Fate of [^{14}C]Photoheptachlor in Rabbits

Muhammad Feroz and M. A. Quddus Khan*

Tissue distribution, elimination, and metabolism of intraperitoneally administered photoheptachlor was studied in male rabbits. Biological half-life of the compound at 0.8 mg/kg dose level was about 70 days. The radioactivity was excreted almost exclusively in urine. Analyses of the urine extracts revealed that all the radioactivity was in the form of four metabolites, none of which corresponded to the parent compound. Gas chromatography–mass spectrometry analysis of one of the metabolites indicates that this metabolite arises by oxidative dechlorination of photoheptachlor and is eliminated both in free and conjugated form. Tissue distribution indicated that maximal amounts of the radiocarbon were present in visceral fat and the residues declined with the passage of time.

The observation that cyclodiene insecticides undergo isomerization (Rosen et al., 1966) has resulted in numerous reports which show that these photoconversions are fairly common and take place not only under laboratory conditions but also in simulated and natural environments (Henderson and Crosby, 1967; Matsumura et al., 1970; Ivie and Casida; 1970, 1971a,b; Benson et al., 1971; Ivie et al., 1972; Turner et al., 1977; Parlar and Korte, 1977). The photoisomers of dieldrin and heptachlor appear to have ecological and health significance (Khan et al., 1974). However, the importance of such photoalterations can be put in true perspective only if their behavior in ecosystems (biomagnification, ecological concentration) and in individual organisms (such as their subacute effect, storage and disposition) are well understood.

Among the photoisomers of cyclodiene insecticides only photodieldrin has received some attention with regard to its fate in animals and in environment (Dailey et al., 1970, 1972; Reddy and Khan, 1974, 1975, 1977a, 1978; Nohynek et al., 1978). Work on photoisomers of other cyclodienes is either lacking or fragmentary at best (Khan et al., 1969; Reddy and Khan, 1977b).

Heptachlor has been used in plant protection on a very wide scale for well over 20 years and is known to be transformed, under a variety of conditions, into photoheptachlor which has consistently been shown to have greater toxicity than the parent compound (Rosen et al., 1969; Georgacakis and Khan, 1971; Podowski et al., 1978), especially to nontarget species. Being a possible environmental contaminant of that significance, studies of the fate of photoheptachlor in the herbivore rabbit were undertaken. The results of these investigations are reported here.

MATERIALS AND METHODS

Animals. Eight-month-old adult albino male rabbits weighing 3.3 to 3.6 kg were used. The animals were kept in 12:12 light:dark regimen at 23 °C and were fed on Rabbit Chow (Purina Chow Co.) and water ad libitum.

Chemicals and Equipment. Photoheptachlor (nonradioactive or carbon-14 labeled) was prepared from heptachlor (1-*exo*,4,5,6,7,8,8-heptachloro-2,3,3a,7a-tetrahydro-4,7-methanoindene) by exposing it in acetone with or without benzophenone (equimolar) to ultraviolet light (Podowski et al., 1978). [^{14}C]Heptachlor, the starting material for [^{14}C]photoheptachlor, had the specific activity of 13.0 mCi/mmol and was a gift from the Velsicol Chemical Corporation, Chicago, Ill. Photoheptachlor was purified by thin-layer chromatography (TLC) and when tested by different TLC systems or gas–liquid chromatography (GLC) was found to be free from impurities.

Other chemicals and solvents used in the study were of reagent or analytical grade. The equipment employed in analyses is described at appropriate places in the text.

Preparation of Solutions. [^{14}C]Photoheptachlor was diluted with cold compound. The solution of photoheptachlor was prepared in corn oil and the specific activity of compound subsequent to dilution was 38.41 μCi/mmol.

Treatment of Animals. Two dose levels for treating the rabbits were used. Three rabbits were intraperitoneally injected with [^{14}C]photoheptachlor at a dose of 25.8 mg/kg. These studies, however, could not be continued because the animals died within 24 h after treatment. Immediately after death, the dead rabbits were analyzed to study the pattern of distribution of radioactivity in various tissues.

The other dose, 2.833 mg/rabbit (fixed dose irrespective of weight of animals), approximating to 0.8 mg/kg, was also intraperitoneally administered to three rabbits. One of the rabbits did not receive any more insecticide but the other two were given additional five doses at the end of week 2, 4, 5, 9, and 11 after the start of the experiment. All doses except the third dose, which was 1.416 mg/rabbit, were the same, i.e., 2.833 mg/rabbit. Treated rabbits elicited toxic symptoms after the first dose but all of them recovered later on.

Urine and feces of treated rabbits were collected daily. A 1-mL portion of urine was taken for the estimation of radioactivity excreted and the remainder was stored in a freezer until analyzed.

Chromatography. Isolation and preliminary characterization of metabolic products was carried out by thin-layer, column, and gas–liquid chromatography. TLC was performed on 0.25-mm thick silica gel G (F-254) plates (Brinkmann Instruments, Des Plaines, Ill.), 1.0 mm PLQF, and 0.25 mm LQDF plates (Pierce Chemical Co., Rockford, Ill.) or 0.5 mm thick Prekotes (Applied Science Laboratories, State College, Pa.) plates.

Column chromatography involved the use of gel filteration on Sephadex LH 20 (bead size: 25 to 100 μm; Sigma Chemical Co., St. Louis, Mo.). The gel was swollen in anhydrous methanol overnight for solvent regain and was packed to a depth of 34 cm in a 1-cm diameter glass column. After a thorough wash with 200 mL of methanol, the mixture of metabolites was added in minimal volume of methanol and was eluted with 250 mL of the same solvent.

GLC was performed on Packard Gas Chromatographs (Series 7300, Packard Instruments, Ill.). One of the gas chromatographs was fitted with a tritium electron capture detector and two 2-mm diameter glass columns: one 3.5-ft

Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Illinois 60680.

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long, containing 3% SE-30 on Gas-Chrom Q, 80–100 mesh, and the other 5-ft long, packed with 3% QF-1 on Chromosorb W-HP, 80–100 mesh. Operating temperature (°C) for this machine were: inlet, 205; column, 190; and detector, 205. Nitrogen flowing at 40 mL/min served as a carrier gas. The other gas chromatograph was fitted with a ^{63}Ni electron capture detector and two 6 ft × 2 mm glass columns packed with 6% SE-52 on 60–80 mesh Chromosorb W-AW, HMDS and 3% OV-101 on 80–100 mesh Chromosorb W-HP. Inlet, column, and detector temperature, respectively, were, 230, 205, and 230 °C. Nitrogen flow rate in SE-52 column was 55 mL and that in the OV-101 column 35 mL/min.

Radioassay. Estimation of radioactivity was done by liquid scintillation counting using a Model 3390 Tri-Carb Scintillation Spectrometer (Packard Instruments).

In tissue distribution work, preweighed samples of tissues were digested in a known volume of Soluene-350 (Packard Instruments) for 6 h at room temperature and then for 6 h at 55 °C in a water bath. The procedure usually resulted in complete digestion of the tissue. A 1-mL aliquot of the digest was counted in 10 mL of Dimilume-30 (Packard Instruments).

Urine samples were counted in the scintillation counting cocktail Insta-Gel (Packard Instruments). A 1-mL sample of urine was diluted with 4 mL of distilled water, to which 15 mL of the cocktail was added.

Radioactivity in organic solvents such as urine and feces extracts was assessed by taking an aliquot, evaporating the solvent, and counting it in 15 mL of Insta-Gel. Zonal scrapings from thin-layer plates were also counted directly in 15 mL of Insta-Gel.

Calibration curves for the determination of the efficiency of the counting systems employed were prepared beforehand and necessary corrections were made. Care was taken to keep chemiluminescence or thermal noise to the minimal. Deductions for the backgrounds were preset on the counter.

X-ray Autoradiography. Radioactive areas on thin-layer plates were detected by exposing them to Eastman Kodak No-screen films No. NS-2T (George W. Brady, Skokie, Ill.). The plates were left in the dark room for at least 2 weeks. Radioactive spots on the plates were then marked and taken for analyses.

Extraction of Metabolites from Urine. As urinary or water-soluble metabolites may be ionic in nature, their partitioning behavior depends on the pH of the system. They are extractable with organic solvents at pH values where ionization is minimal. Rabbit urine was appreciably alkaline, pH 9.2. For initial work in establishing a satisfactory extraction procedure, three samples, each a 250-mL portion, were extracted with an equal volume of either chloroform, diethyl ether, or ethyl acetate, and their efficiency was determined by the difference of radioactive contents in aliquots of urine prior to and after extraction. Also aliquots of organic extracts were counted to keep a check. These experiments indicated that chloroform extracted 32%, diethyl ether 43%, and ethyl acetate 76% of the radioactivity in urine. Second extraction with these solvents yielded only a negligible amount of the radioactivity. To find if the extraction could be improved, a 200-mL portion of urine was acidified with 10 mL of concentrated HCl to pH 1.7 and extracted with 210 mL of ethyl acetate. It resulted in a strong suspension which was broken by the addition of 15 mL of isopropyl alcohol. Estimates of radioactivity in this extract showed that this step extracted just as much radioactivity as without acidification. Besides, it favored partitioning of urinary pigments into the organic phase to an intolerably high level. Therefore, in all subsequent work urine was extracted with ethyl acetate. Occasional checks indicated the extractability of radioactivity in urine to range from 60 to 80%. The unextractable 20 to 40% of the radioactivity was not followed further.

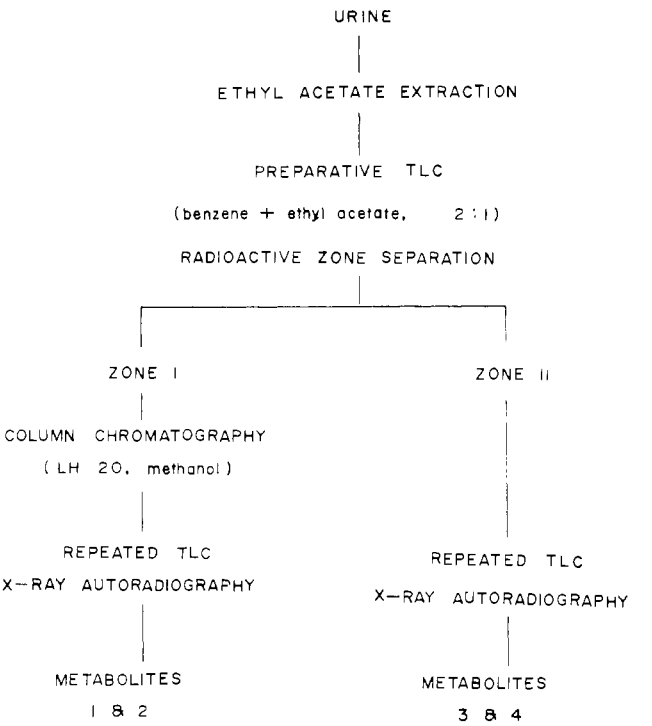

Figure 1. Flow chart of extraction and purification procedure of urinary metabolites of photoheptachlor in rabbit.

Ethyl acetate extracts were evaporated on a rotary evaporator and the concentrates processed for analysis as shown in Figure 1.

Extraction of Feces. Daily collected feces were air-dried at room temperature, weighed, and pulverized in a mortar and pestle to a powdery form. The material was then extracted with acetone (100 mL for every 10 g of feces) by a vigorous stirring with a magnetic stirrer for 1 h. Radioactivity in aliquots of the extracts was then assessed.

Derivatization. Derivatization of the metabolites was carried out with Tri-Sil "Z" (Pierce Chemical Co., Rockford, Ill.) in small reaction tubes. Petroleum ether solution of metabolites was evaporated to dryness under nitrogen, the tubes were sealed, and the derivatizing agent was introduced to restore the starting volume. The vials were allowed to stand for about 15 min at room temperature and then directly injected into gas chromatographs.

Spectroscopy. Gas-liquid–mass spectroscopy was performed on a Hewlett-Packard, Model 5982A, mass spectrometer. The column used and other operating conditions are described elsewhere (Feroz and Khan, 1978).

RESULTS AND DISCUSSION

Elimination. Urinary elimination of the administered radiocarbon is shown in Figure 2, which indicates that a single 0.8 mg/kg dose results in a steady elimination of the label. Biological half-life of photoheptachlor at this dose level appears to be around 70 days.

Acetone extracts of feces during the period of study did not show even trace amounts of radioactivity at any time, suggesting that the disposition of photoheptachlor takes place exclusively through the kidneys. This mode of

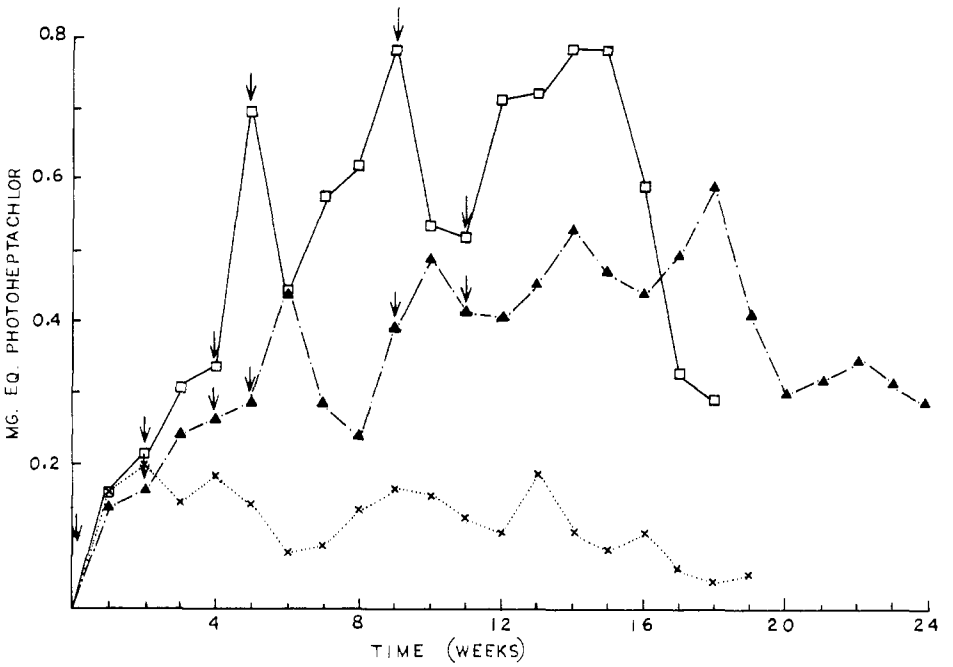

Figure 2. Urinary elimination of radiocarbon by rabbits treated with single or multiple doses of photoheptachlor. Arrows indicate doses.

excretion resembles that reported for photodieldrin in rabbit and monkey (Reddy and Khan, 1975; Nohynek et al., 1978) but differs from that of photodieldrin in rat (Dailey et al., 1970) where the major part of the label is passed through feces. It should, however, be pointed out that complete absence of radioactivity in feces of rabbits treated with [^{14}C]photoheptachlor may be due to two reasons: one that the specific activity of the compound was very low and two that acetone extracts of feces contain high content of greenish pigments causing considerable quenching which consequently could mask low-level scintillations. In such a case, trace amounts of radioactivity would escape detection simply because of the technique employed here, but this possibility was not verified. Excretion of nearly 90% of the dose in urine over 19 weeks suggests that biliary excretion of photoheptachlor or its metabolites in rabbits, if at all present, is relatively unimportant.

Two rabbits initially given the same dose as the one discussed above were, 2 weeks later, used for the multiple dose program. It may be mentioned that these rabbits passed much less urine than the rabbit treated with a single dose, in which urination was profuse throughout the period of study. Consistently the animal treated with a single dose passed three to five times more urine than the others given multiple doses. However, this abnormality in the rabbit treated with single dose did not seem to affect the metabolism of the pesticide because, in absolute terms, comparable amounts of radioactivity were eliminated irrespective of the volume of urine voided. For example, the rabbit given single dose excreted 164.4 and 201.1 μg equiv of [^{14}C]photopheptachlor, respectively, during the first and second week after treatment while the corresponding estimates for the second rabbit were 159.4 and 215.4 and those for the third being 139.7 and 158.9. Such comparisons were not possible after 2 weeks because the dosing schedule diverged after the end of week 2 onward.

The plots in Figure 2 also show average radioactivity excreted every week by the two rabbits receiving multiple doses over the period of study. Between these, one rabbit was always slightly more active than the other in eliminating the label. It will also be seen from Figure 2 that after the first 4 weeks there was a clear trend toward reaching an equilibrium with a plateau representing the period of maximum output. These rabbits remained active in eliminating the radioactivity after dispensing the last dose but started to slow down elimination toward the termination of the experiment.

Table I. Residual Radioactivity in Various Tissues of Rabbits Treated with [^{14}C]Photoheptachlor

tissue	concentration (ppm) at weeks after last dose			
	0.1[a]	7[b]	13[b]	19[c]
kidney	5.87	1.57	0.33	trace
liver	5.11	3.12	2.25	0.58
fat	112.71	10.20	6.32	0.55
lungs	2.47	2.07	trace	0.00
heart	1.00	1.24	trace	0.00
spleen	26.64	1.06	0.00	0.00
urinary bladder	1.87	0.00	trace	0.00
gall bladder	6.39	trace	0.77	0.00
stomach	4.44	0.21	0.00	0.00
duodenum	18.22	0.93	0.00	trace
testes	0.39	0.09	0.00	0.00
muscles	0.17	0.00	0.59	trace
skin	0.07	0.00	0.00	0.26
spinal cord	0.91	0.43	0.00	0.00
duodenal contents		0.00	0.00	0.00
blood		0.00	trace	0.00
bile	1.85	trace	0.00	trace
brain	0.71	0.51	0.25	0.00

[a] Single dose (25.8 mg/kg); all rabbits died within 24 h and the values represent concentration at the time of death. [b] Six doses. [c] Single dose (0.8 mg/kg).

Tissue Distribution. The pattern of distribution of radioactivity in tissues of rabbits treated with single or multiple doses of [^{14}C]photoheptachlor for different periods of time is shown in Table I. Radioactivity in visceral fat far exceeded that in any other organ which was not an unexpected observation. With 25.8 mg/kg doses, generally abdominal viscera contained more radioactivity than the thoracic organs or other organs away from abdomen and it may have been due to intraperitoneal delivery of the compound as these organs were in direct contact with the

Table II. R_f Values of Various Metabolites[a] of Photoheptachlor in Rabbit Urine Using Various Solvent Systems

compd or metab.	A[b,c]	B	C	D	E	F
photoheptachlor	0.58	0.65	0.64	0.74	0.69	0.75
4	0.38	0.44	0.56	0.62	0.68	0.76
3	0.19	0.35	0.51	0.56	0.62	0.71
2	0.00	0.00	0.10	0.05	0.47	0.68
1	0.00	0.00	0.05		0.29	0.50

[a] Values varied slightly with the type of the thin-layer plates used. Those with a binder gave lower R_f values as compared to those without it. [b] A, hexane-ethyl acetate (3:1); B, benzene-ethyl acetate (2:1); C, ethyl acetate (pure); D, chloroform-acetic acid (9:1); E, chloroform-methanol (7:3); F, propanol-ethyl acetate-water (3:2:1). [c] Three runs.

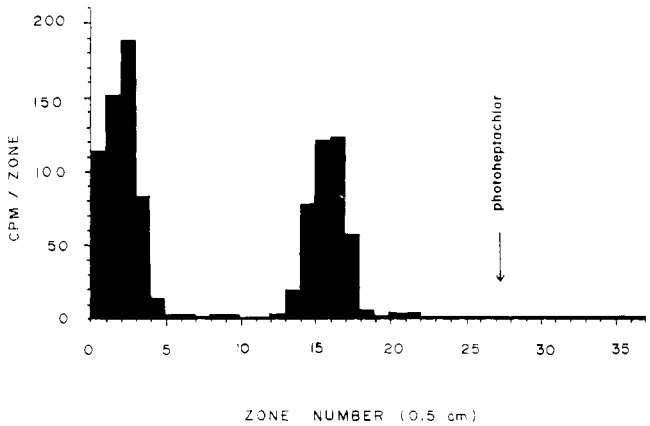

Figure 3. Thin-layer chromatographic profile of ethyl acetate extract of urine of rabbits treated with photoheptachlor (solvent system: benzene–ethyl acetate, 2:1). The arrow shows the position of photoheptachlor in this system.

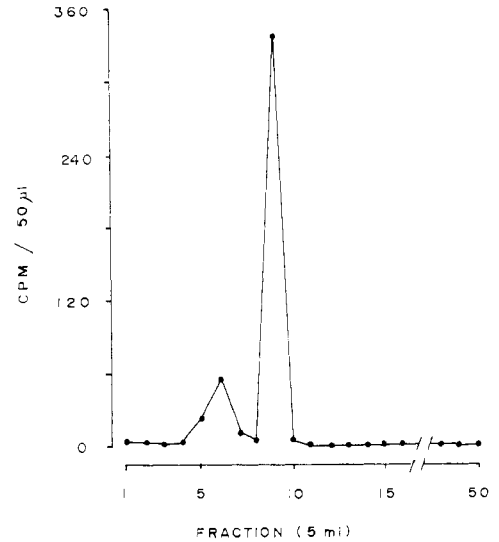

Figure 4. Separation of urinary metabolites 1 and 2 of photoheptachlor on a Sephadex LH 20 column eluted with methanol.

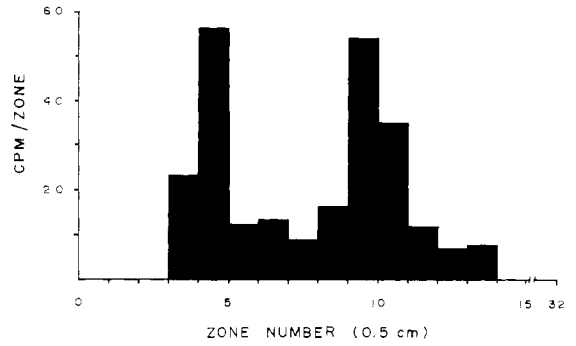

Figure 5. Profile of thin-layer chromatogram showing separation of urinary metabolites 3 and 4 in rabbits receiving photoheptachlor (solvent system: hexane–ethyl acetate, 3:1; three runs).

insecticide. From among the extra-abdominal organs, the lungs seem to accumulate relatively high amounts of radioactivity, which also appears to be the case with photodieldrin treatment of rats (Dailey et al., 1972).

The rabbit given a single dose at 0.8 mg/kg was analyzed for the residual activity in various organs at the end of 19 weeks (Table I). Most of its organs were clear of the radiocarbon. Only small but detectable amounts were present in fat and liver.

The two rabbits receiving multiple doses were sacrificed at 7 and 13 weeks after the administration of the last dose. The levels of radioactivity in tissues of the former were higher than in those of the latter.

Metabolism. The chromatographic profile of ethyl acetate extracts of urine (Figure 3) shows that all the radioactivity extractable from urine was in the form of derivatives of photoheptachlor. In this step radioactivity appeared in the form of two broad zones: one staying near the base (zone I) and the other around the middle of the chromatoplate (zone II). Radioactivity present in the two zones was almost equal during the first week but later on comparatively more could be detected in zone I. For instance, values of ratios of zone I to zone II for one rabbit during the first four consecutive weeks were 1, 1.31, 1.39, and 1.86.

After scraping, the two zones were separately processed. Zone I contained high amounts of pigments. It was eluted from a Sephadex LH-20 column with anhydrous methanol at a flow rate of about 0.1 mL/min. The eluate was collected in fifty 5-mL fractions and a 50-µL aliquot from each was tested for radioactivity. The results (Figure 4) show that this zone could be resolved into two components. One of these leaving the column in the fifth to seventh fraction was buried in urinary pigments. It was present in relatively small amounts, being almost absent in some extracts, and was designated metabolite 1. The other coming out of the column immediately after metabolite 1 in a sharp peak constituted the bulk of the radioactivity in the fraction and was almost free of extraneous materials; it was designated metabolite 2. Attempts for further purification of the two metabolites involved the use of repeated TLC in various solvent systems of varying polarities. In all these systems (Table II), the two metabolites behaved as single compounds. Metabolite 1, being in small amounts and associated with urinary pigments, has not so far been procured in pure form, but metabolite 2 was purified for characterization.

Zone II also revealed the presence of two metabolites fairly close to each other in most of the TLC systems used (Table II, Figure 5). They were, however, separable in hexane–ethyl acetate (3:1) after multiple runs. The one with the lower R_f value was designated as metabolite 3 and the other with the higher R_f value as metabolite 4. Of these, metabolite 4 was obtained in pure form.

As to the nature of metabolic products, conventional cochromatographic techniques could not be employed

Table III. GLC Behavior of Photoheptachlor and Its Urinary Metabolites

compd or metab.	retention time, min QF-1	SE-30	SE-52	OV-101
photoheptachlor	0.79	2.36	5.67	3.31
photoheptachlor-S[a]	0.79	2.36	5.67	3.31
4	1.87	4.92	11.10	5.66
4-S[a]	1.08	4.13	8.26	4.49
2-H[b]	1.87	4.92	11.10	5.66
2-HS[a]	1.08	4.13	8.26	4.49

[a] Tri-Sil "Z" treated. [b] Metabolite released by acid hydrolysis.

because of the lack of reference standards or routes of synthesis and probably the present work was the maiden attempt in characterization of photoheptachlor metabolites. By referring back to Table II, it would be observed that both metabolites 1 and 2 are extremely polar which provided a clue that they may be conjugates. Two small samples of purified metabolite 2 were, therefore, incubated with either 5 mL of 1 N HCl or 5000 units of bovine β-glucuronidase (Sigma Chemical Co.) in 5 mL of 0.2 M acetate buffer, pH 4.8, for 24 h at 37 °C. At the end of the incubation period both incubates were extracted with 10 mL of diethyl ether. About 86% of the radioactivity in the HCl incubate partitioned into the organic phase, whereas only 2% radioactivity was present in the ether extract from β-glucuronidase incubate, which could, however, be extracted with ethyl acetate. These experiments indicated that metabolite 2 is a conjugate. Exact nature of the conjugate is being investigated. (Incidentally, this set of experiments also explained poor performance of diethyl ether to extract radioactivity from urine as pointed out in the Materials and Methods section. Also, metabolites 1 and 2 could not be stripped with diethyl ether from scrapings of silica gel chromatoplates, while ethyl acetate effectively removed them. On the other hand, metabolites 3 and 4 could be eluted from gel scrapings with both solvents. A method for the separation of metabolites 1 and 2 from metabolites 3 and 4 can thus easily be evolved without resorting to initial TLC for zone separation. First extraction with diethyl ether would remove metabolites 3 and 4 from the urine and a second extraction with ethyl acetate would allow removal of metabolites 1 and 2.)

Further analysis of the ether extract of acid hydrolysate by TLC indicated that the radioactivity now occupied the same position as metabolite 4. It was, thus, inferred that metabolite 4 is a free form of the conjugate metabolite 2.

GLC retention times of metabolite 4 are shown in Table III. Derivatization of this metabolite as trimethylsilyl ether changed its GLC behavior, indicating that it had at least one hydroxl group. GLC characteristics of metabolite 2 after release by acid hydrolysis are identical with metabolite 4, confirming that metabolite 2 is a conjugate of metabolite 4. Both metabolite 2 and 4 were stable at ambient temperature for at least 4 months.

GC–MS analysis of metabolite 4 is given in Figure 6. The compound showed a parent ion at m/e 352, indicating that one of the chlorines of photoheptachlor was replaced by a hydroxyl group. Two alternate structures deducible from the fragmentation pattern of the molecule are shown in Figure 7. Of these, the one having a hydroxyl group on the hexachloronorbornane part of the molecule appears to be more probable due to the total absence of a peak at 270. Further studies are under way.

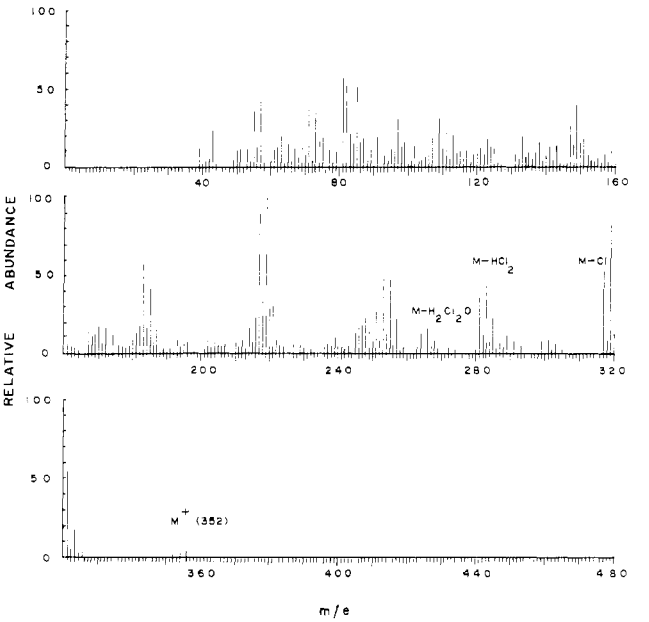

Figure 6. Mass spectrum of metabolite 4.

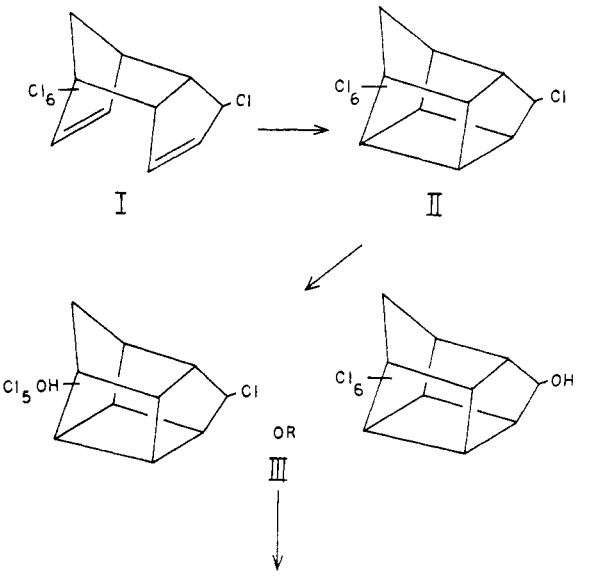

Figure 7. Pathway of formation of photoheptachlor (II) from heptachlor (I) and one of the possible routes of metabolism of photoheptachlor in rabbits. Structure III shows alternate configurations of metabolite 4.

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Comparative in Vitro Metabolism of Tetrachlorvinphos by the Soluble Fraction (105000*g*) from Sheep, Pig, and Cow Liver Homogenates

M. Humayoun Akhtar* and Thomas S. Foster

The organophosphorus insecticide tetrachlorvinphos was incubated with the soluble fraction (105000*g*) from homogenates of sheep, pig, and cow livers. Dealkylation to a water-soluble metabolite desmethyl tetrachlorvinphos by a reduced glutathione dependent enzyme system was the major route of degradation. The rate of dealkylation followed the order cow ≃ sheep > pig. The water-soluble metabolite was further metabolized very effectively by the enzyme system in the soluble fraction. The metabolites 2,4,5-trichlorophenacyl chloride, 2,4,5-trichloroacetophenone, 1-(2,4,5-trichlorophenyl)ethanol, and 2-chloro-1-(2,4,5-trichlorophenyl)ethanol were positively identified. The rate of production of these metabolites did not vary greatly between species.

The metabolism of tetrachlorvinphos has been investigated in the rat and dog (Akintonwa and Hutson, 1967) and in the dairy cow (Gutenmann et al., 1971). In vitro studies with the supernatant (1000000*g*) from mammalian livers (mouse, rat, rabbit, and pig) have been reported (Hutson et al., 1972). More recently, Akhtar and Foster (1977) reported in vitro studies with the soluble fraction (105000*g*) from chicken liver homogenates. This study examines the soluble fractions (105000*g*) from the livers of sheep, pig, and cow for species differences in the mode and rate of degradation of the insecticide and reports the relative amounts of metabolites produced.

EXPERIMENTAL SECTION

Chemicals. Pesticide and reagent grade solvents were used. ^{14}C-labeled tetrachlorvinphos (I), desmethyl tetrachlorvinphos (II), 2,4,5-trichlorophenacyl chloride (III), 2,4,5-trichloroacetophenone (IV), 1-(2,4,5-trichlorophenyl)ethanol (V), and 2-chloro-1-(2,4,5-trichlorophenyl)ethanol (VI) were prepared using a previously published procedure (Akhtar and Foster, 1977). *S*-(2,4,5-Trichlorophenacyl)glutathione was synthesized by the technique reported by Akhtar (1978).

Enzyme Preparation. Portions of livers were removed immediately after slaughter from pregnant Yorkshire sows approximately 2 years old, pregnant ewes from a new synthetic crossbred line approximately 3 years old, and cows approximately 4 years old, and were washed in distilled water and placed on crushed ice. The soluble fraction (105000*g*) was prepared in ice-cold 0.134 M phosphate buffer, pH 7.4 (8 g/40 mL), in a glass–Teflon homogenizer as described by Akhtar and Foster (1977). The precipitate (microsomes) after centrifugation at 40000 rpm (10500*g*) was washed by resuspending in buffer and centrifuging at 40000 rpm for a further 30 min. The resultant precipitate was the microsomal preparation and was resuspended in one-fourth of the original volume of phosphate buffer.

In Vitro Incubations. Incubations with the soluble fraction were carried out in a water-bath in glass-stoppered Erlenmeyer flasks (50 mL) at 37.5 °C under nitrogen. The incubation mixtures consisted of enzyme preparation (4.5 mL), reduced glutathione (0.5 mL), and [*vinyl*-^{14}C]-tetrachlorvinphos (122 μg). Reduced glutathione (GSH) was prepared just prior to addition by mixing 0.0027 g (8.7 × 10^{-6} mol) of glutathione with 0.5 N NaOH (0.05 mL) and neutralizing with 0.134 M phosphate buffer, pH 7.4 (0.45 mL). The reactions were carried out in duplicate and were terminated by addition of acetone.

Reactions with microsomal preparations were carried out in glass-stoppered Erlenmeyer flasks (25 mL) under O_2/CO_2 (95:5) at 37.5 °C. The volume of the reaction mixture was 3 mL and contained enzyme preparation (2.4 mL),

Animal Research Institute, Agriculture Canada, Research Branch, Ottawa, Ontario, K1A 0C6.

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