CONCLUSIONS

As described in a previous paper on aldrin-^{14}C application to soil (Kohli *et al.*, 1973), hydrophilic metabolites were detected in the edible parts of sugar beets, not looked for in any routine analyses before, and exceeding the residues of aldrin and dieldrin. In this paper, such residues were detected, too, in most of the wheat, maize, and soil samples, but in the case described here, only trace amounts were found in the edible parts, the grains. Similarly, the total residues in wheat grain were negligible after *seed treatment*, but residues occurred in straw, low stems, roots, and soil.

Whereas a comparison of these data for hydrophilic metabolites with those found in real field experiments is not possible, the aldrin and dieldrin data are in line with former field data, in the case of soil application. The residues found after seed application, however, were somewhat higher than in agricultural practice, for, with radioactive compounds, it was not possible to use exactly the same application method as in practical seed dressing.

ACKNOWLEDGMENT

We thank K. I. Beynon, J. Yates, and A. N. Wright of Woodstock Agricultural Centre, Sittingbourne, U.K., for their kind cooperation.

LITERATURE CITED

Klein, W., Kohli, J., Weisgerber, I., Korte, F., *J. Agr. Food Chem.* **21**, 152 (1973).

Kohli, J., Zarif, S., Weisgerber, I., Klein, W., Korte, F., *J. Agr. Food Chem.* **21**, 855 (1973).

Received for review November 23, 1973. Accepted March 25, 1974.

Metabolism of Chlordane in Rats

Jerry R. Barnett[1] and H. Wyman Dorough*

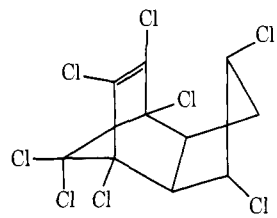
The fate of HCS-3260-^{14}C (3:1 *cis*-chlordane and *trans*-chlordane) and the individual isomers was studied in rats. Single oral doses of the compounds were almost completely (>90%) eliminated after 7 days. Females excreted more of the dose, 5–6%, in the urine than did the males, 2–3%. *cis*-Chlordane was eliminated more rapidly, 70% after 24 hr, than the trans isomer, 60% after 24 hr. Approximately 15% of the radiocarbon in the 0–24-hr feces was as the administered compound, but none was detected in the urine. The metabolites in the excreta were formed predominantly by dechlorination and various degrees of hydroxylation of the cyclopentane ring. Levels of residues in the fat of rats after being fed 1, 5, and 25 ppm of HCS-3260-^{14}C in the diet for 56 days were approximately three times the parts per million level in the diet. In liver, kidney, brain, and muscle, the levels were $\frac{1}{8}$, $\frac{1}{10}$, $\frac{1}{25}$, and $\frac{1}{50}$ that of the concentration in the feed. Oxychlordane was the major ^{14}C residue in the tissues, ranging from 50% of the radiocarbon in the kidney to about 90% in the fat. Feeding *trans*-chlordane gave higher residue levels in the tissues than *cis*-chlordane, the increase being primarily in higher oxychlordane concentration. Oxychlordane was the most persistent residue in the tissues after the chlordane was removed from the diet.

Although chlordane is one of the older and most commonly used chlorinated hydrocarbon insecticides, its metabolism in animals has received little attention until recently. This stems, in part, from the fact that technical chlordane is composed of a complex mixture of components including the cis and trans isomers of the insecticide. Furthermore, ^{14}C-labeled pure *cis*- and/or *trans*-chlordane has not been generally available. Poonawalla and Korte (1971) did investigate the metabolism of *trans*-chlordane-^{14}C in rabbits, demonstrating that the compound was rapidly metabolized and excreted.

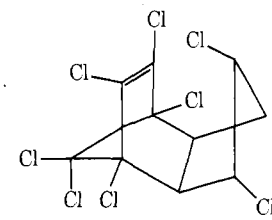
Lending new interest to the fate of chlordane in animals was the development of a high purity chlordane by Velsicol Chemical Corp. This product is composed of 98+% of a 3:1 mixture of *cis*- and *trans*-chlordane and is designated as HCS-3260 by the manufacturer. This designation will be used to denote high-purity chlordane in this report. The components of HCS-3260 and technical chlordane as determined by gas–liquid chromatography (glc) are illustrated in an earlier report from our laboratory (Dorough *et al.*, 1972).

Department of Entomology, University of Kentucky, Lexington, Kentucky 40506.

[1] Present address: Nelson Research and Development Co., Irvine, Calif. 92664.



cis-chlordane



trans-chlordane

One metabolite whose significance appears to increase with each new report on the fate of chlordane is oxychlordane (1-*exo*,2-*endo*-4,5,6,7,8,8a-octachloro-2,3-*exo*-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene). This metabolite is formed from both *cis*- and *trans*-chlordane in animals and indications are that it is a persistent storage product (Schwemmer *et al.*, 1970; Polen *et al.*, 1971; Street and Blau, 1972; Dorough and Hemken, 1973).

In the current paper, a study of the fate of HCS-3260-^{14}C and of the individual pure isomers in rats is presented. The data reflect the fate of the materials in animals treated with single oral doses and in animals fed the insecticides in the diet.

METHODS AND MATERIALS

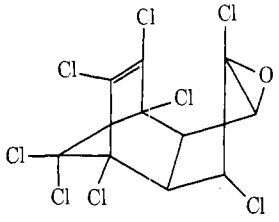
Chemicals. Radioactive materials used in this study were as follows: HCS-3260-^{14}C, 3:1 mixture of *cis*- and

trans-chlordane-^{14}C, sp act. 10.9 mCi/mmol; pure *cis*-chlordane-^{14}C, sp act. 10.9 mCi/mmol; pure *trans*-chlordane-^{14}C, sp act. 6.26 mCi/mmol; pure oxychlordane-^{14}C (extracted from fat of sacrificed rats), sp act. 4.9 mCi/mmol. The radiochemical purity of the chlordanes (4,5,6,7,8-^{14}C) was greater than 99% as determined by tlc and glc.

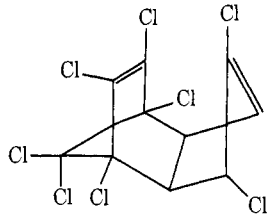
Thin-Layer Chromatography. Merck silica gel F_{254} precoated chromatoplates were used in all phases of this study. For one-dimensional work, the plates were developed in either a 1:1 mixture of benzene–ethyl acetate or a 9:1 mixture of hexane–ethyl acetate. When two-dimensional chromatography was employed, both were used in conjunction.

Radioautography was used to detect radiocarbon on the plates. Nonradioactive standards were located by spraying the plates with a 5% diphenylamine solution in acetone and then exposing them to ultraviolet light (Ivie and Casida, 1971). Quantitation of the radioactive areas was accomplished by direct scintillation counting of the tlc gel. When necessary, ^{14}C-labeled metabolites were removed from the gel by extraction with methanol.

Gas Chromatography. Because *cis*- and *trans*-chlordanes were not completely separated on tlc, these compounds were extracted from the gel and analyzed by glc. For this purpose, a Varian Aerograph Model 1700 chromatograph equipped with an electron capture detector was used. The column, 6 ft × 2 mm glass, was packed with 5% OV-101 on Gas Chromosorb Q, 100–120 mesh. Operating temperatures for the injector, column, and detectors were 215, 200, and 225°, respectively. Nitrogen, 5 psi at the inlet, served as the carrier gas. Under these conditions, *cis*-chlordane had a retention time of 7.5 min and *trans*-chlordane a retention time of 6.7. Two other compounds analyzed by glc analysis were oxychlordane and dichlorochlordene which had retention times of 5.6 and 4.9 min.



oxychlordane



dichlorochlordene

Treatment and Sampling. *Single Dose.* The rats used in all experiments were Sprague-Dawley albino animals weighing 200–250 g. Each radiolabeled material was dissolved in 0.5 ml of corn oil and administered orally with a feeding needle (Popper and Sons, New York, N. Y.). The animals were held individually in metabolism cages and the feces and urine collected separately.

Four male and four female rats were treated with HCS-3260-^{14}C at dosage rates of 0.05, 0.2, and 1.0 mg/kg. The same number of animals were treated with the individual isomers, *cis*- or *trans*-chlordane-^{14}C, but only one dosage level, 0.2 mg/kg, was used for each isomer. Oxychlordane-^{14}C was administered to only two female rats at a dose of 0.2 mg/kg because of the limited supply of this material. One of the animals was sacrificed 1 day after treatment and the other after 7 days. In all other cases, two males and two females were sacrificed at each of the aforementioned times after treatment.

Continuous Feeding. HCS-3260-^{14}C, *cis*-chlordane-^{14}C, and *trans*-chlordane-^{14}C were diluted with the corresponding nonradioactive material to make a final specific activity of 4.9 mCi/mmol. The feed was prepared by adding an acetone solution of the insecticide to a commercial laboratory rat diet and mixing until radioassay of small aliquots of feed demonstrated that a homogenous mixture had been attained.

HCS-3260 was fed to female rats at 1, 5, and 25 ppm in the diet for a maximum of 56 days, and then the remaining animals were returned to a normal ration for up to 56 days. Two rats were sacrificed and tissue samples collected for analysis at 2-week intervals throughout the experimental period. Urine and feces were collected every 24 hr. Male rats were fed a diet containing 5 ppm of HCS-3260-^{14}C for 56 days and then two animals were sacrificed. Also, one rat was sacrificed on the 28th and 56th day after the HCS-3260 source was removed.

Female rats were fed *cis*- or *trans*-chlordane at 25 ppm in the diet for 14 days and then returned to untreated feed for an additional 14 days. Urine and feces were collected daily throughout the study whereas tissue samples were taken after 14 days on treatment and again 14 days after treatment was terminated. For each isomer, two rats were sacrificed at each sampling interval.

Urine. The radiocarbon content of the urine was monitored by direct counting of 1-ml samples in 10 ml of Scintisol-Complete liquid scintillation counting medium (Isolab, Inc., Akron, Ohio). To evaluate the nature of the ^{14}C residues, one drop of concentrated hydrochloric acid was added to 5 ml of urine (final pH about 2) and the mixture extracted three times with 20-ml portions of ethyl acetate. After drying over anhydrous sodium sulfate, the solvent was evaporated and the residue analyzed by tlc.

Feces. Each feces sample was thoroughly mixed in a plastic bag and samples of about 0.4 g were combusted using a Beckman Biological Materials Oxidizer. The gaseous oxidation products were trapped in 20 ml of a 2:1 mixture of 2-methoxyethanol and 2-aminoethanol. Four milliliters of the trap mixture was monitored for radiocarbon content in 15 ml of scintillation cocktail.

Five-gram samples of the feces were extracted for 15 hr with a soxhlet apparatus employing 200 ml of a 1:1 mixture of methanol and chloroform. The solvent was concentrated and analyzed on tlc. That residue remaining in the extraction thimble was air-dried and ground, and portions were monitored for total radiocarbon by combustion.

Tissues. For each animal sacrificed, samples of the brain, muscle, liver, kidney, and fat were removed for radioassay. Except for fat, tissue samples weighing 400–500 mg were combusted; 100–120-mg samples of fat were combusted.

To determine the nature of the residues in the fat, 0.5 g was minced and extracted twice with 30 ml of hexane in a Brinkmann Polytron homogenizer. After filtering, the residue and filter paper were combusted to determine the radiocarbon in the undissolved solids. The hexane extract was reduced in volume and spotted on tlc.

One gram of muscle, kidney, or liver tissue was homogenized in 5 ml of water and the mixture extracted three times with 20 ml of ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and spotted on tlc. The aqueous portion was centrifuged and the radiocarbon content of the water layer and tissue solids was determined.

Stability of Chlordanes in Feed and Excreta. Experiments were conducted wherein the stabilities of HCS-3260-^{14}C and oxychlordane-^{14}C in feed and excreta were determined. Methods of analysis to determine the extent of degradation during incubations with feces and urine were as described above. The feed was subjected to the same analysis as the feces. Each of the radioactive materials was mixed with feed or fresh control feces and allowed to stand overnight. Also, the compounds were added to 25 ml of control urine and allowed to incubate at room temperature for 2 days.

HCS-3260 Metabolism in the Rabbit. A brief examination of the metabolism of HCS-3260 by rabbits was investigated. A male rabbit weighing 2.5 kg was allowed to eat *ad libitum* feed fortified with 25 ppm of HCS-3260-^{14}C for

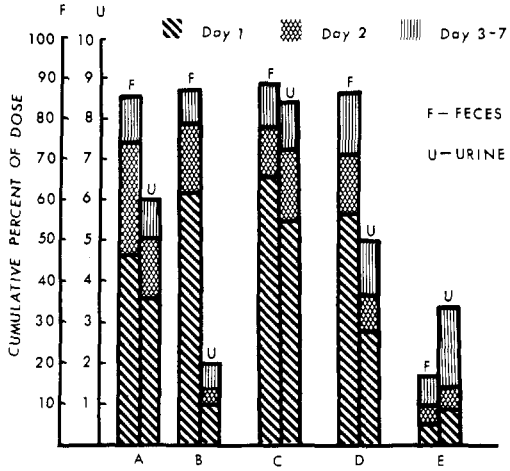


Figure 1. Excretion of chlordane-^{14}C equivalents from rats treated with single oral doses, 0.2 mg/kg, of radioactive: (A) HCS-3260, females; (B) HCS-3260, males; (C) *cis*-chlordane, females; (D) *trans*-chlordane, females; or (E) oxychlordane, females.

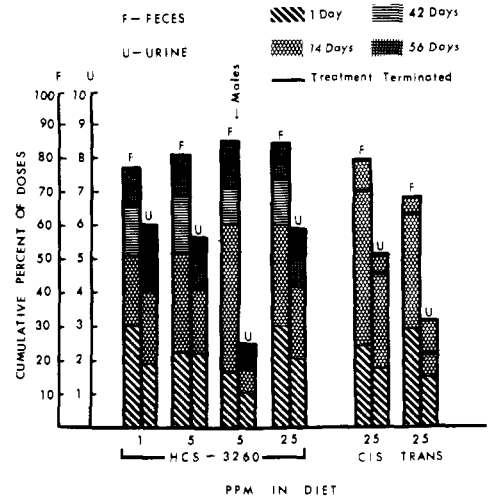


Figure 2. Excretion of radiocarbon from rats fed HCS-3260-^{14}C in the diet at 1, 5, and 25 ppm for 56 days, and from rats fed *cis*- or *trans*-chlordane-^{14}C in the diet at 25 ppm for 14 days. Unless otherwise indicated, all animals were female.

2 days. The animal was returned to a normal ration for 5 days and sacrificed, and tissue samples were collected for analysis. For the length of the experiment, daily urine and feces samples were collected separately. All analyses were carried out exactly as described for the rat study.

RESULTS AND DISCUSSION

Stability. There was no detectable decomposition of HCS-3260-^{14}C when mixed with rat feed and held under ambient conditions for up to 8 weeks. Likewise, HCS-3260-^{14}C and oxychlordane-^{14}C were stable when incubated with feces and urine for periods representing the maximum times the materials would be in these substrates in the unfrozen state. These data demonstrate that the chlordane metabolites reported in this investigation were formed in the animal rather than being generated in the feed or in the excreta after being voided from the body.

Excretion. Over 90% of single oral doses of HCS-3260, *cis*-chlordane, and *trans*-chlordane was eliminated from the rats in the excreta by 7 days after treatment (Figure 1). The feces was the major route of elimination in both female and male rats. A small difference was noted in the amount of the dose excreted in the urine of females and males (Figure 1A and B). A total of 6% of the dose was voided in the urine of the females and 2% in the males.

Data in Figure 1 represent only the treatment of rats with 0.2 mg/kg of HCS-3260, but do reflect the excretion patterns of the 0.05 and 1.0 mg/kg doses. For example, the total eliminations of these latter doses for females and males were 91 and 88% in the feces and 6.2 and 1.4% in the urine of rats treated with 0.5 mg/kg, and 90 and 94% in the feces and 5.5 and 2.7% in the urine of rats treated with 1.0 mg/kg of HCS-3260.

Slightly more of the *cis*-chlordane dose was excreted by female rats than was the *trans*-chlordane (Figure 1C and D). Although both the urine and feces contained a greater percentage of the *cis*-chlordane dose than the *trans*-chlordane treatment, the differences were not great enough to indicate a strikingly different metabolic pathway of the two isomers in the animals.

Excreta of rats treated with a single oral dose of oxychlordane contained only 21% of the administered dose after 7 days. The feces contained about 18% and the urine a little over 3%. The potential for this metabolite of chlordane to accumulate in the body of animals would certainly be greater than the *cis*- and *trans*-chlordane.

The patterns of elimination of ^{14}C-labeled HCS-3260, *cis*-chlordane, and *trans*-chlordane when administered in the diets of rats are shown in Figure 2. After 56 days on the HCS-3260-^{14}C diet, the urine voided by females during this period contained approximately 6% of the total radiocarbon consumed. This was true for feeding levels of 1, 5, and 25 ppm in the diet. In the feces, greater elimination of the doses occurred as the level of HCS-3260 in the diet increased. After 56 days on treatment, the total amount of the consumed radioactivity eliminated in the feces was 70% at the 1-ppm level, 75% at the 5-ppm level, and 80% at the 25-ppm level. This probably means that efficiency of absorption of the compound from the digestive tract decreased as the dosage level increased. Male rats fed HCS-3260 at 5 ppm eliminated somewhat more of the consumed insecticide than the females, 80 *vs.* 75% after 56 days, but less in the urine, 2.3 *vs.* 5.7% after 56 days. This trend was indicated earlier in the single-dose studies.

At all three feeding levels, and for both females and males, the excretion of HCS-3260-^{14}C equivalents declined abruptly when the insecticide source was removed from the diet. Less than 10% of the consumed radiocarbon was eliminated in the excreta during a 56-day period following termination of treatment.

Feeding the pure *cis*- and *trans*-chlordane separately in the diet at 25 ppm for 14 days showed that the cis isomer was more effectively eliminated from the rats than the trans isomer (Figure 2). Total elimination after 14 days of treatment was 75% of the consumed radioactivity in the case of the *cis*-chlordane and about 65% for the *trans*-chlordane. Although the difference is not great, the data do indicate that in long-term exposure situations, the trans isomer would contribute a relatively greater amount to the body burden of the exposed animal than would the cis isomer.

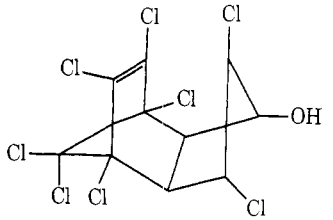
Tlc analysis of extracts of feces showed the presence of eight radioactive areas on the gel when the solvent system was 1:1 benzene–ethyl acetate (Table I, system I). The areas were designated metabolites A–G, *cis*-chlordane, and *trans*-chlordane. The identities of the *cis*- and *trans*-chlordanes were confirmed by glc and mass spectral analysis. Tlc and glc analyses of the feces extract failed to show the presence of oxychlordane or its apparent intermediate, dichlorochlordene.

Metabolite G, when isolated from tlc system I and rechromatographed in system II, was resolved into three components. One product accounted for over 90% of the mixture. Each of the materials reacted with acetic anhydride to form a product with a different R_f value, indicating that the metabolites were hydroxylated. Mass spectral analysis of the major product showed an ion at m/e 422

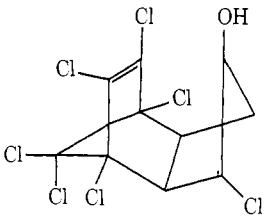
Table I. R_f Values of Chlordane, Certain of Its Analogs, and of Its Metabolites Formed in Rats

Compd or metabolite	Tlc solvent system	
	I, 1:1 benzene–ethyl acetate	II, 9:1 hexane–ethyl acetate
Oxychlordane	0.95	0.83
Dichlorochlordene	0.93	0.79
cis-Chlordane	0.84	0.73
trans-Chlordane	0.84	0.71
G	0.79	0.14
F	0.67	0
E	0.45	0
D	0.40	0
C	0.23	0
B	0.09	0
A	0	0

which was assumed to be the parent ion. This molecular weight corresponds to a monohydroxylated form of chlordane of the type designated as hydroxychlordane.



hydroxychlordane

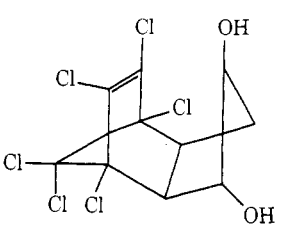


chlordene chlorohydrin

Metabolite F, a rather minor component of the feces, cochromatographed two-dimensionally with a known sample of the chlordene chlorohydrin. Reaction of metabolite F with acetic anhydride gave two products in about equal concentrations. One had the same retention time on glc as did the acetate of the chlorohydrin. The other had a retention time the same as a minor component of the acetylated standard. Based on this information, metabolite F was considered to consist in part of the chlordene chlorohydrin, pictured above, and of a material of similar structural conformation.

Metabolites E and D also were low in concentration in the feces extract and limited information relative to their chemical identity was obtained. Both metabolites when acetylated yielded two products detectable by glc analysis. The acetylated products of the metabolites had different retention times and, in each case, one product gave a peak height four times greater than the second product. The lesser intense peaks had shorter retention times in both cases. Mass spectral analysis of partially purified metabolites D and E indicated that the molecular weight of each was 404, suggesting that the metabolites were monochloro, dihydroxy derivatives of chlordane.

Metabolite C formed two products when acetylated. These acetylated products had identical glc characteristics of standard samples of the acetates of *cis*- and *trans*-chlordenediol. Each of these standards gave two peaks on glc having the same retention times and, therefore, the configuration of the metabolite was not established. However, the evidence strongly indicated that metabolite C was the *cis*- and/or *trans*-dihydroxychlordane derivative.



dihydroxychlordane

Metabolite B reacted with acetic anhydride to produce a single compound with a higher R_f value in tlc system I (Table I). However, metabolite B, or its acetylated form, could not be isolated in sufficient quantities in the purified form for meaningful glc or mass spectral analysis. The R_f value of metabolite B in tlc system I suggests that the compound might be a trihydroxylated chlordene.

Metabolite A, that material remaining at the origin of tlc system I, moved as a single band when rechromatographed in a 75:15:10 (v/v) mixture of chloroform, methanol, and acetic acid. Heating metabolite A at 60° for 1 hr in methanol containing 2% HCl converted approximately 60% of the radiocarbon to metabolites B–G. Their relative concentrations were similar to that observed in the original feces extract. It appears, then, that metabolite A was a conjugated form of the hydroxylated metabolites of chlordane.

Feces excreted daily by animals treated with single oral doses of the insecticides were analyzed. However, the feces of the animals fed the materials in the diet were composited on a weekly basis prior to analysis. The results of these analyses for selected samples are shown in Table II and are representative of all results obtained. Both *cis*- and *trans*-chlordane were excreted in the feces and formed the same series of metabolites in similar concentrations when administered separately. No major differences were noted between the nature of metabolites in the excreta of female and male animals.

Oxychlordane, administered as a single oral dose, was excreted intact in the feces, but was not detected as a metabolite in the feces of animals treated with HCS-3260 or the individual isomers. Metabolites B, E, F, and G were not present in the feces of the oxychlordane-treated animals but were present in all other fecal samples along with metabolites A, C, and D.

The relative percentages of the metabolites in the feces were similar in both the single dose and continuous feeding studies. These relative concentrations changed, however, as the interval after exposure increased. In the single dose study, the monohydroxylated metabolites (F, G) constituted less of the total residues with time and the dihydroxylated materials (C, D, E) increased. In the continuous feeding study, there was a gradual reduction in the percentage of the radiocarbon in the feces composed of nonconjugated metabolites when the insecticide source was removed. Seven weeks after terminating the feeding of HCS-3260-^{14}C, 60% of the metabolites in the feces was as metabolite A and unextracted radioactivity.

Because of the small quantities of radioactive residues in the urine, only that from the high dosage level animals was analyzed. Generally, the nature of the metabolites in the urine was the same as that in the feces (Table III). However, oxychlordane was detected in the urine of rats fed 25 ppm of HCS-3260, but was not detected in any of the feces samples. Characterization of the oxychlordane in the urine was based on both tlc and glc information. For the other metabolites, only tlc analysis was utilized.

Tissues. Total ^{14}C residues in the tissues of rats treated with single doses of the insecticides are shown in Table IV. As expected from the excretion data, the levels of residues were generally low except in the fat. In the comparative aspects of the study, it was found that the level of residues in the fat was considerably higher in the females than in the males but was slightly lower in the other tissues. Also, treatment with *trans*-chlordane resulted in higher concentrations of residues in the tissues than *cis*-chlordane.

The reduction in the level of residues in the tissues at 7 days as compared to the 1-day values varied with the compound administered and the tissue involved. The sex of the animal did not appear to greatly influence the rate of dissipation. Treatment with oxychlordane resulted in

Table II. Radioactive Components of the Feces of Rats Treated with HCS-3260-^{14}C, *cis*-Chlordane-^{14}C, *trans*-Chlordane-^{14}C or Oxychlordane-^{14}C

	% of total ^{14}C in feces sample from females (F) and males (M)										
	Single oral dose, 0.2 mg/kg							Continuous feeding, ppm in diet[b]			
	HCS-3260		*cis*-Chlordane		*trans*-Chlordane		Oxychlordane,	HCS-3260		*cis*-Chlordane,	*trans*-Chlordane,
Metabolite and time[a]	F	M	F	M	F	M	F	F	M	F	F
cis-Chlordane											
1	9	12	15	12	0	0	0	11	8	15	0
7	0	0	0	0	0	0	0	0	0		
trans-Chlordane											
1	5	6	0	0	15	17	0	5	8	0	13
7	0	0	0	0	0	0	0	0	0		
Oxychlordane											
1	0	0	0	0	0	0	44	0	0	0	0
7	0	0	0	0	0	0	8	0	0		
Metabolite G											
1	20	13	17	18	28	21	0	21	17	15	12
7	15	11	14	15	23	18	0	18	13		
Metabolite F											
1	7	3	6	6	6	5	0	3	2	4	2
7	0	0	0	0	0	0	0	2	0		
Metabolite E											
1	9	5	7	2	4	2	0	7	5	5	3
7	13	8	15	10	15	10	0	1	1		
Metabolite D											
1	9	5	12	5	5	3	23	8	9	6	9
7	25	18	19	21	14	15	37	7	4		
Metabolite C											
1	18	17	13	19	7	8	4	16	19	12	14
7	25	27	20	29	13	18	11	8	12		
Metabolite B											
1	2	8	4	7	3	9	0	5	4	7	12
7	0	0	0	0	0	0	0	1	1		
Metabolite A											
1	9	20	14	17	22	19	14	9	14	23	19
7	14	22	22	24	21	27	29	15	22		
Unextracted											
1	12	13	12	12	10	16	15	21	21	13	16
7	8	14	10	21	14	12	15	42	40		

[a] Single oral dose, days after treatment; continuous feeding, weeks after treatment was terminated, composite sample/week. [b] Data from female and male rats fed 5 ppm of HCS-3260-^{14}C in the diet for 56 days and then returned to normal ration for 56 days. *cis*- and *trans*-chlordane were fed at 25 ppm for 14 days and returned to normal ration for 14 days. Therefore, the 7-week posttreatment data were not obtained.

Table III. Radioactive Components of the Urine of Female rats Treated with HCS-3260

	% of total ^{14}C in sample	
Metabolite	Single oral dose, 1.0 mg/kg[a]	Continuous feeding, 25 ppm in diet[b]
cis-Chlordane	0.0	1.7
trans-Chlordane	0	0.5
Oxychlordane	0	0.2
Metabolite G	3.9	1.4
Metabolite F	0.9	0.4
Metabolite E	0.3	0.1
Metabolite D	3.2	2.4
Metabolite C	1.1	1.8
Metabolite B	21.8	13.9
Metabolite A	46.9	50.2
Unextracted	21.9	27.4

[a] Urine voided 0–24 hr after treatment. [b] Urine voided during final 7 days of a 56-day feeding period.

more persistant residues in the fat than treatment with the other chlordane compounds.

Feeding HCS-3260-^{14}C to female rats at 1, 5, and 25 ppm for 56 days resulted in chlordane-^{14}C equivalent in the fat at levels three–four times that in the diet (Figure 3). The residue levels had not plateaued at the end of the treatment period regardless of the concentration of HCS-3260 consumed by the rats. Once the HCS-3260 was removed from the diet, the residues declined steadily for 4 weeks, at which time the concentrations were reduced by approximately 60%. During the following 4 weeks, the residues declined only slightly.

Accumulation of ^{14}C residues in the muscle, brain, liver, and kidney was much less than in the fat. Of these tissues, the liver contained the highest levels of residues and after 56 days of treatment the parts per million concentration in the liver was $\frac{1}{8}$ that fed in the diet. At the same time, the residues in the kidney, brain, and muscle were at concentrations of $\frac{1}{10}$, $\frac{1}{25}$, and $\frac{1}{50}$ that of the level of HCS-3260 in the feed. Dissipation of the residues from these tissues after the treatment period ended followed the same general pattern as that observed in the fat.

Male rats fed 25 ppm of HCS-3260 for 56 days had less chlordane-^{14}C equivalents in the tissues than females fed the same diet (Table V). For example, the fat of the males contained 15 ppm and that of the females 20 ppm. Dissipation of the residues from tissues after returning the animals to normal rations was greater in males than in the females.

Female rats on a diet containing 25 ppm of either *cis*- or *trans*-chlordane for 14 days showed greater quantities of residues in the tissues with the *trans*-chlordane (Table V). The largest difference occurred in the fat where animals

Table IV. Residues in Tissues of Rats Treated with a Single Oral Dose of HCS-3260, *cis*-Chlordane, *trans*-Chlordane, or Oxychlordane

Treatment, sex, and dose, mg/kg	ppm of ^{14}C equivalents of administered compd at indicated days after treatment									
	Brain		Muscle		Liver		Kidney		Fat	
	1	7	1	7	1	7	1	7	1	7
HCS-3260										
Female, 0.05	0.01	0.00	0.00	0.00	0.02	0.00	0.12	0.00	0.14	0.09
Male, 0.05	0.01	0	0.01	0	0.02	0	0.01	0.01	0.09	0.06
Female, 0.2	0.01	0	0.01	0	0.05	0.01	0.03	0.01	0.50	0.33
Male, 0.2	0.01	0	0.02	0	0.08	0.02	0.08	0.02	0.35	0.24
Female, 1.0	0.07	0.02	0.04	0.01	0.43	0.05	0.16	0.05	3.71	2.00
Male, 1.0	0.04	0.02	0.03	0.01	0.57	0.11	0.36	0.09	2.58	1.19
cis-Chlordane										
Female, 0.2	0.01	0	0	0	0.07	0.01	0.02	0.01	0.32	0.22
Male, 0.2	0.01	0	0.01	0	0.07	0.01	0.05	0.01	0.21	0.14
trans-Chlordane										
Female, 0.2	0.02	0	0.02	0.01	0.08	0.04	0.05	0.02	0.66	0.55
Male, 0.2	0.04	0	0.04	0.01	0.11	0.02	0.01	0.02	0.51	0.39
Oxychlordane										
Female, 0.2	0.03	0.01	0.02	0	0.03	0.01	0.02	0.01	0.54	0.50

Table V. Residues in Tissues of Male Rats Fed 5 ppm of HCS-3260-^{14}C and in Females Fed 25 ppm of *cis*- or *trans*-Chlordane-^{14}C in the Diet

Treatment and days	ppm of ^{14}C equivalents of administered compound				
	Muscle	Brain	Kidney	Liver	Fat
HCS-3260, males					
On treatment, 56	0.42	0.91	0.55	0.68	14.73
Off treatment, 28	0.36	0.04	0.15	0.10	3.67
Off treatment, 56	0.17	0.03	0.08	0.05	2.49
cis-Chlordane, females					
On treatment, 14	0.18	0.34	1.10	1.48	29.49
Off treatment, 14	0.08	0.17	0.40	0.36	18.46
trans-Chlordane, females					
On treatment, 14	0.31	0.58	1.30	1.93	43.51
Off treatment, 14	0.15	0.33	0.57	0.72	31.63

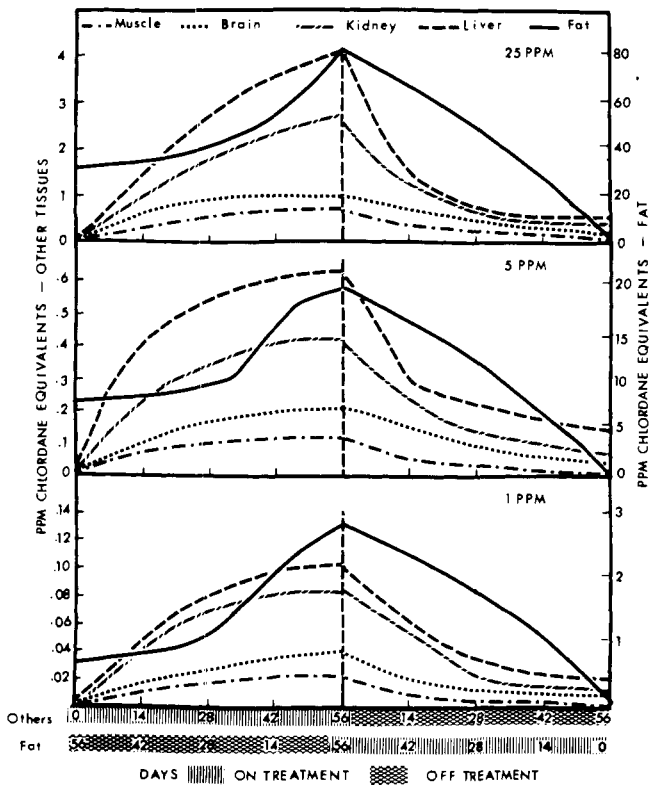


Figure 3. Residues in the tissues of rats fed HCS-3260-^{14}C in the diet at 1, 5, and 25 ppm for 56 days and for a 56-day period after the insecticide was removed from the diet.

fed the *trans*-chlordane contained 44 ppm and those fed *cis*-chlordane had 29 ppm in their tissue. There was no appreciable difference in the percentage reduction of residues from tissues sampled 14 days after the treatments with *cis*- and *trans*-chlordane were terminated.

Evaluations of the nature of the ^{14}C residues in tissues of rats treated with single doses of the chlordane-^{14}C or fed the materials in the diet showed that oxychlordane was a major component in all tissues. This metabolite accounted for 53 and 63% of the radiocarbon in the fat of females and males sacrificed 24 hr after a single oral dose, 1 mg/kg, of HCS-3260 (Table VI). Treatment with the individual isomers showed that a greater percentage of the radiocarbon in the fat was composed of oxychlordane following treatment with *trans*-chlordane.

Thirty to forty per cent of the ^{14}C residues in the liver and kidney of the HCS-3260 treated rats was oxychlordane (Table VI). In these tissues, residues of a water-soluble and unextractable nature constituted a considerable portion of the total, approximately 60% of the residues in the liver and 40% in the kidney. Residues of this nature were negligible in the fat. Both *cis*- and *trans*-chlordane residues were detected in all three tissues. Although variations were noted in the relative concentrations of individual metabolites in the tissues of females and males, sex did not appear to influence the proportion of the different residues in the tissues.

Essentially all (>99%) of the radioactive residues in the fat of female rats treated with a single oral dose of oxychlordane-^{14}C was as the administered compound (Table VI). Concentrations of ^{14}C residues in other tissues were too low to determine their nature.

Table VI. Nature of ^{14}C Residues in Tissues of Rats 24 hr after Treatment with a Single Oral Dose of HCS-3260-^{14}C, *cis*-Chlordane-^{14}C, *trans*-Chlordane-^{14}C, or Oxychlordane-^{14}C

Treatment	% of total ^{14}C in sample as				
	cis-Chlordane	*trans*-Chlordane	Oxychlordane	Water solubles	Unextracted
			Fat		
HCS-3260, 1 mg/kg					
Females	38.1	7.6	53.4	0.0	0.9
Males	28.6	8.3	63.0	0	0.1
cis-Chlordane, 0.2 mg/kg					
Females	30.2	0	69.5	0	0.3
Males	33.5	0	66.3	0	0.2
trans-Chlordane, 0.2 mg/kg					
Females	0	16.4	83.5	0	0.1
Male	0	12.7	87.0	0	0.3
Oxychlordane, 0.2 mg/kg					
Females	0	0	99.6	0	0.4
			Liver		
HCS-3260, 1 mg/kg					
Females	8.8	2.9	31.9	43.2	13.2
Males	5.3	1.4	34.7	39.6	19.0
			Kidney		
HCS-3260, 1 mg/kg					
Females	14.7	4.5	37.8	36.3	6.7
Males	15.9	6.4	40.6	29.7	7.4

Table VII. Chemical Nature of ^{14}C Residues in Tissues of Rats Fed HCS-3260-^{14}C in the Diet for 56 Days

Metabolite and days	% of ^{14}C in sample at indicated ppm in the diet[a]								
	Muscle,	Liver		Kidney		Fat		
	25	5	25	5	25	1	5	25
On treatment								
cis-Chlordane								
14	17.6	15.4	13.0	8.6	9.2	15.9	10.5	15.4
56	6.3	5.8 (3.2)	3.9	4.4 (7.6)	3.2	11.5	7.1 (7.0)	9.6
trans-Chlordane								
14	8.3	4.7	3.8	3.2	2.4	3.5	2.2	3.1
56	3.2	1.8 (1.6)	1.1	1.7 (1.7)	2.3	4.0	1.1 (1.4)	1.0
Oxychlordane								
14	63.9	49.4	40.6	43.4	41.1	79.5	86.9	81.1
56	78.9	65.9 (55.2)	66.3	53.2 (48.1)	48.8	84.0	91.3 (91.0)	89.0
Dichlorochlordene								
14	0	0	0	0	0	0	0	0
56	0	0 (2.4)	0	0 (4.1)	0	0	0 (0)	0
Water solubles								
14	4.7	16.6	21.3	30.7	25.6	0	0	0
56	5.7	16.0 (19.0)	13.6	23.8 (23.4)	20.7	0	0 (0)	0
Unextracted								
14	5.5	13.9	21.3	14.1	21.7	1.1	0.4	0.4
56	5.9	10.5 (18.6)	15.1	16.9 (15.1)	25.0	0.5	0.5 (0.6)	0.4
Off treatment[b]								
cis-Chlordane								
14	1.7	1.8	1.0	0	0	3.6	1.3	3.9
28	0	0	0	0 (0)	0	0	0 (0)	0
trans-Chlordane								
14	0.5	0	0	0	0	1.0	0.3	0.8
28	0	0	0	0 (0)	0	0	0 (0)	0
Oxychlordane								
14	90.9	65.9	77.4	61.9	67.8	95.1	97.7	94.7
28	96.0	91.8	90.6	87.3	85.0	99.5	99.6 (99.9)	99.3
Water solubles								
14	2.1	17.1	10.0	16.8	17.5	0	0	0
28	1.5	4.7	4.5	4.7	2.1	0	0 (0)	0
Unextracted								
14	4.8	15.2	11.6	21.3	14.7	0.3	0.7	0.6
28	2.5	3.5	4.9	17.4	12.9	0.5	0.4 (0.1)	0.4

[a] Values in parentheses are for male animals, all others are for females. [b] Results of the 56-day samples are not shown since they were essentially the same as the 28-day data presented here.

Data presented in Tables VII and VIII demonstrate that the nature and proportions of residues in the tissues of rats fed HCS-3260 and *cis*- or *trans*-chlordane were similar to those found in the single dose studies. The only major difference was that oxychlordane accounted for an increasingly larger percentage of the ^{14}C residues as time on treatment increased. As this occurred, the percentage of the residues as *cis*- and *trans*-chlordane declined. In fat, where residues were the highest, oxychlordane was on the order of 80% of the total after 14 days on treatment and approximately 90% after 56 days. As was the case in the single dose studies, a greater percentage of the resi-

Table VIII. Nature of ^{14}C Residues in Tissues of Female Rats Fed 5 ppm of Radioactive *cis*- or *trans*-Chlordane-^{14}C in the Diet for 14 days

Tissue and metabolite	% of ^{14}C in tissue after 14 days on and off indicated treatment			
	cis-Chlordane		*trans*-Chlordane	
	On	Off	On	Off
Fat				
cis-Chlordane	26.5	9.8	0.0	0
trans-Chlordane	0	0	5.3	1.2
Oxychlordane	72.4	89.3	94.1	98.0
Unextracted	1.1	0.9	0.6	0.8
Liver				
cis-Chlordane	7.7	3.5	0	0
trans-Chlordane	0	0	4.1	0
Oxychlordane	40.4	64.3	59.0	78.0
Water solubles	30.1	17.6	17.7	11.1
Unextracted	21.8	14.6	19.2	10.9
Kidney				
cis-Chlordane	5.0	3.9	0	0
trans-Chlordane	0	0	2.3	0.9
Oxychlordane	32.7	51.6	57.1	68.2
Water solubles	28.8	14.7	18.2	10.3
Unextracted	33.5	29.8	22.4	20.6

Table IX. Nature of Radiocarbon in Tissues and Excreta of Male Rabbits Fed HCS-3260-^{14}C in the Diet at 25 ppm for 2 Days

Metabolite	% of total ^{14}C in sample[a]				
	At ppm in tissue[b]				
	Liver, 0.44	Kidney, 0.28	Fat, 0.78	Urine	Feces
cis-Chlordane	1.2	0.9	9.4	0.0	29.8
trans-Chlordane	0.9	0.3	2.7	0	16.5
Oxychlordane	53.1	18.6	85.0	0	0
Dichlorochlordene	4.4	3.6	0	0	0
Metabolite G	0	0	0	10.4	4.2
Metabolite F	0	0	0	1.1	2.0
Metabolite E	0	0	0	0.4	1.7
Metabolite D	0	0	0	2.4	0.9
Metabolite C	0	0	0	1.4	1.7
Metabolite B	0	0	0	9.9	2.9
Metabolite A	0	0	0	36.6	18.9
Water solubles	20.6	41.7	0	37.8	0
Unextracted	19.8	34.9	2.9	0	21.4

[a] Tissues collected 5 days after last day of treatment; samples of excreta represent that voided for 3 days after treatment was initiated. [b] Muscle = 0.02, brain = 0.03.

dues in tissues was as oxychlordane when *trans*-chlordane was fed than with *cis*-chlordane (Table VIII).

The effect of removing the insecticide from the diet after 56 days was that oxychlordane gradually become almost the sole ^{14}C residue in the tissues (Tables VII and VIII). In fat, it was 95+% of the residue by 14 days and 99+% by 28 days after treatment stopped (Table VII). Residues of the *cis*- and *trans*-chlordane dissipated to very low levels during the first 2 weeks off treatment, and were not detected in any of the tissues sampled at subsequent time intervals. Those residues characterized as water soluble and unextractable were detected in the muscle, liver, and kidney for 56 days after treatment terminated, but at declining levels at each sacrificed time. Livers and kidneys of male rats contained low levels of dichlorochlordene. Otherwise, the male rats did not differ from the females insofar as the nature and relative concentrations of metabolites were concerned (Table VII).

Rabbit Study. Cumulative elimination of radiocarbon from a male rabbit fed 25 ppm of HCS-3260-^{14}C for 2 days was equivalent to 54% of the consumed insecticide by 24 hr after treatment was terminated; 21% was voided in the feces and 33% in the urine. At the end of the experiment, 5 days off treatment, the values had increased to 28% in the feces and 37% in the urine. The elimination of this large quantity of the consumed radiocarbon in the urine was vastly different than that voided in the urine of rats. Urine of male rats seldom contained over 3% of any treatment, either single or multiple.

With the exception of the presence of *cis*- and *trans*-chlordane in the feces, the metabolites in the urine and feces appeared to be the same (Table IX). A greater percentage of the urinary ^{14}C was as very polar metabolites (74% as combined metabolite A and water solubles) than in the feces where 40% was as combined metabolite A and unextracted radioactivity. The polar materials in the urine accounted for the majority of the difference between the urinary ^{14}C excreted by rabbits and rats. This suggests that the conjugative metabolism system was more efficient in rabbits than in rats.

Excretion of chlordane-^{14}C by the rabbit used in this study was similar to that reported by Poonawalla and Korte (1971) for rabbits which were fed *trans*-chlordane-^{14}C for 10 weeks. There, 23% of the doses was excreted in the feces and 47% in the urine. As in the current study, all of the radiocarbon in the urine was composed of metabolites. The authors reported that all of the radiocarbon was removed from the urine by ether extractions before and after treatment with sulfuric acid. Two metabolites were in extracts of the natural urine and two in the extract of acidified urine; their relative concentrations were not reported. One of the metabolites in the extract of natural urine was identified as the chlorohydrin of chlordene and the identity of the other was proposed as the dihydroxychlordene derivative. These are comparable to metabolites F and C as tentatively identified in the present study and, as shown in Table IX, are among the lesser metabolites. Possibly, the apparent difference in the two studies is related to the difference in compounds administered, *trans*-chlordane *vs.* HCS-3260, and/or to the treatment period, 10 weeks *vs.* 2 days.

The nature of the ^{14}C residues in the tissues of the rabbit fed HCS-3260 (Table IX) was the same as in tissues of rats. As with the male rats, dichlorochlordene was detected in the liver and kidney of the male rabbit.

ACKNOWLEDGMENT

The authors are grateful to Velsicol Chemical Corporation for providing the radioactive materials and metabolite standards used in this study.

LITERATURE CITED

Dorough, H. W., Hemken, R. W., *Bull. Environ. Contam. Toxicol.* **10**, 208 (1973).
Dorough, H. W., Skrentny, R. F., Pass, B. C., *J. Agr. Food Chem.* **20**, 42 (1972).
Ivie, G. W., Casida, J. E., *J. Agr. Food Chem.* **19**, 405 (1971).
Polen, P. B., Hester, M., Benziger, J., *Bull. Environ. Contam. Toxicol.* **5**, 521 (1971).
Poonawalla, N. H., Korte, F., *J. Agr. Food Chem.* **19**, 467 (1971).
Schwemmer, B., Cochran, W. P., Polen, P. B., *Science* **169**, 1087 (1970).
Street, J. C., Blau, S. E., *J. Agr. Food Chem.* **20**, 395 (1972).

Received for review December 26, 1973. Accepted February 19, 1974. This study was supported, in part, by Regional Research Project S-73 and the Velsicol Chemical Corporation.