Evaluation of Environmental Distribution and Fate of Hexachlorocyclopentadiene, Chlordene, Heptachlor, and Heptachlor Epoxide in a Laboratory Model Ecosystem

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The distribution and fate of [^{14}C]heptachlor and the organochlorine compounds closely associated with it in manufacture and use, hexachlorocyclopentadiene, chlordene, and heptachlor epoxide, have been evaluated in food chain organisms in two laboratory model ecosystems and in vitro by sheep liver microsomes. Chlordene and heptachlor undergo epoxidation rapidly and are also hydroxylated at C_1 to form the corresponding hydroxy analogs. Heptachlor epoxide, however, is highly stable in biological systems. The rates of conversion and degradation of these compounds are influenced by microsomal oxidases, photolysis, and chemical hydrolysis. The relative balance of the epoxidation, an intoxication, and hydroxylation, a detoxication, determines the magnitude of persisting residues in the environment.

The cyclodiene insecticides heptachlor, chlordane, aldrin, dieldrin, endrin, endosulfan, and mirex are produced from a common intermediate hexachlorocyclopentadiene or "hex", a perchlorinated highly reactive diene, C_5Cl_6. The annual United States production of hexachlorocyclopentadiene is not a matter of public record but cannot be less than about 50 million lb, as the estimated annual production of the cyclodiene insecticides is: chlordane, 25 million; aldrin, 10 million; heptachlor, 6 million; endosulfan, 2 million; and dieldrin, endrin, and mirex, <1 million lb (Lawless et al., 1972). Additional amounts of hexachlorocyclopentadiene are used in the manufacture of flame retardants such as dodecachlorotetracyclopentalene (mirex) (U.S. Patent 2,671,045, 1954).

There is substantial concern about the environmental pollutant properties of heptachlor and chlordane, which are widely used as soil insecticides for the control of termites, ants, white grubs, cutworms, wireworms, and corn rootworms. The Mrak Commission (1969) has recommended restrictions on the use of these persistent pesticides. Heptachlor epoxide, a more stable and persistent oxidation product formed environmentally (Gannon and Bigger, 1958) and in animals by microsomal oxidation (Davidow and Radomski, 1953) is ubiquitous. In the FDA Market Basket Survey heptachlor epoxide has been found in 11–13% of the samples, providing an average daily human intake of 0.00002 to 0.00005 mg/kg (Duggan and Corneliussen, 1972). Heptachlor epoxide has been found in human adipose tissues at mean values up to 0.29 ppm (Hayes et al., 1965) and in 97% of the individuals examined (Wyllie et al., 1972). Heptachlor epoxide was found in all of 53 samples of human milk in a Pennsylvania study with an average value of 0.16 ppm (Kroger, 1972). In the National Soils Monitoring Program (Wiersma et al., 1972) heptachlor epoxide was found in Illinois at 25.4% of sample sites, at a mean level of 0.02 ppm with a range of 0.01–1.08 ppm. These residues are viewed with concern as heptachlor and heptachlor epoxide have produced a high incidence of carcinomas when fed in the diet of mice (Carter, 1974).

The other chlorinated cyclodienes discussed in this paper are associated with the manufacture and use of both heptachlor and chlordane, hexachlorocyclopentadiene representing the first stage and chlordene the second stage in their manufacture. Technical chlordane has been stated to contain about 1% hexachlorocyclopentadiene and about 21.5% chlordene isomers, together with about 10% heptachlor (Brooks, 1974). Therefore, the model ecosystem evaluation of the four chlorinated compounds hexachlorocyclopentadiene, chlordene, heptachlor, and heptachlor epoxide is an effort to investigate the comparable environmental degradative pathways, bioaccumulation, and biodegradation of the important environmental contaminants incident to the use of heptachlor and chlordane, from raw materials to environmental transformation products.

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MATERIALS AND METHODS

^{14}C-Radiolabeled hexachlorocyclopentadiene, radiopurity 98%, was purchased from Mallinckrodt. ^{14}C-Radiolabeled chlordene or 4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, mp 154° (lit. 155°; Riemschneider, 1951), was synthesized by heating together 12 μl (20.8 mg) of [^{14}C]hexachlorocyclopentadiene and 30 μl (21 mg) of cyclopentadiene, freshly distilled from dicyclopentadiene, for 2 hr at 70°. The product had a millimolar radioactivity of 3.32 mCi/mmol with a radiopurity of >98% after thin-layer chromatography (TLC) and radioautography.

^{14}C-Radiolabeled heptachlor or 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene was prepared by dissolving approximately 28 mg of [^{14}C]chlordene in 200 μl of carbon tetrachloride, adding 1 mg of Fuller's earth (200 mesh) as catalyst and saturating with Cl_2 gas for 5 min. After filtration, the product was purified by preparative thin-layer chromatography (TLC) to give the purified heptachlor, mp 94° (lit. 95–96°; U.S. Patent 2,576,666, 1951), millimolar radioactivity 1.54 mCi/mmol, with a radiopurity of >98% after TLC and radioautography.

^{14}C-Radiolabeled heptachlor epoxide or 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene was prepared by oxidation of 16.7 mg of [^{14}C]heptachlor with 19 mg of potassium dichromate in a mixture of acetic acid (0.8 ml) and sulfuric acid (0.2 ml) (Belgian Patent 609,938, 1962) giving 14.7 mg of crude product. Preparative TLC on silica gel with *n*-heptane–acetone (98:2) gave 7.2 mg of [^{14}C]heptachlor epoxide, mp 142–147° (lit. 157–158°, U.S. Patent 3,118,913, 1964), millimolar radioactivity 0.226 mCi/mmol, radiopurity >99% after TLC and radioautography.

Model metabolites were synthesized as standards for chromatography. 1-Hydroxychlordene or 1-hydroxy-4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, mp 194–196° (lit. 197–201°; U.S. Patent 2,528,656, 1950), was prepared by allowing 1-bromochlordene (U.S. Patent 2,528,655, 1950) to react with potassium carbonate in aqueous dioxane.

The epoxide, 1-hydroxy-2,3-epoxy-4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, was prepared by oxidation of 1-hydroxychlordene with 40% peracetic acid and sodium acetate in chloroform (Sauers et al., 1965). Although an accurate melting point could not be ob-

Table I. Properties of Cyclodiene Studied

Compound	R_f[a]	H_2O solubility, ppm	Mouse oral LD_{50}, μg/g	*Musca domestica* topical LD_{50}, μg/g	*Culex* larva LC_{50}, ppm
Hexachlorocyclopentadiene	0.73	0.805	505	565	2.3
Heptachlor	0.67	0.056[b]	100–162[c]	2.25	0.0054
Chlordene	0.66	0.773	>1000	100	0.45
Heptachlor epoxide	0.57	0.35[b]	46.6–61.3[c]	1.0	0.0064
Chlordene epoxide	0.56	1.359	1000	26.5	0.275
1-Hydroxychlordene	0.20	1.231	>1000	>500	7.5
1-Hydroxychlordene epoxide	0.16	2.741	>1000	>500	11.5

[a] TLC on silica gel with cyclohexane–diethyl ether (80:20, v/v). [b] Park and Bruce (1968). [c] Rat oral LD_{50}, male and female (Hayes, 1963).

tained, the epoxide was purified by sublimation at 130° (1.0 mm) and the structure confirmed by spectral analysis. Chlordene epoxide, 2,3-epoxy-4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, mp 215–220° (lit. 231–233°; U.S. Patent 2,583,569, 1952) was also prepared by oxidation of chlordene with 40% peracetic acid. NMR, infrared, and mass spectra fully confirmed the assigned structures of all these compounds. Some of their chemical and biological properties are shown in Table I.

Model Ecosystem Studies. Two types of model ecosystem experiments were conducted with the radiolabeled cyclodienes. In the model aquatic ecosystem (Lu and Metcalf, 1975) the ^{14}C-labeled compounds were added directly to the water at approximately 0.1 ppm and allowed to pass through a food web of plankton, daphnia (*Daphnia magna*), mosquito larva (*Culex pipiens quinquefasciatus*), fish (*Gambusia affinis*), alga (*Oedogonium cardiacum*), and snail (*Physa* sp.). The transfer and degradation were observed over a 3-day period at 80 ± 1°F (26.7°C). In the model ecosystem studies (Metcalf et al., 1971; Metcalf, 1974) 5.0 mg of the ^{14}C-labeled compounds was topically applied from acetone solution to *Sorghum vulgare* plants growing on the terrestrial portion, simulating an agriculture application of 1.0 lb/acre. The plants were consumed by the salt marsh caterpillar larvae (*Estigmene acrea*), and the ^{14}C-labeled products entered the terrestrial portion as fecal products, leaf frass, etc. The organisms in the aquatic portion were the same as listed for the model aquatic system and the radiolabeled products were allowed to pass through the system over a 33-day period at 80 ± 1°F (26.7°C) with a 12-hr diurnal cycle and 5000 ft-c illumination.

At the conclusion of both sets of experiments the radioactivity in water was extracted in diethyl ether and in the various organisms, in acetone, and evaluated as total in parts per million, and for relative amounts of degradation products by TLC, radioautography, and liquid scintillation counting of the ^{14}C-labeled spots. The residual activity in the extracted substrates was determined by total combustion analysis as $^{14}CO_2$ using the Schöniger oxygen flask technique (Kelly et al., 1961). The identification of the metabolites was confirmed by cochromatography and gas–liquid chromatography (GLC).

Microsomal Metabolism. A standard sheep liver microsome preparation (Wilson et al., 1975) was used to evaluate the degradation of [^{14}C]chlordene, [^{14}C]heptachlor, and [^{14}C]heptachlor epoxide. Approximately 0.1 mg of each compound was dissolved in acetone and coated on pavement marking glass beads and exposed to 1.6 mg of microsomal protein suspended in 0.05 *M* Tris-HCl buffer (pH 7.4) with NADPH (5.0×10^{-4} *M*), G-6-P (2.5×10^{-3} *M*), $MgCl_2$ (7.5×10^{-3} *M*), and G-6-P dehydrogenase (1 unit). After 30 min of incubation at 39°, the reaction was stopped by acetone and the products were extracted with diethyl ether, dried with anhydrous sodium sulfate, and then concentrated. The concentrated extract was evaluated by TLC, radioautography, and liquid scintillation counting of the radiolabeled products.

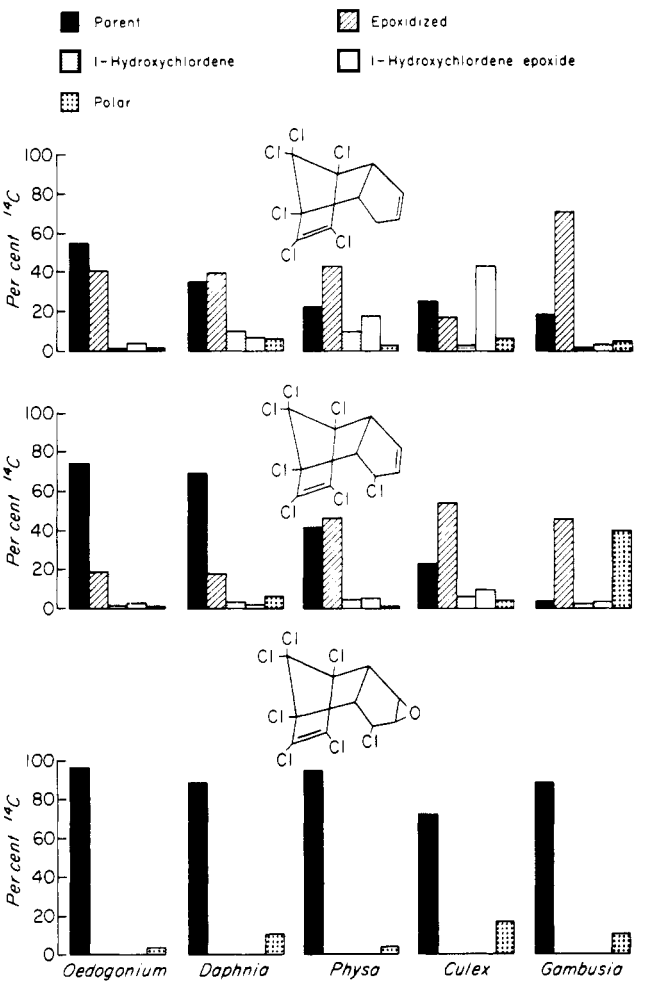

Figure 1. Relative detoxification capacities of key organisms of model aquatic ecosystem, following treatment with radioactive compounds: chlordene, heptachlor, and heptachlor epoxide.

DISCUSSION OF RESULTS

Model Ecosystem Studies. The results of the uptake and biotransformation of [^{14}C]chlordene, [^{14}C]heptachlor, and [^{14}C]heptachlor epoxide in the 3-day aquatic system are shown in Figure 1, demonstrating the comparative biochemical transformations occurring after bioconcentration.

Table II. Distribution of Hexachlorocyclopentadiene and Its Degradation Products in a Model Ecosystem

	Hexachlorocyclopentadiene equivalents, ppm				
	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Total extractable ^{14}C	0.00978	0.2450	0.7746	0.4772	0.2618
Hexachlorocyclopentadiene (R_f 0.73)[a]	0.00024	0.0818	0.3922	0.2230	0.1076
Unknown I (R_f 0.60)	0.00018	0.0658	0.1912	0.1682	0.0710
Unknown II (R_f 0.28)					0.0098
Unknown III (R_f 0.20)	0.00004	0.0052	0.0252	0.0214	0.0244
Unknown IV (R_f 0.12)	0.00014	0.0026	0.0352	0.0332	0.0220
Polar (R_f 0.0)	0.00168	0.0896	0.1308	0.0314	0.0270
Unextractable	0.00750	0.0094	0.0814	0.0104	0.0982

[a] TLC with cyclohexane–diethyl ether (80:20, v/v).

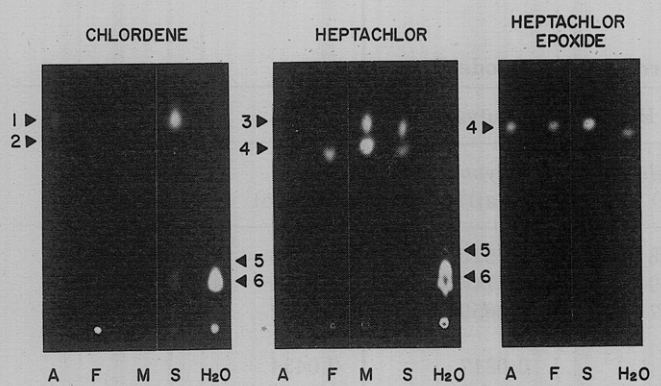

Figure 2. Radioautograms of environmental fates of [^{14}C]chlordene, [^{14}C]heptachlor, and [^{14}C]heptachlor epoxide from model ecosystem: (A) alga, (F) fish, (M) mosquito larva; (S) snail; (1) chlordene; (2) chlordene epoxide; (3) heptachlor; (4) heptachlor epoxide; (5) 1-hydroxychlordene; and (6) 1-hydroxychlordene epoxide.

All three compounds are highly lipophilic and total bioconcentration values in snail and fish, respectively, were: chlordene, 4167–465; heptachlor, 1841–1143; and heptachlor epoxide, 2781–1324.

Chlordene was rapidly epoxidized in all organisms with the highest activity in fish, where the epoxide comprised 71% of total extractable ^{14}C (Figure 1). Chlordene thus resembled aldrin with which epoxidation was correlated with the phyllogenetic position of the organism and dieldrin comprised 80% of the total ^{14}C from fish in a similar experiment (Lu and Metcalf, 1975). Hydroxylation of chlordene at C_1 formed products comprising 4% (alga) to 47% (mosquito) of total extractable ^{14}C. In heptachlor, however, the allylic moiety (CH=CHCHCl) substantially activated C_1–Cl to hydrolysis forming 1-hydroxychlordene which presumably was conjugated to form 40% of the extractable ^{14}C in fish. 1-Hydroxychlordene was also readily epoxidized to 1-hydroxy-2,3-epoxychlordene which was present in relatively small amounts. The most important metabolic change with heptachlor was rapid formation of the epoxide which comprised 45 to 54% of the extractable ^{14}C in snail, mosquito, and fish (Figure 1).

Heptachlor epoxide, without the activating allylic structure, was relatively inert, much like dieldrin (Metcalf et al., 1973) and the parent compound comprised 72–95% of the extractable ^{14}C in the various organisms.

The results of the 33-day terrestrial–aquatic model system studies are shown in Tables II–V and in the radioautographs of Figure 2.

Hexachlorocyclopentadiene reached a maximum level of 0.031 ppm in the water phase after 14 days and decreased to 0.016 ppm at 33 days. The compound showed considerable environmental stability and was stored as 33% of extractable ^{14}C in alga (ecological magnification or EM 340), 50% in snail (EM 929), 46% in mosquito (EM 1634), and 41% in fish (EM 448). The substantial volatility of the compound appeared to contribute to the relatively low amounts of total ^{14}C in the various organisms (compare Tables II–V). Although none of the trace degradation products present were identified, the extent of biodegradation can be estimated from the relative percentages of unextractable ^{14}C: alga, 4%; snail, 10%; mosquito, 2%; and fish, 27% (Table II).

Chlordene reached a maximum of 0.057 ppm in water after 5 days and decreased to 0.022 ppm at 33 days. Unmetabolized chlordene was stored as 61% of extractable ^{14}C in alga (EM 11,094), 71% in snail (EM 53,038), 42% in mosquito (EM 2038), and 7.8% in fish (EM 1122). In the fish, chlordene 2,3-epoxide, 16% of extractable ^{14}C (EM 2240), was the major component. The 1-hydroxychlordene 2,3-epoxide comprised 41% of the ^{14}C in the water and was stored as 21% of extractable ^{14}C in alga, 13% in snail, and about 10% in mosquito and fish. As suggested by its much higher water solubility (Table I), this metabolite showed low levels of bioconcentration (E.M. alga 46, snail 120, fish 16). The data of Table III in conformity with the 3-day results show that chlordene is more readily epoxidized in vivo than hydroxylated (Figure 1). The unextractable ^{14}C was 51% of the total in alga, 13% in snail, 37% in mosquito, and 43% in fish.

Heptachlor reached a maximum of 0.091 ppm in water after 3 days and the total ^{14}C declined to 0.035 ppm at 33 days. However, the water phase was still toxic to daphnia and mosquito and the experiment was extended to 71 days when the water concentration was equivalent to 0.024 ppm. Of three fish added at 68 days, one died in convulsions. Heptachlor was stored intact as 79% of extractable ^{14}C in alga (EM 20,730), 41% in snail (EM 37,153), 30% in mosquito (EM 31,403), and 6% in fish (EM 3820). However, much of the heptachlor was also oxidized to heptachlor 2,3-epoxide (Table IV) which comprised 22% of the extractable ^{14}C in alga (EM 894), 37% in snail (EM 5075), 49% in mosquito (EM 7300), and 79% in fish (EM 7760). Hydrolysis to produce 1-hydroxychlordene was responsible for about 9% of the total extractable ^{14}C in snail, 10% in mosquito, and 2.3% in fish. The unextractable ^{14}C was 32% of total in alga, 5.5% in snail, 7.1% in mosquito, and 43% in fish.

Heptachlor epoxide reached 0.059 ppm in the aqueous phase after 3 days and declined to 0.011 ppm at 33 days. This compound was unusually stable in the model system and the concentration in the water killed daphnia and mosquito larvae throughout the course of the experiment,

Table III. Distribution of Chlordene and Its Degradation Products in a Model Ecosystem

	Chlordene equivalents, ppm				
	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Total extractable ^{14}C	0.01818	1.8166	7.4722	0.4914	1.4306
Chlordene (R_f 0.66)[a]	0.00010	1.1094	5.3038	0.2038	0.1122
Chlordene epoxide (R_f 0.56)	0.00010	0.0970	0.3632	0.1438	0.2240
Unknown I (R_f 0.31)	0.00006		0.1038		0.0152
1-Hydroxychlordene (R_f 0.20)	0.00042	0.0694	0.2906	0.0240	0.0306
1-Hydroxychlordene epoxide (R_f 0.16)	0.00844	0.3882	1.0066	0.0480	0.1374
Unknown II (R_f 0.05)	0.00018		0.1450		
Polar (R_f 0.0)	0.00348	0.1526	0.2592	0.0718	0.9112
Unextractable	0.00540	1.9142	1.1190	0.2888	1.0870

[a] TLC with cyclohexane–diethyl ether (80:20, v/v).

Table IV. Distribution of Heptachlor and Its Degradation Products in a Model Ecosystem

	Heptachlor equivalents, ppm				
	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Total extractable ^{14}C	0.02225	0.8448	2.7515	3.1258	2.0603
Heptachlor (R_f 0.64)[a]	0.00003	0.6219	1.1146	0.9421	0.1146
Heptachlor epoxide (R_f 0.56)	0.00021	0.1877	1.0659	1.5332	1.6293
Unknown I (R_f 0.43)	0.00002		0.0217	0.0434	
Unknown II (R_f 0.37)	0.00001		0.1142	0.0328	
Unknown III (R_f 0.32)	0.00005		0.0490	0.0244	
1-Hydroxychlordene (R_f 0.21)	0.00040		0.0597	0.0791	0.0471
1-Hydroxychlordene epoxide (R_f 0.14)	0.00659		0.2066	0.2694	0.1211
Unknown IV (R_f 0.07)	0.00036		0.0272	0.0763	0.1010
Unknown V (R_f 0.03)	0.00026		0.0055	0.0244	
Polar (R_f 0.0)	0.00677	0.0352	0.0871	0.1007	0.0472
Unextractable	0.00755	0.4079	0.1646	0.2363	1.5479

[a] TLC with cyclohexane–diethyl ether (80:20, v/v).

Table V. Distribution of Heptachlor Epoxide and Its Degradation Products in a Model Ecosystem

	Heptachlor epoxide equivalents, ppm			
	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Gambusia* (fish)
Total extractable ^{14}C	0.00638	2.2620	101.9105	8.8809
Heptachlor epoxide (R_f 0.63)[a]	0.00125	2.0618	83.0774	6.1100
1-Hydroxychlordene epoxide (R_f 0.16)	0.00036	0.0800	8.8663	1.7114
Polar (R_f 0.0)	0.00200	0.1202	9.9668	1.0595
Unextractable	0.00277	1.1602	0.1110	0.8554

[a] TLC with cyclohexane–diethyl ether (80:20, v/v).

which was extended to 43 days (water concentration 0.0074 ppm). Heptachlor epoxide does not contain the reactive allylic moiety of heptachlor and is therefore much more stable environmentally, as heptachlor epoxide comprised 91% of the extractable ^{14}C in alga (EM 1649), 82% in snail (EM 66,462), and 69% in fish (EM 4888) (Table V). Hydrolysis of C_1–Cl to produce 1-hydroxy-2,3-epoxychlordene formed only about 3.5% of extractable ^{14}C in alga, 8.7% in snail, and 19% in fish. The unextractable ^{14}C comprised 34% of the total in alga, 1% in snail, and 9% in fish. The model ecosystem behavior of heptachlor epoxide is therefore quantitatively similar to that of dieldrin (Metcalf et al., 1973; Sanborn and Yu, 1973).

Heptachlor Transformations in Water. Water sam-

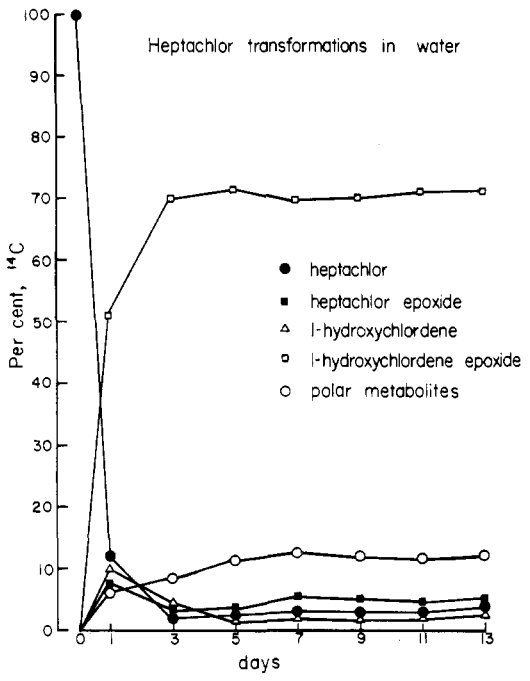

Figure 3. Radiolabeled heptachlor transformations in the water phase of a model ecosystem.

ples were taken at 1, 3, 5, 7, 9, 11, and 13 days from the [^{14}C]heptachlor-treated model ecosystem and the relative amounts of the various transformation products determined by TLC and radioisotope counting as shown in Figure 3. Transformation of heptachlor into 1-hydroxychlordene and especially 1-hydroxychlordene epoxide was relatively rapid and indicated the speed with which hydrolysis of C_1–Cl occurs in water under photolytic catalysis. The relatively small proportion of heptachlor epoxide formed suggests that this compound is formed environmentally largely in vivo by microsomal multifunction oxidase action.

Degradation by Salt Marsh Caterpillar. The results of feeding 50 μg of ^{14}C-labeled chlordene, heptachlor, and heptachlor epoxide, incorporated with 200 mg of synthetic moth medium (Vail et al., 1967), are shown in Table VI. Chlordene was largely converted to chlordene 2,3-epoxide in body homogenate, and excreted largely as 1-hydroxychlordene epoxide and conjugates in feces. Heptachlor was largely epoxidized to heptachlor epoxide in body homogenate and excreted as 1-hydroxychlordene epoxide in feces. Heptachlor epoxide was recovered intact as 95% of the ^{14}C in body homogenate and 97% in feces. These data clearly show that heptachlor forms 1-hydroxychlordene by hydrolysis followed by oxidation to 1-hydroxychlordene epoxide.

Degradation by Sheep Liver Microsomes. As shown in Table VII, sheep liver microsomal protein readily epoxidized chlordene to chlordene epoxide and hydroxylated chlordene to 1-hydroxychlordene. Heptachlor under identical conditions is oxidized to heptachlor epoxide, but only small percentages of 1-hydroxychlorodene or 1-hydroxychlordene epoxide were formed indicating the relative stability of the C_1–Cl bond to microsomal oxidation. Heptachlor epoxide was very stable to microsomal oxidation and no identifiable products were formed.

Biomass Recovery. The total recovery of ^{14}C in the various organisms of the terrestrial–aquatic model ecosystems is shown in Table VIII. The increasing percentages of recovery from hexachlorocyclopentadiene < chlordene < heptachlor < heptachlor epoxide will be noted. The ordering is that suggested by the relative stability of the individual compounds as shown in Figure 1 and Tables II–V.

Table VI. Metabolism of [^{14}C]Chlordene, [^{14}C]Heptachlor, and [^{14}C]Heptachlor Epoxide by Salt Marsh Caterpillar, *Estigmene acrea*

	Body homogenate	Feces extraction
A. Chlordene		
Total ^{14}C, %	40.44	59.56
Chlordene (R_f 0.64)[a]	10.88	8.61
Chlordene epoxide (R_f 0.56)	26.31	1.36
1-Hydroxychlordene (R_f 0.23)	0.06	1.07
1-Hydroxychlordene epoxide (R_f 0.16)	2.24	16.11
Unknown I (R_f 0.07)	0.01	0.26
Unknown II (R_f 0.05)		0.32
Unknown III (R_f 0.03)	0.02	0.85
Polar (R_f 0.0)	0.92	30.98
B. Heptachlor		
Total ^{14}C, %	47.54	52.46
Heptachlor (R_f 0.66)[a]	17.98	22.66
Heptachlor epoxide (R_f 0.56)	28.18	4.73
1-Hydroxychlordene (R_f 0.20)	0.10	0.33
1-Hydroxychlordene epoxide (R_f 0.15)	0.73	7.23
Unknown I (R_f 0.10)		0.50
Unknown II (R_f 0.05)		0.38
Polar (R_f 0.0)	0.55	16.63
C. Heptachlor epoxide		
Total ^{14}C, %	33.23	66.77
Heptachlor epoxide (R_f 0.56)[a]	31.71	64.88
Polar (R_f 0.0)	1.52	1.89

[a] TLC with cyclohexane–diethyl ether (80:20, v/v).

CONCLUSIONS

The model ecosystem studies show that heptachlor behaves environmentally somewhat like aldrin (Metcalf et al., 1973) in its rapid epoxidation to the very stable heptachlor epoxide. However, unlike aldrin which is stored as dieldrin in the organisms of the model ecosystem, in amounts comprising 86 to 96% of the total extractable ^{14}C, heptachlor has an alternate pathway for degradation to 1-hydroxychlordene which increases water solubility about 20-fold (Table I) and thus reduces the opportunity for bioconcentration. However, once heptachlor epoxide is formed by photolytic action on plant surfaces, by bacterial action, or by microsomal oxidation in organisms it is very stable biologically because the allylic moiety is no longer present to promote hydrolysis to 1-hydroxychlordene 2,3-epoxide. The comparative in vivo stabilities of heptachlor epoxide and dieldrin are shown by the relative percentages of unextractable ^{14}C, e.g. 9 and 16%, respectively, in fish (Sanborn and Yu, 1973).

The data in this paper agree generally with degradative pathways for heptachlor found in soils (Bowman et al., 1965; Carter and Stringer, 1970) and produced by soil microorganisms (Miles et al., 1969, 1971). There are two major pathways: (1) epoxidation to heptachlor epoxide and (2) hydrolysis to 1-hydroxychlordene. Pathway 1 is an intoxication reaction producing the substantially more toxic heptachlor epoxide (Table I) which is very persistent in the environment and bioaccumulative in plant and animal tissues (Figure 1, Table III). Heptachlor epoxide appears to have approximately the same environmental stability as dieldrin

Table VII. Metabolism of [^{14}C]Chlordene, [^{14}C]Heptachlor, and [^{14}C]Heptachlor Epoxide by Sheep Microsomal Preparation

	% ^{14}C		
	Chlordene	Heptachlor	Heptachlor epoxide
Heptachlor (R_f 0.68)[a]		86.16	
Chlordene (R_f 0.65)	46.18		
Heptachlor epoxide (R_f 0.58)		6.37	97.66
Chlordene epoxide (R_f 0.56)	7.09		
Unknown I (R_f 0.46)		3.29	
Unknown II (R_f 0.40)	5.80		
1-Hydroxychlordene (R_f 0.19)	2.57	0.74	
1-Hydroxychlordene epoxide (R_f 0.16)	30.42	1.44	
Unknown III (R_f 0.03)	1.65		
Polar (R_f 0.0)	6.29	2.00	2.34

[a] TLC with cyclohexane–diethyl ether (80:20, v/v).

Table VIII. Biomass Recovery of Compounds from Organisms of a Model Ecosystem

	Alga	Snail	Mosquito	Fish
		Hexachlorocyclopentadiene		
% recovery ^{14}C in solution	0.027	0.0125	0.0164	0.116
% recovery total ^{14}C	0.0012	0.00054	0.00071	0.0050
% biomass recovery of ^{14}C lost from solution	0.37			
		Chlordene		
% recovery ^{14}C in solution	0.14	0.30	0.010	0.56
% recovery total ^{14}C	0.011	0.024	0.0082	0.040
% biomass recovery of ^{14}C lost from solution	1.51			
		Heptachlor		
% recovery ^{14}C in solution	0.038	0.49	0.129	0.36
% recovery total ^{14}C	0.0064	0.082	0.022	0.060
% biomass recovery of ^{14}C lost from solution	1.93			
		Heptachlor Epoxide		
% recovery ^{14}C in solution	0.78	1.63	*a*	2.31
% recovery total ^{14}C	0.063	0.133	0.0	0.19
% biomass recovery of ^{14}C lost from solution	5.5			

[a] Killed throughout the course of the experiment.

(Metcalf et al., 1973). Pathway 2 is a true detoxication reaction producing a much less acutely toxic 1-hydroxychlordene (Table I) which is conjugatable and not readily bioaccumulative (Tables III and IV). The relative balance of these two reactions in the total environment determines the magnitude of persisting residues. The epoxidation of heptachlor appears to be largely a microsomal oxidation reaction taking place in vivo especially in the tissues of animals (Figure 1; Tables IV and VI) and the hydrolysis of heptachlor appears to be largely the result of chemical hydrolysis, which takes place very rapidly in water and is presumably accelerated by photolysis (Figure 3; Table VII). In soils, the relative amounts of heptachlor epoxide and 1-hydroxychlordene formed 1 to 3 years after treatment apparently depend upon soil type. 1-Hydroxychlordene can be oxidized to 1-hydroxychlordene epoxide by soil microorganisms (Miles et al., 1971) and by microsomal oxidases in vitro (Table VII) and in vivo (Table III). The 1-hydroxychlordene epoxide is a true detoxication product of heptachlor (Table I) and is not highly bioaccumulative (Table IV). As an epoxide, however, its role in carcinogenesis needs investigation.

Although Miles et al. (1971) found evidence of the reduction of heptachlor to chlordene by anaerobic soil bacteria, this reaction is of little or no consequence aerobically, at least under model ecosystem conditions (Table IV) where no chlordene was detected.

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Simplified Spectrophotometric Analysis of Copper from Cupric Sulfide Synthesized in Porcine Fecal Matter

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The presence of cupric sulfide in fecal matter of pigs fed copper sulfate was investigated. The method involves the preliminary isolation of cupric sulfide from soluble copper and oxidation of the sulfide ion with the concurrent dissolution of the insoluble copper. This technique permits the direct analysis of copper from cupric sulfide by atomic absorption spectrophotometry. The method described establishes the feasibility of this approach for obtaining accuracy and precision for the estimation of low amounts of copper from cupric sulfide in fecal matter.

Several reports summarized by Braude (1965) indicate that copper is an effective growth stimulus for pigs. With animals fed copper levels, concern with environmental contamination from soluble copper in fecal matter increases. This contamination, however, may not pose a problem if appreciable amounts of cupric sulfide are present in fecal matter. Cupric sulfide possibly may be synthesized in the intestinal tract by microorganisms. The very low K_{sp} of cupric sulfide (8.5×10^{-45}) indicates that a very high hydrogen ion concentration is not sufficient to dissolve appreciable amounts of it. Copper bound in this form is insoluble and lessens the contamination problem. The necessity for a precise and accurate method for the quantitative estimation of this compound from fecal matter is of prime importance. The proposed method considers the separation of cupric sulfide from soluble copper in fecal matter and uses a combination of nitric–perchloric–sulfuric acid reagents for sample decomposition and the complete dissolution of copper from cupric sulfide. The preparation of the sample for atomic absorption spectrometry requires about 1 hr. Groups of samples can be run simultaneously. Once the insoluble copper is in solution, atomic absorption spectrophotometry offers a sensitive solution for the estimation of copper in low concentrations.

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EXPERIMENTAL SECTION

Standard Curve. A standard copper solution was prepared by dissolving 0.19645 g of $CuSO_4 \cdot 5H_2O$ and diluting to 500 ml with deionized water. Aliquots of this solution (0.25 to 15 ml) were placed in a 100-ml volumetric flask, 8 ml of 0.3 *N* HCl and 2 ml of concentrated H_2SO_4 were added, and the solution was brought to volume with deionized water. Working standards cover a range from 0.25 to 15 μg of copper. Absorbance was measured at 3247 Å with a Model 503 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). A slit setting of 4 (7 Å spectre band width) and a copper hollow cathode lamp utilized at 15 mA were used. The air–acetylene flame was maintained at operating pressures of 30 psi for air and 8 psi for acetylene. Flowmeter settings were regulated to deliver 21.5 l./min of air and 3.5 l./min of fuel to a 4-in. single slot burner.

Cupric Sulfide Determinations. [*Caution:* Conduct determination in well ventilated hood. $HClO_4$ contact with concentrated H_2SO_4 may be explosive. Refer to "Notes on Perchloric Acid and Its Handling in Analytical Work" (1969).] A 200-mg lyophilized sample was placed in a 250-ml digestion flask to which was added 20 ml of 0.3 *N* HCl (analytical reagent grade) and heated to 40° while the flask was swirled to wet the sample. Soluble copper (sample filtrate) was quantitatively removed by filtering through Whatman no. 42 filter paper and washing under vacuum. The paper containing the cupric sulfide (sample residue) was transferred to the original 250-ml volume flask. Concentrated nitric acid (15 ml), 2 ml of concentrated sulfuric