Metabolism of *cis*-[^{14}C]Chlordane in the American Cockroach, *Periplaneta americana* (L.)

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The American cockroach, *Periplaneta americana*, topically treated with *cis*-[^{14}C]chlordane showed extensive metabolism of this compound to more than 25 products. Of these, 1,2-dihydroxydihydrochlordene, 1,2-dihydroxyheptachlor, 1-hydroxy-2,3-epoxychlordene, chlordene chlorohydrin, chlordene epoxide, heptachlor epoxide, heptachlor, oxychlordane, and 1,2-dichlorochlordene were chemically characterized. Pathways and significance of formation of these metabolites are discussed.

It is well known that organochlorine pesticides have been common environmental contaminants and being generally lipophilic accumulate in biological systems producing a variety of ecological and health effects. This, and the emergence of increasing number of insect strains resistant to these chemicals, has resulted in a decline in the usage of organochlorine compounds not only in the United States but also in other parts of the world. Among these insecticides, chlordanes have been applied in the field on a very wide scale and over a prolonged period of time. But, unfortunately, studies of the metabolism of chlordanes have been carried out during only the last few years. Such studies merit more attention because sometimes metabolic products such as oxychlordane rather than the parent compound, are the cause of harmful effects.

Literature review of the work on metabolism of chlordanes in animals shows that almost all studies concern vertebrates, especially rats (Poonawalla and Korte, 1964, 1971; Schwemmer et al., 1970; Lawrence et al., 1970; Polen et al., 1971; Street and Blau, 1972; Barnett and Dorough, 1974; Tashiro and Matsumura, 1977; Brimfield et al., 1977). Among invertebrates only one isolated study on earthworms has been carried out (Chio and Sanborn, 1976). The fate of chlordanes in insects, against which they have been used, has not been studied at all! The objective of these studies, therefore, was to gather information on the extent and nature of biotransformations of chlordane in a target insect species, the American cockroach, *Periplaneta americana*, and to find out whether they differed from those reported in mammals.

MATERIALS AND METHODS

Insects. The insects used, *Periplaneta americana*, were obtained from two sources. Dr. B. Greenberg of this Department very kindly provided the insects for initial work from a colony he maintains. The later part of the study was carried out on insects generously supplied by the Department of Entomology, University of Illinois at Urbana-Champaign. The insects in this laboratory were kept at 28 °C and 50% relative humidity in an environmental chamber with 12:12 h light/dark photoperiods. Food for the roaches comprised mixed vegetables, bread crumbs, and rat chow (Purina Chow Co.). Only adult insects of all ages were used.

Chemicals. The pesticide employed in the work, *cis*-chlordane (1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), was uniformly carbon labeled in the hexachloronorbornene moiety (New England Nuclear Corporation, Boston, MA). It was a gift from the Velsicol Chemical Corp., Chicago, IL. The compound had specific activity 10.9 mCi/mmol and was reported to be 97+% pure. Tests indicated the compound to be about 99% pure. The impurities were removed by thin-layer chromatography (TLC). Subsequent tests using various TLC systems and gas–liquid chromatographic (GLC) columns showed it to be a single compound.

Small samples of all reference compounds, except 1,2-dihydroxydihydrochlordene, were provided by the Velsicol Chemical Corp. The standard for 1,2-dihydroxydihydrochlordene was kindly supplied by Dr. R. Beeman of Dr. F. Matsumura's Laboratory (Department of Entomology, University of Wisconsin, Madison, WI).

The solvents and reagents used were of reagent or analytical grade. Details of instruments and equipment along with their operating conditions appear in relevant sections of the article.

Treatment of Insects. The insects were immobilized by chilling and treated with the insecticide as follows: (i) Fifteen males and 15 females were treated with 3 μg of *cis*-[^{14}C]chlordane/insect in 2.5 μL of acetone. The insecticide was applied on the mesothoracic sterna of the insects which were held individually without food or water. Three males and three females were withdrawn for analysis at 0, 6, 12, 24, and 48 h after treatment. None of the insects showed toxic symptoms. This part of the work was undertaken to compare the sexes in metabolic activity toward the compound and its distribution in various fractions.

(ii) Three males and three females were injected with 3 μg of *cis*-[^{14}C]chlordane in 4 μL of ethanol. The insects were maintained as those in treatment i. The purpose of this experiment was to find out the effect of mode of delivery of the pesticide on its metabolism.

(iii) Eighteen females treated with 12 μg of *cis*-[^{14}C]-chlordane/insect applied topically in 10 μL of acetone were divided in six groups (three insects/group) for analysis at 0, 12, 24, 48, 72, and 96 h posttreatment time intervals. This dose level evoked toxic symptoms in some of the insects. One insect in each of the 48- and 72-h groups showed severe tremors of appendages and uncoordinated movements and two of the insects in the 96-h group were moribund. Food and water were not provided to the insects during the experimental time. This treatment provided data on the relative amounts of the various metabolites at different time intervals.

(iv) Four groups each of 25 females were treated with 8 μg of *cis*-[^{14}C]chlordane/insect, delivered topically in 20 μL of acetone–ethanol (1:1). The insects were held for 9 days during which period there was 23% mortality. Food and water was not provided during the initial 24 h but subsequently feeding was resumed. The objective of this treatment was to collect metabolites in amounts suitable for characterization.

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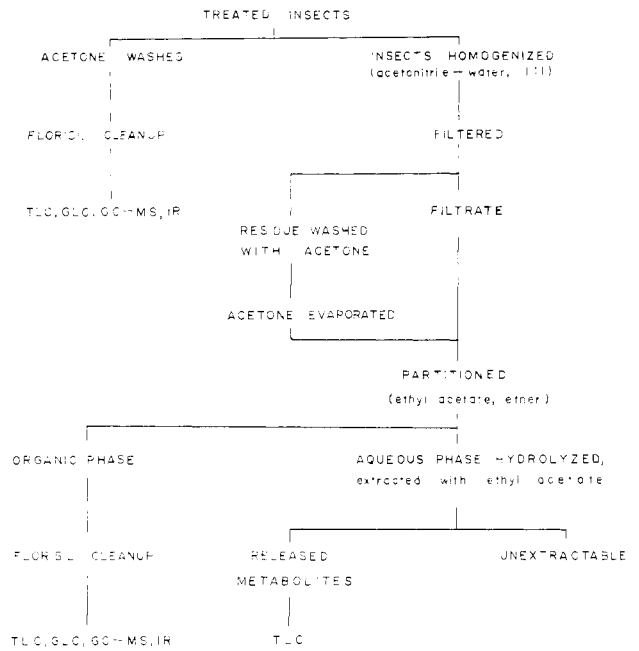

Figure 1. Flow diagram of procedure employed for extraction of *cis*-chlordane and its metabolites from treated cockroaches.

Washes and Extraction Procedure. The insects topically treated with 3 μg of *cis*-[^{14}C]chlordane were washed with 4 mL (2 × 2 mL) of acetone and the radioactivity recovered in these washes provided a measure of the disappearance of radiocarbon and thereby penetration.

The cockroaches treated with 12 μg of *cis*-[^{14}C]chlordane were washed with 12 mL (5 + 4 + 3 mL) of acetone. The washes for the three insects in each batch were pooled (total 36 mL), and an aliquot of that was radioassayed to give an estimate of the radioactivity remaining outside the body. The washes were evaporated, cleaned, and analyzed.

Figure 1 outlines the general extraction procedure. After acetone washes, the topically treated cockroaches were homogenized in a 1:1 mixture of acetonitrile and water (5 mL for each insect). The homogenate was filtered and the residue was again homogenized in the acetonitrile–water mixture. The residue still retained appreciable amounts of radioactivity which, however, could be stripped with four successive washes with acetone. Acetone extract was evaporated on a rotary evaporator to near dryness. The residue of this fraction was taken in the acetonitrile–water filtrate which was then partitioned twice against an equal volume of ethyl acetate and then twice against diethyl ether. The organic extracts were pooled and evaporated on a rotary evaporator to a viscous residue which was cleaned up and analyzed as detailed below.

After estimation of the remaining radioactivity in the aqueous phase, it was hydrolyzed with HCl (pH 2) at 80 °C for 6 h, followed by extraction with ethyl acetate (twice the volume of the aqueous phase). The organic extracts were evaporated and subjected to further analysis by TLC.

Chromatography. All fractions, except those in the preliminary work, involved cleanup on deactivated, 60 to 100 mesh, Florisil (Fisher Scientific Co., Fair Lawn, NJ). A glass column (1 cm diameter) packed with the adsorbent to a depth of 34 cm and covered with a 3-cm layer of anhydrous sodium sulfate was used. The column was prewetted with 50 mL of anhydrous ethyl ether and the sample was applied in minimal volume of the same solvent. Elution was carried out with 100 mL of each of the solvents in the following order: anhydrous diethyl ether, acetone, and methanol. Free metabolites along with unchanged *cis*-chlordane were eluted with diethyl ether while the conjugate(s) was removed with acetone and more successfully with methanol.

For TLC 0.25-mm silica gel G, F-254 plates (Brinkmann Instrument Co., Des Plaines, IL) were used. Repeated TLC (three to five times) was necessary for obtaining metabolites without interfering materials.

GLC analyses were done on Packard Gas Chromatographs Series 7300 (Packard Instruments Co., IL) as described elsewhere (Feroz and Khan, 1977). One of the gas chromatographs was outfitted with tritium electron-capture detector and two 2 mm i.d. glass columns, one of which was 3.5 ft long and packed with 3% SE-30 on 80–100 mesh Gas-Chrom Q and the other was 5 ft long packed with 3% QF-1 on 80–100 mesh Chromosorb W-HP. The machine was operated at 205, 190, and 205 °C temperatures, respectively, for inlet, column, and detector. Nitrogen which flowed at 40 mL/min through the SE-30 and at 30 mL/min through the QF-1 column served as a carrier. The other gas chromatograph was fitted with ^{63}nickel electron-capture detector and two 6 ft long (2 mm i.d.) glass columns which were packed with 6% SE-52 on Chromosorb W-AW, HMDS, 60–80 mesh, and 3% OV-101 on 80–100 mesh Chromosorb W-HP. Flow rate of nitrogen was 55 mL for SE-52 and 35 mL/min for the OV-101 column.

Derivatization. To test the presence of hydroxyl groups in the metabolic products, a small volume of petroleum ether solution of the material was evaporated in Reacti-Vials (Pierce Chemical Co., Rockford, IL) under a gentle stream of dry nitrogen. The vials were sealed with screw caps and Tri-Sil 'Z' (Pierce Chemical Co.) was added to make the starting volume. The solution was then directly injected into the gas chromatographs.

Spectroscopy. GC–mass spectrometry (GC–MS) was performed on a Hewlett-Packard Model 5982A quadrupole mass spectrometer. Data output from the mass spectrometer was monitored with a Model 5934A Dual Disc Data System. The mass spectrometer was operated at 70 eV. The column used was a 6 ft × 2 mm glass column packed with 4% OV-101 on Gas-Chrom Q, 80–100 mesh. Column temperature was maintained at 220 °C. The gas chromatograph was interfaced with the mass spectrometer by a jet separator for helium which served as carrier.

Infrared spectra of the metabolites were obtained on a Perkin-Elmer IR spectrophotometer, Model 257, using micropellets of KBr.

Detection and Estimates of Radioactivity. X-ray autoradiography and radiometry was done as described elsewhere (Feroz and Khan, 1977). Radioactive areas on the TLC plates were detected by exposing the developed plates to Eastman Kodak No-Screen X-ray films NS-5T or NS-2T (bought from Alphatek Corporation, Chicago, IL, or G. W. Brady, Skokie, IL) for 3 to 4 weeks.

All quantitative estimates of the radioactivity were done on a Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co.), Model 3390. Aqueous samples (5 mL) were mixed with 15 mL of liquid scintillation cocktail Insta-Gel (Packard Instrument Co.) and counted.

Estimate of the radioactivity in the residue was made after digesting 0.1 g of the material in 1.0 mL of Soluene-350 (Packard Instrument Co.) for about 12 h at room temperature and then at 55 °C for 3 to 6 h. The digested residue was then mixed with 10 mL of the scintillation liquid Dimilume-30 (Packard Instrument Co.). These samples were left in the counter for about 12 h to avoid chemiluminescence and thermal noise.

Sometimes radioactivity in the spots on chromatoplates was estimated by taking silica gel scrapings directly in 15

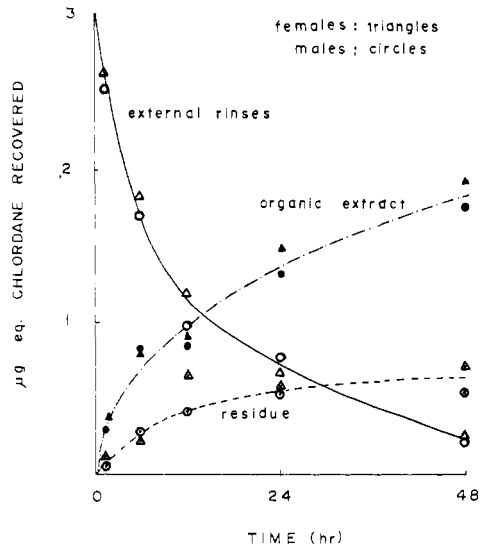

Figure 2. Plots of radioactivity in different fractions of cockroaches treated with 3 μg of *cis*-[^{14}C]chlordane at various posttreatment times.

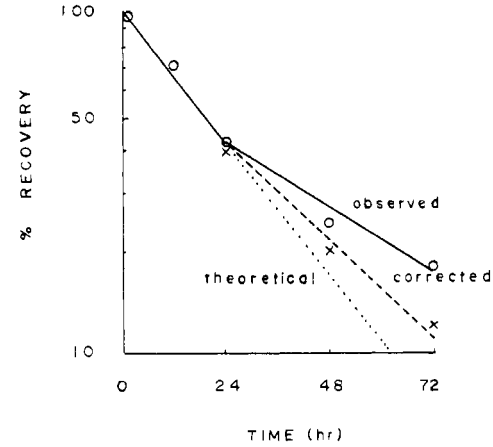

Figure 3. Plots of radioactivity in acetone rinses of female cockroaches treated with 12 μg of *cis*-[^{14}C]chlordane/insect (for details see text).

mL of Insta-Gel and counting them. Radioactivity in organic solvents was measured by evaporating small samples in scintillation vials and adding scintillation cocktail.

Corrections for the background and quenching were made in all radioassays.

RESULTS AND DISCUSSION

Penetration. Quantitative estimates of radiocarbon recovered in acetone rinses, organic extracts, and residue of the male and female cockroaches treated topically with 3 μg of *cis*-chlordane/roach at various intervals of time are presented in Figure 2. There was a steady loss of radiocarbon from the surface of the insects with concomitant increase in the organic extracts and solids. The amount of radioactivity in the residue showed a tendency to level off around 12 h after treatment, indicating that at this dose level binding of chlordane or its products to nonspecific component(s) becomes maximal sometime during 6 to 12 h after treatment. (Radioactivity in the aqueous fraction was negligible and is not shown in the figure.) The figure also shows that there was virtually no difference between male and female insects with regard to the total radioactive contents in various fractions. Plots of the radioactivity recovered in acetone washes in semi-log form (log percent radioactivity present in washes against time) indicated a biphasic pattern of penetration of chlordane. Although further analysis of the recovered radioactivity in this set of experiments was not carried out, studies at 12 μg treatment level revealed that the biphasic nature of the curve may well be an experimental artifact. Figure 3 elucidates the point, giving log percent recoveries of the radioactivity on external surface of insects treated with 12 μg of chlordane at 0, 12, 24, 48, and 72 h after treatment. Again, the loss of insecticide from the surface of the treated cockroaches was biphasic. Analyses of the external rinses by TLC and autoradiography indicated that considerable amount of the recovered radioactivity was in the form of the compounds other than *cis*-chlordane. At 24, 48, and 72 h conversion products amounted to, respectively, 2.8, 8.9, and 28.2% of the recovered radiocarbon. When corrections for these are made in the plots, the resulting curve fits fairly close to the theoretical line drawn by extrapolating the initial phase. Nevertheless, even corrected plots fall short of the theoretical line. Among several possibilities which may account for the discrepancy is that some of the pesticide outside the body of the insects may actually be the parent compound excreted unchanged and the extent of that amount would be difficult to determine. Cockroaches appear to be active in eliminating apolar compounds (for instance oxychlordane which tends to be stored in adipose tissues in mammals was present in appreciable amounts in external rinses). The matter is further complicated when one considers the possibility of resorption of excreted metabolites, especially those of a polar nature for it has been shown (Olson and O'Brien, 1963; Brueger and O'Brien, 1965) that polar compounds penetrate the cockroach cuticle faster than the apolar ones. The whole biodynamic picture becomes increasingly complex with progressing posttreatment time, and observations are merely net results of operating variables.

Relying on the values at initial time intervals when factors other than penetration may be relatively less effective, it appears that penetration of *cis*-chlordane in cockroach follows a monophasic course. For the 12-μg treatment, the half-time for penetration is approximately 19 h.

Metabolism. Pilot experiments on metabolism of *cis*-chlordane in cockroaches treated with 3 μg/insect indicated that females were more active than males in metabolizing the pesticide. Although total organosoluble radioactivity at 24-h posttreatment time was almost equal in males and females (Figure 2), TLC analyses followed by radiometry showed that transformed products in the females constituted 7.1% of the fraction compared to 4.9% in the males. Similar differences between the two sexes of cockroaches in the metabolism of other cyclodiene insecticides have been recorded (Nelson and Matsumura, 1973). Apart from the quantitative difference, the qualitative pattern in the male and female insects was identical. The route of administration of chlordane, i.e., whether topical or by injection, did not result in any noticeable qualitative difference. Since the main purpose of the work was to characterize the metabolic products of chlordane, only topically treated female insects were included in all further studies since yield of metabolites was higher in this sex and mode of application of the pesticide did not affect their nature.

Results of the gross analyses of female insects treated with 12 μg of *cis*-chlordane/insect are summarized in Table I. It will be seen that recovery of the applied radiocarbon exceeded 80% at all time intervals. Presence of more radioactivity in external rinses of the insects at 96 h as compared to those at 72 h and reverse relationship in organic and aqueous fractions seems anomalous. As al-

Table I. Distribution of Radioactivity in Various Fractions of Female Cockroaches Treated with 12 μg of *cis*-[^{14}C]Chlordane/Roach[a]

time, h	μg of equivalent *cis*-[^{14}C]chlordane in external rinses	organic extract	aqueous extract	total	% recov.
zero	11.42	0.55	0.00	11.97	99.75
12	8.72				
24	5.12	6.18	0.07	11.37	94.75
48	2.96	6.87	0.19	10.02	83.50
72	2.16	9.18	0.24	11.58	96.50
96	3.59	6.02	0.19	9.80	81.66

[a] Of three insects for each time interval, one insect in each of the 48-h and 72-h groups showed toxic symptoms and two in the 96-h group were moribund.

ready mentioned, the explanation probably lies in the observation that this dose level was rather high and two of the three treated insects in the 96-h batch were severely poisoned.

The metabolic picture of chlordane in the cockroach is shown in Figure 4. Quantitative estimates of the products are listed in Table II. R_f values of reference standards appear in Table III and those of the metabolites in Table IV. Further comparison of the available reference standards with the products extracted from cockroach was carried out by GLC; and the results are shown in Tables V and VI. Figure 5 shows structural formulae of some of

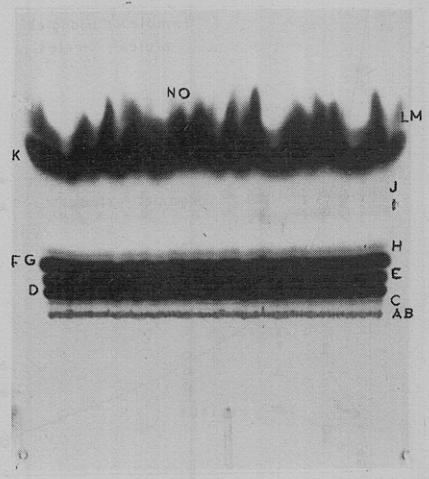

Figure 4. Photograph of an X-ray autoradiograph of a thin-layer chromatoplate (silica gel G, F-254 developed with hexane/ethyl acetate, 9:1) depicting the metabolic fate of *cis*-[^{14}C]chlordane in external washes of cockroaches. Letters correspond to metabolite designation.

the reference standards/metabolites. Following is a brief description of the major metabolites:

Metabolite A. A polar metabolite present in small amounts (Table II) was identified by TLC and GLC as 1,2-dihydroxydihydrochlordene (Tables III–VI).

Table II. Relative Amounts of Various Metabolites in External Rinses and Organic Extracts of Female Cockroaches Treated with 12 μg of *cis*-[^{14}C]Chlordane at Various Posttreatment Times[a]

metabolite	identity	% of radioactivity in external rinses at (h)[b] 24	48	72	96	% of radioactivity in organic extract at (h)[b] 24	48	72	96
P, A, B[c]	conjugate; 1,2-dihydroxydihydrochlordene; 1,2-dihydroxyheptachlor	0.61	2.54	8.15	5.80	0.41	2.30	4.11	7.41
C	1-hydroxy-2,3-epoxychlordene	0.21	0.62	0.99	1.01	0.38	0.72	1.36	0.39
D	chlordene chlorohydrin	0.36	1.89	9.11	9.69	2.64	11.78	15.24	11.59
E	unknown	0.13	0.88	0.87	1.00	0.25	1.25	1.32	0.00
F, G[d]	unknown; unknown	0.35	1.32	5.08	4.89	2.60	7.57	7.49	8.42
H	unknown	0.01	0.21	0.79	0.37	0.20	0.58	0.69	1.13
I	chlordene epoxide	0.00	0.06	0.02	0.01	0.11	0.04	0.05	0.00
J	heptachlor expoxide	0.00	0.02	0.07	0.12	0.04	0.17	0.17	0.18
K	*cis*-chlordane	97.21	91.13	71.81	75.63	92.02	73.24	67.59	66.11
L, M[e]	oxychlordane; unknown	1.10	1.25	3.03	1.40	1.21	2.31	1.69	4.62
N, O[f]	heptachlor; 1,2-dichlorochlordene	0.01	0.10	0.09	0.07	0.08	0.04	0.28	0.14

[a] The analyses carried out prior to Florisil cleanup. [b] For recoveries of the dose applied see Table I. [c] Further analysis showed that the ratio of metabolites P, A, and B was 20:1:6, respectively. [d] F/G present in approximately 1:1.2 ratio. [e] Approximate ratio of L/M 20:1. [f] Approximate ratio of N/O was 1:1.

Table III. R_f Values of Reference Compounds in Various Solvent Systems[a]

compound	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1,2-dihydroxydihydrochlordene	0.00	0.05	0.02	0.00	0.03	0.04	0.00	0.00	0.11	0.25	0.05	0.10
1,2-dihydroxyheptachlor	0.005	0.05	0.09	0.00	0.08	0.07	0.00	0.00	0.26	0.35	0.11	0.16
1-*exo*-hydroxy-2-chlorochlodene	0.08	0.19	0.26	0.00	0.28	0.12	0.00	0.02	0.51	0.50	0.25	0.46
chlordene chlorohydrin	0.06	0.15	0.24	0.00	0.25	0.12	0.00	0.01	0.50	0.52	0.24	0.43
1-hydroxy-3-chlorochlordene	0.08	0.17	0.27	0.01	0.28	0.13	0.00	0.01	0.51	0.53	0.26	0.46
1-hydroxychlordene	0.05	0.12	0.22	0.01	0.24	0.08	0.00	0.01	0.47	0.49	0.22	0.43
1-hydroxy-2,3-epoxychlordene	0.04	0.13	0.16	0.00	0.20	0.11	0.00	0.01	0.44	0.48	0.19	0.42
chlordene epoxide	0.36	0.45	0.49	0.17	0.49	0.43	0.09	0.18	0.62	0.62	0.60	0.67
heptachlor epoxide	0.41	0.48	0.52	0.24	0.49	0.47	0.11	0.24	0.65	0.62	0.63	0.66
oxychlordane	0.51	0.58	0.57	0.43	0.55	0.55	0.27	0.38	0.65	0.65	0.67	0.66
chlordene	0.53	0.57	0.57	0.54	0.54	0.55	0.46	0.48	0.64	0.61	0.67	0.67
heptachlor	0.54	0.58	0.57	0.51	0.56	0.58	0.42	0.47	0.64	0.62	0.66	0.66
2-chlorochlordene	0.53	0.55	0.57	0.52	0.59	0.51	0.43	0.49	0.64	0.62	0.66	0.67
1,2-dichlorochlordene	0.56	0.59	0.60	0.53	0.58	0.57	0.43	0.50	0.65	0.65	0.68	0.68
cis-chlordane	0.45	0.52	0.55	0.42	0.53	0.50	0.30	0.41	0.67	0.62	0.67	0.66
trans-chlordane	0.44	0.52	0.54	0.42	0.52	0.50	0.30	0.40	0.65	0.62	0.66	0.67

[a] I, hexane/ethyl acetate (9:1); II, hexane/ethyl acetate (4:1); III, hexane/ethyl acetate (3:1); IV, hexane/methylene chloride (4:1); V, hexane/methyl ethyl ketone (4:1); VI, hexane/ethyl acetate/benzene (18:3:1); VII, cyclohexane/chloroform (9:1); VIII, cyclohexane/chloroform (4:1); IX, benzene/ethyl acetate (2:1); X, benzene/ethyl acetate (1:1); XI, chloroform pure; XII, chloroform/acetone (9:1).

Table IV. R_f Values of Different Metabolites in Various Solvent Systems[a]

metabolite	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
A	0.00	0.03	0.06	0.00	0.06	0.03	0.00	0.00	0.10	0.24	0.06	0.12
B	0.00	0.03	0.06	0.00	0.06	0.03	0.00	0.00	0.19	0.34	0.06	0.16
C	0.03	0.11	0.23	0.00	0.23	0.09	0.00	0.02	0.33	0.51	0.23	0.39
D	0.04	0.17	0.26	0.01	0.23	0.12	0.00	0.02	0.49	0.53	0.21	0.46
E	0.09	0.22	0.32	0.01	0.34	0.14	0.00	0.02	0.55	0.60	0.25	0.49
F	0.15	0.34	0.42	0.04	0.39	0.24	0.02	0.03	0.55	0.63	0.28	0.51
G	0.15	0.34	0.42	0.04	0.39	0.24	0.02	0.03	0.55	0.63	0.31	0.51
H	0.18						0.00	0.02		0.52		
I	0.26				0.44		0.04			0.60	0.61	0.64
J	0.32				0.44		0.10			0.60	0.61	0.65
K	0.41	0.53	0.58	0.39	0.53	0.50	0.31	0.41	0.65	0.64	0.67	0.68
L	0.51	0.59	0.60	0.38	0.57	0.56	0.27	0.37	0.68	0.64	0.67	0.68
M	0.51	0.58	0.60	0.34	0.57	0.56	0.23	0.32	0.68	0.64	0.67	0.68
N	0.55	0.62	0.66	0.49	0.57	0.60	0.44	0.50	0.68	0.64	0.69	0.72
O	0.55	0.62	0.66	0.49	0.57	0.60	0.44	0.50	0.68	0.64	0.69	0.72

[a] Solvent systems same as in Table III.

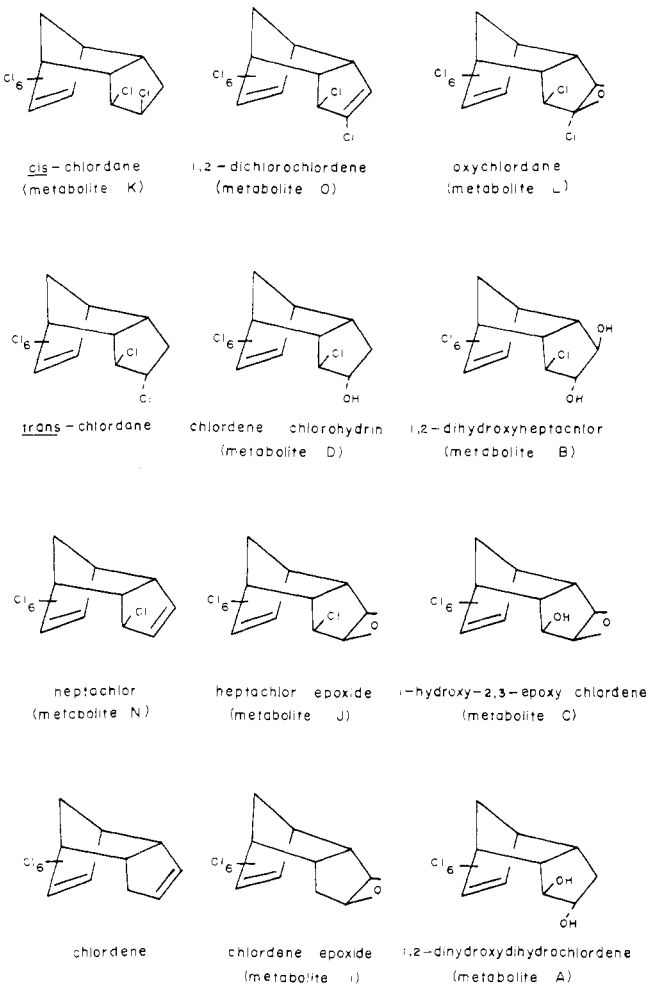

Figure 5. Structural formulae of some reference compounds/metabolites.

Metabolite B. A polar product, close in thin-layer behavior to metabolite A, was identified as 1,2-dihydroxyheptachlor (Tables III–VI). This metabolite was about six times more abundant than A (Table II).

Metabolite C was a minor metabolite (Table II) with behavior corresponding to 1-hydroxy-2,3-epoxychlordene (Tables II–VI).

Metabolite D was the most abundant metabolite (Table II) of which approximately 100 μg was isolated in pure form. Cochromatographic analysis indicated that it was either chlordene chlorohydrin or 1-*exo*-hydroxy-2-chlorochlordene (Tables III, V) since the two reference

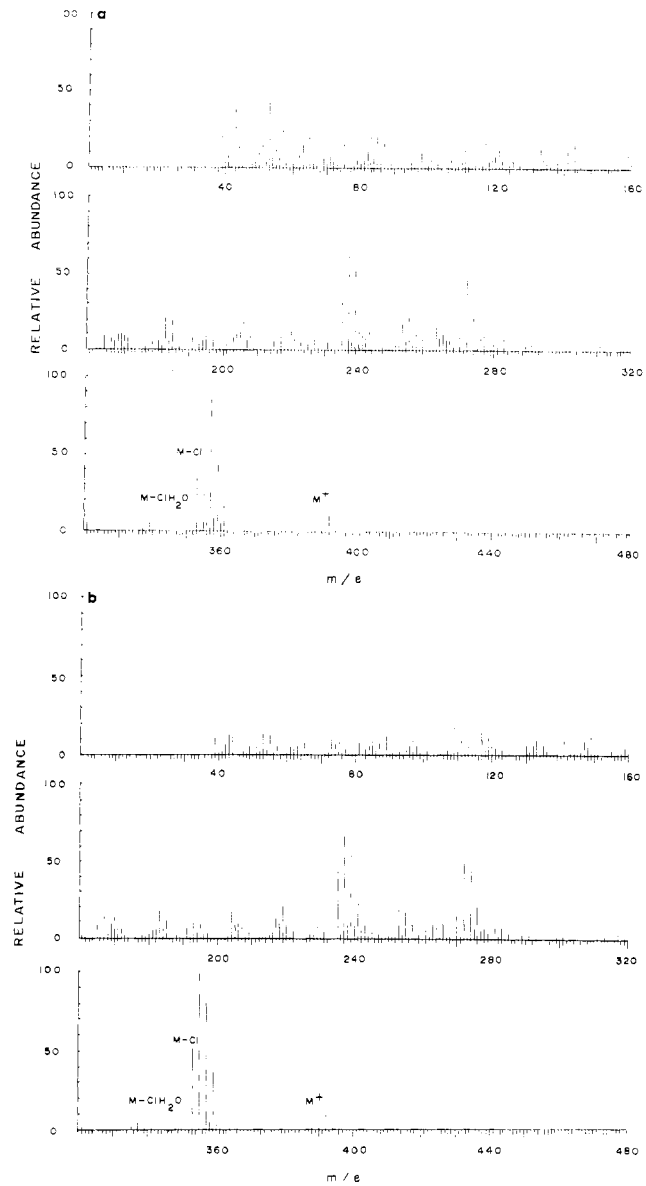

Figure 6. Comparison of mass spectrum of metabolite D (a) with that of chlordene chlorohydrin (b).

standards were exactly similar in all TLC systems and GLC columns employed. Further analysis by GC–MS (Figure 6) showed a parent ion at m/e 388 which was comparable to chlordene chlorohydrin. Infrared spectrum (Figure 7) of the metabolite is likewise comparable to that

Table V. Gas-Liquid Chromatographic Behavior of Reference Compounds

compound	retention time, min[a]							
	QF-1		SE-30		SE-52		OV-101	
	U	D	U	D	U	D	U	D
1,2-dihydroxydihydrochlordene	2.56	1.47	5.56	8.26	ND[b]	5.67	5.90[c]	7.08
1,2-dihydroxyheptachlor	2.95	1.87	7.57	11.51	ND[b]	18.66	8.97[c]	11.81
1-*exo*-hydroxy-2-chlorochlordene	1.67	1.28	4.52	5.70	9.45	9.68	5.25	6.14
chlordene chlorohydrin	1.67	1.27	4.52	5.70	9.45	9.68	5.25	6.14
1-hydroxy-3-chlorochlordene	1.57	0.98	4.57	4.82	10.31	12.75	5.43	5.66
1-hydroxychlordene	0.98	0.69	2.65	3.05	5.62	6.85	3.18	3.30
1-hydroxy-2,3-epoxychlordene	1.47	1.28	3.44	4.33	6.00	8.03	3.93	5.43
chlordene epoxide	0.79	0.79	2.16	2.16	4.25	4.25	2.59	2.59
heptachlor epoxide	1.28	1.28	3.34	3.34	6.37	6.37	4.01	4.01
oxychlordane	1.08	1.08	3.49	3.49	6.37	6.37	3.77	3.77
chlordene	0.49	*d*	1.47	1.47	2.71	2.71	1.88	1.88
heptachlor	0.59	0.59	2.16	2.16	3.77	3.77	2.59	2.59
2-chlorochlordene	0.59	0.59	2.16	2.16	3.77	3.77	2.59	2.59
1,2-dichlorochlordene	0.68	0.68	2.95	2.95	5.19	5.19	3.30	3.30
cis-chlordane	1.38	1.38	4.33	4.33	8.26	8.26	4.72	4.72
trans-chlordane	1.18	1.18	3.83	3.83	7.55	7.55	4.48	4.48

[a] Operating conditions given in the text. U indicates retention times without Tri-Sil 'Z' derivatization or treatment and D gives retention times of the compounds derivatized or treated with Tri-Sil 'Z'. [b] Not detectable. [c] Detector response very poor. [d] Buried in solvent.

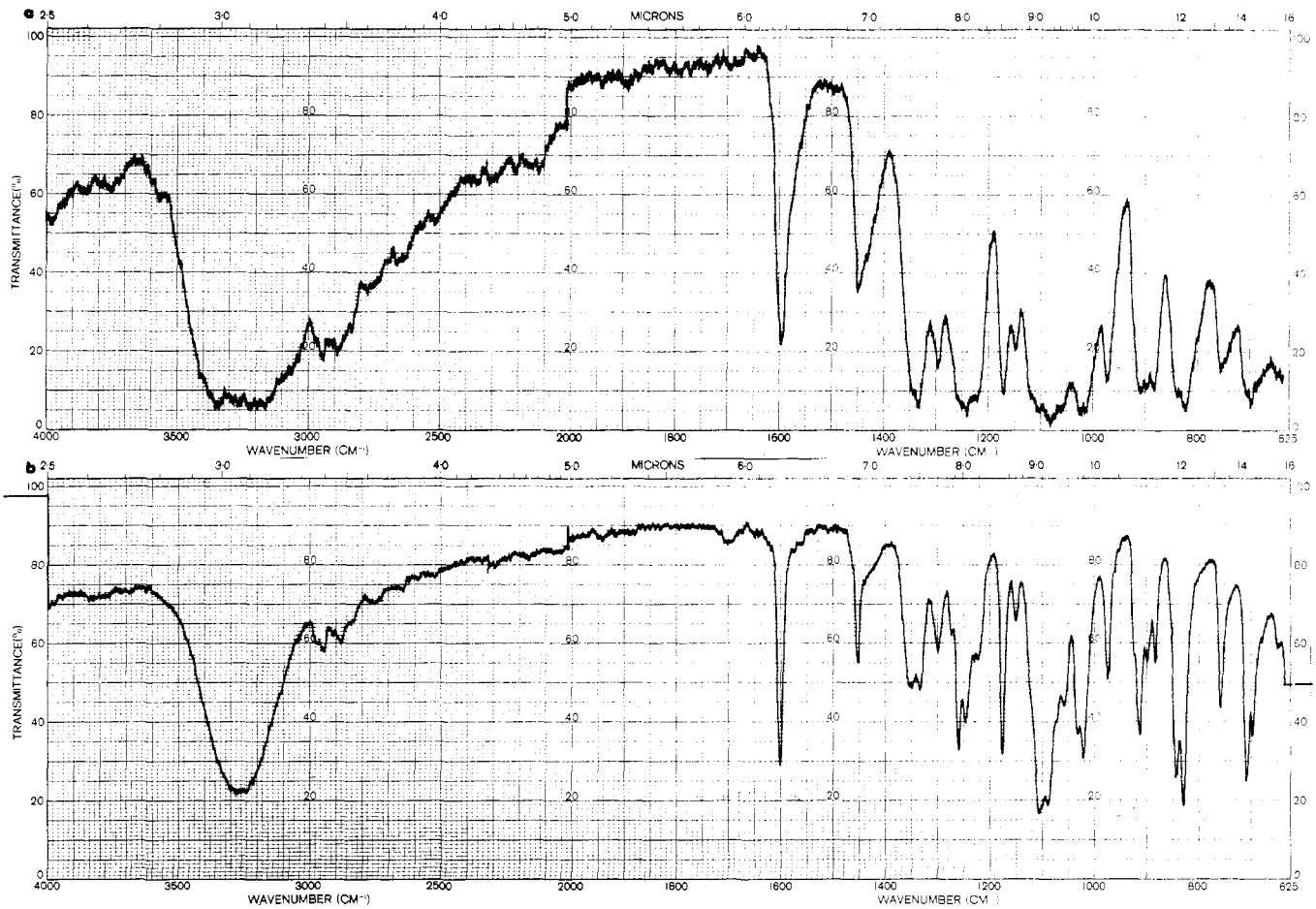

Figure 7. IR spectra of metabolite D (a) and chlordene chlorohydrin (b).

of authentic reference standard of chlordene chlorohydrin.

Metabolite E, present in fairly good amount (Table II), did not compare with any of the reference samples. However, a different retention time after derivatization was suggestive of its hydroxylated form (Table VI). Its behavior on thin-layer chromatoplates indicated it to be a monohydroxy product (Table IV).

Metabolites F and G. The two major metabolites, together second in abundance (Table II) only to chlordene chlorohydrin, initially could not be separated from each other on TLC plates and they were taken to be a single compound; but GLC analysis revealed the presence of two overlapping peaks, one being 1.2 times the other. Extension of analysis by TLC showed they could, however, be separated in solvent system XI (Tables III, IV) after multiple development. Neither their TLC characteristics nor GLC behavior correspond to any of the reference compounds. Since derivatives of the compounds exhibit different retention times, it was presumed that both of them were monohydroxy products of chlordane.

Table VI. GLC Behavior of Metabolic Products of *cis*-Chlordane

	retention time, min[a]							
	QF-1		SE-30		SE-52		OV-101	
metabolite	U	D	U	D	U	D	U	D
A	2.56	1.47	5.51	8.26	ND[b]	5.67	5.90	7.08
B	2.95	1.87	7.57	11.51	ND[b]	18.66	8.97[c]	11.81
C	1.47	1.28	3.44	4.33				
D	1.67	1.27	4.52	5.70	9.45	9.68	5.25	6.14
E	2.36	1.77	6.79	8.75	10.62	14.64	7.08	8.03
F	1.57	1.47	3.93	5.01	7.79	8.74	4.37	5.43
G	1.18	0.89	3.59	4.23	6.61	7.08	3.78	4.49
H			5.51	7.28	8.73	9.21	7.32	9.45
I	0.79[d]		2.16[d]					
J	1.28[d]		3.34[d]					
K	1.38	1.38	4.33	4.33	8.26	8.26	4.72	4.72
L	1.08	1.08	3.49	3.49	6.37	6.37	3.77	3.77
M	0.84	0.84	2.46	2.46	4.48	4.48	2.71	2.71
N	0.59	0.59	2.16	2.16	3.77	3.77	2.59	2.59
O	0.68	0.68	2.95	2.95	5.19	5.19	3.30	3.30

[a] As in Table V. [b] As in Table V. [c] As in Table V. [d] Excessive interfering materials.

Metabolite H. The product was present in small amounts (Table II) and did not match any of the reference standards. Trimethylsilyl ether of the compound showed different retention times (Table VI) pointing to its hydroxylated nature.

Metabolite I. The metabolite was present in trace amounts (Table II) and associated with lipids. On the basis of TLC and GLC behavior it was tentatively identified as chlordene epoxide.

Metabolite J. The component was present in extremely small amounts (Table II) and could not be freed from lipids in amounts allowing extensive analysis. TLC and GLC characteristics (Tables III–VI) of this metabolite indicate that it was heptachlor epoxide. The identification is tentative.

Metabolite K was identified by TLC and GLC (Tables III–VI) as unchanged *cis*-chlordane. Mass spectral analysis showed a parent ion at m/e 410, thus confirming its nature.

Metabolite L was also a major product (Table II) present both in organic extract and in external rinses, corresponding to oxychlordane. The GC–MS spectra (Figure 8) and IR tracings (Figure 9) of oxychlordane standard compare with this product.

Metabolite M. In TLC analysis the minor metabolite (Table II) was closely associated with oxychlordane from which it was separated in solvent systems IV, VII, and VIII (Table IV). Identity of the compound remains unknown.

Metabolite N. This metabolite was present in small amounts (Table II) and was present along with the product N in a single spot. GLC analysis indicate it to be heptachlor (1-chlorochlordene; Figure 5) or its isomer 2-chlorochlordene. The techniques used were unable to resolve the two isomers (Tables III–VI).

Metabolite O was also present in small amount (Table II) and was identified as 1,2-dichlorochlordene by TLC and GLC (Tables III–VI).

Metabolite P. In addition to the major metabolic products, conjugate(s) eluted from Florisil columns with acetone and methanol represented a sizable portion (Table II) of the radioactivity. Precise nature of the conjugate(s) is under investigation.

Apart from the compounds reported above there were at least ten other compounds present in trace amounts. Most of these were polar in nature and were associated with metabolites A to G. Scrutiny of the organic extracts of hydrolyzed aqueous fractions by X-ray autoradiography showed the presence of five compounds. Identity of these metabolites will be reported separately.

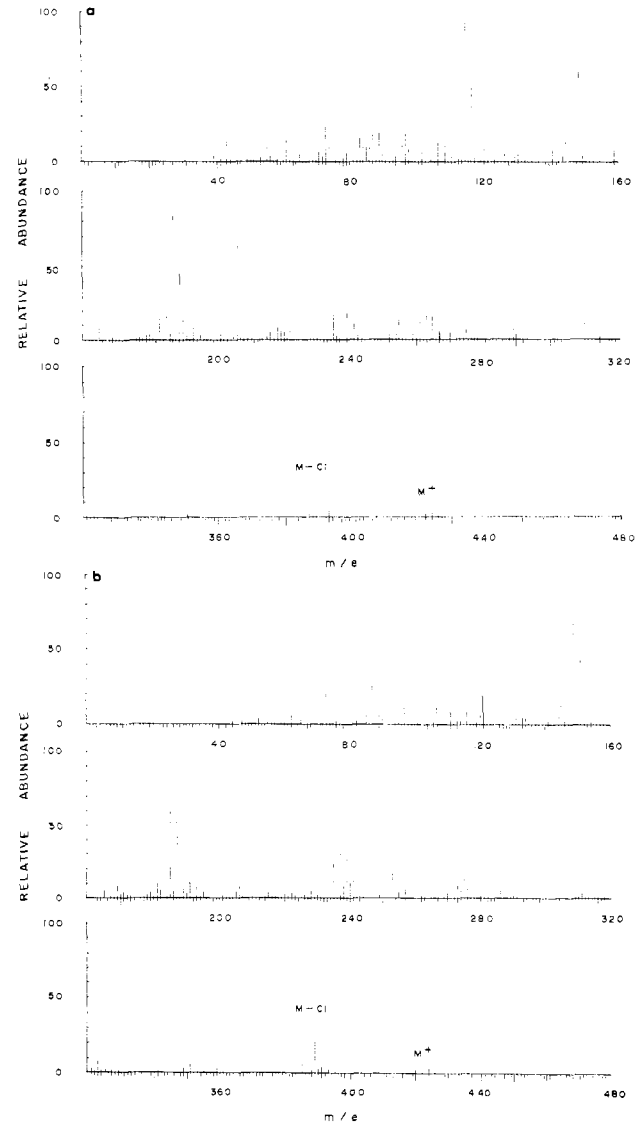

Figure 8. Mass spectra of metabolite L (a) and oxychlordane (b).

The number of transformation products of *cis*-chlordane is far more than those reported in mammals (Poonawalla and Korte, 1971; Barnett and Dorough, 1974; Tashiro and Matsumura, 1977; Brimfield et al., 1977). One probable reason for the observed difference may be the clean-up

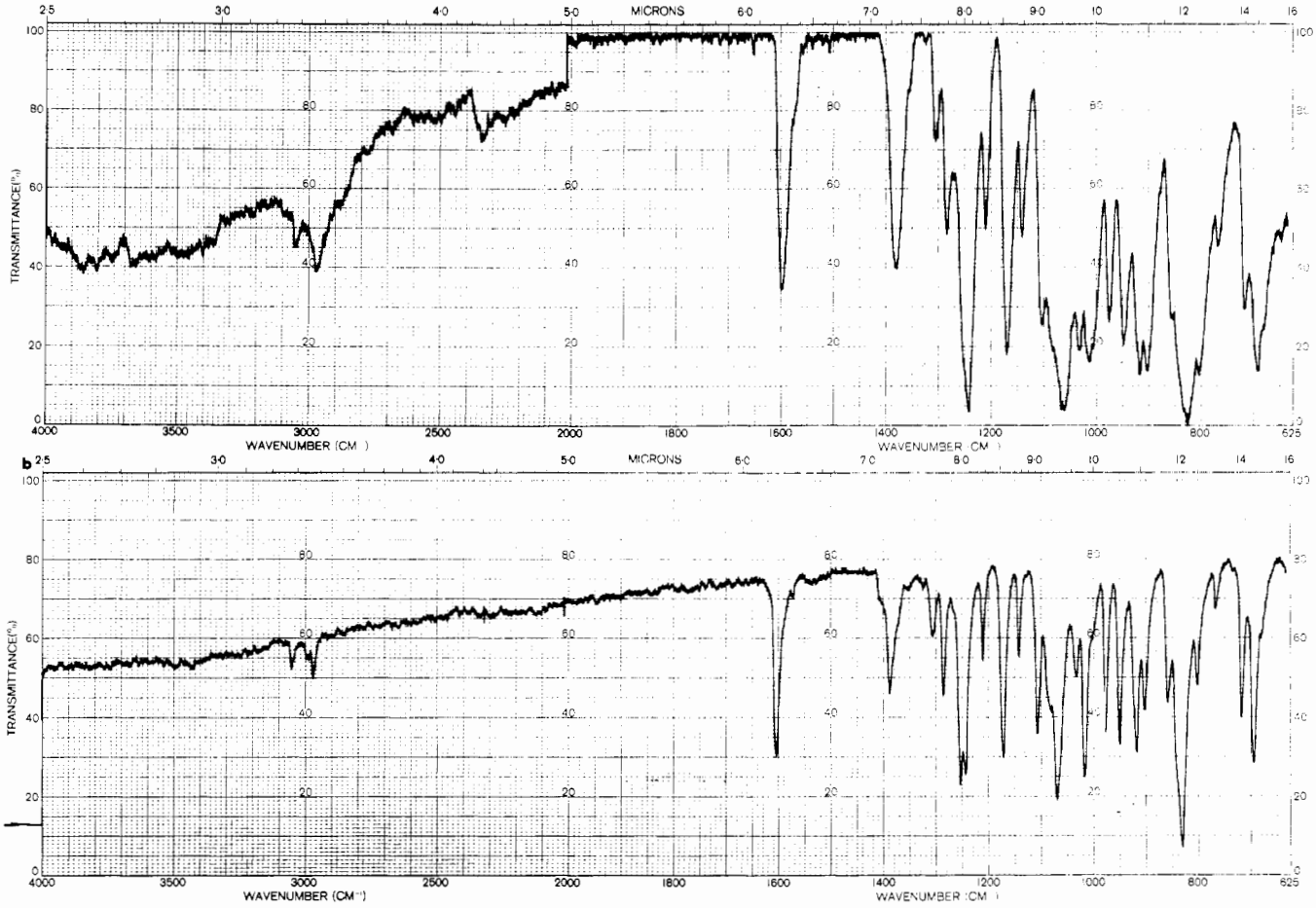

Figure 9. IR spectra of metabolite L (a) and oxychlordane (b).

process, which, of necessity, in mammalian excreta or tissue extracts should be more thorough, and minor products could be lost in that course.

From the nature of the products detected in the cockroach it appears that this insect species is capable of converting chlordane molecule into the forms recorded in mammals. Presence of heptachlor (or 2-chlorochlordene) and 1,2-dichlorochlordene shows desaturation as observed in mammalian species (Street and Blau, 1972). Formation of oxychlordane, heptachlor epoxide, and chlordene epoxide represent epoxidation. If epoxidation proceeds through the formation of 1,2-dichlorochlordene, this route of metabolism of *cis*-chlordane in the roaches is very active, since the amount of oxychlordane relative to 1,2-dichlorochlordene was very large. For instance, in one analysis 1,2-dichlorochlordene and oxychlordane were present in a ratio of 1:23. Also oxychlordane was present in fairly large amounts in the treated insects (Table II).

Degradation of chlordane by hydroxylation mechanism(s) is the most active pathway in these insects. Metabolites A through G all have one or more hydroxyl groups.

Detection of heptachlor, heptachlor epoxide, 1-hydroxy-2,3-epoxychlordene, and 1,2-dihydroxyheptachlor forms a heptachlor metabolic chain observed in various organisms (Brooks, 1974) although the possibility of its being fortuitous cannot be ruled out. Two successive oxidative attacks, one on the carbon–hydrogen bond at C_3 and the other at the carbon–chlorine bond at C_2 of chlordane molecule, would yield dihydroxyheptachlor without the formation of heptachlor and its epoxide.

Finally, a word about elimination of oxychlordane in cockroach may not be irrelevant. Whereas mammals tend to store this product (Schwemmer et al., 1970; Polen et al., 1971; Street and Blau, 1972; Dorough and Hemken, 1973; Barnett and Dorough, 1974), it seems that the cockroach can easily excrete it. It was observed that external rinses and organic extracts of the insects had almost equal amounts of oxychlordane (Table II).

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Effects of Sulprofos and Its Sulfoxide and Sulfone Metabolites on Laying Hens Fed the Compounds in the Diet

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The organophosphate insecticide sulprofos (Bolstar, BAY NTN 9306, *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate) and its sulfoxide and sulfone metabolites were added to the diet of White Leghorn laying hens as a 2:5:3 mixture of sulprofos/sulfoxide/sulfone. At the highest treatment level (150 ppm), the treated diet was apparently highly unpalatable, and feed consumption was greatly reduced; loss of weight and drop in egg production resulted. At treatment levels of 50 ppm or lower in the diet, these effects were reduced or nonexistent. Serum cholinesterase (ChE) activities were significantly decreased in birds fed diets containing as little as 2.7 ppm of the insecticide mixture, but ChE activity increased quickly after the birds were transferred to untreated feed. Analysis of tissues and eggs from birds maintained on the treated diet for 28 days showed no detectable residues (<0.05 ppm) of sulprofos or its intact ester metabolites, except in skin and fat of some of the birds fed at the highest treatment level. Histopathological examination of selected tissues from the treated birds showed no lesions.

The organic phosphate insecticide sulprofos (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate, Bolstar, BAY NTN 9306 of Mobay Chemical Corp., Kansas City, MO) shows high insecticidal activity against certain phytophagous insects, particularly insecticide-resistant strains of *Heliothis* sp., yet its mammalian toxicity is well below that of many of the organic phosphate insecticides in current use. We have previously reported on the environmental behavior of sulprofos, including its photochemistry (Ivie and Bull, 1976), its fate in cotton plants and soil (Bull et al., 1976), and its metabolic behavior in laboratory rats (Bull and Ivie, 1976) and a lactating cow (Ivie et al., 1976). Among other findings, these studies revealed that the sulfoxide and sulfone analogues of sulprofos are two of its major environmental and metabolic transformation products. In the current studies, a mixture of sulprofos, sulprofos sulfoxide, and sulprofos sulfone was added to the diets of laying hens to permit evaluation of the interactions of these compounds with the birds and to determine the potential for appearance of residues in meat and eggs.

MATERIALS AND METHODS

Chemicals. The chemicals used for treatment of the birds were analytical standard grade samples of sulprofos and its sulfoxide (*O*-ethyl *O*-[4 -(methylsulfinyl)phenyl] *S*-propyl phosphorodithioate) and sulfone (*O*-ethyl *O*-[4-(methylsulfonyl)phenyl] *S*-propyl phosphorodithioate) derivatives. Additional compounds used in the residue analysis phase of the study included sulprofos oxygen analogue (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorothioate), the oxygen analogue sulfoxide (*O*-ethyl *O*-4-[methylsulfinyl)phenyl] *S*-propyl phosphorothioate), and the oxygen analogue sulfone (*O*-ethyl *O*-[4-(methylsulfonyl)phenyl] *S*-propyl phosphorothioate. Each of these chemicals was supplied by the Mobay Chemical Corp.

Treatment. This study was made up of two parts, experiment I and experiment II. In experiment I, 34-week-old White Leghorn hens (Ideal Poultry Co., Cameron, TX) that were in full egg production were used. Thirty hens were randomly divided into five groups of six birds each but were housed in individual laying cages. Water was available at all times. Two hundred grams of treated feed was placed in the feed trough before each bird, and the feed consumed each day was measured by weighing the amount of residual feed in the trough. This was then discarded and was replaced with 200 g of fresh treated feed.

The five groups were provided feed containing the following levels of sulprofos/sulfoxide/sulfone mixture: 0, 5, 15, 50, and 150 ppm; the chemical mixture consisted of 20% sulprofos, 50% sulfoxide, and 30% sulfone. This ratio of sulprofos/sulfoxide/sulfone was chosen because it approximates the relative concentrations of these three products seen as residues in sulprofos-treated crops that are used in the manufacture of poultry feeds (Flint, 1978). To minimize the possibility of chemical degradation of the insecticide mixture in the treated feed, it was freshly prepared each week, and the feed samples were stored frozen until immediately before they were placed before the birds.

The feed for each treatment level was prepared in 9.0-kg batches to provide sufficient feed for each group of birds for 1 week (200 g bird^{-1} day^{-1}). Preparation of feed for the 5 ppm treatment level is described below as an example of the mixing procedure. Sulprofos (9.0 mg), sulfoxide (22.5 mg), and sulfone (13.5 mg) were dissolved in 15 mL

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