steric Effects in Organic Chemistry" (M. S. Newman, Ed.), p. 619, Wiley, New York, 1956.