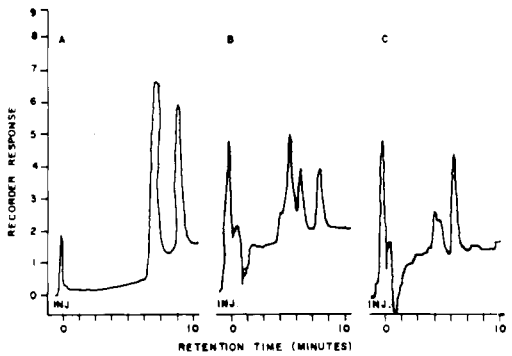

Figure 7. Representative chromatogram of standard oxadiazon and methoxy derivative (a) and hexane partition solvent from acetonitrile–hull extract of oxadiazon treated peanut plants (b) and hexane partition solvent from acetonitrile–hull extract of untreated peanut plants (c) harvested at 131 days.

of the ^{14}C residue remained, with benzene used as developing solvent for TLC. From a different sample, methanol and water (80:20, v/v) extracted 81% of the ^{14}C from peanut hulls (Figure 4).

About 20% of ^{14}C was partitioned into hexane and chromatographed as four or more metabolites, including phenol and alcohol metabolites (II and V) at concentrations approximating 0.01 to 0.04 ppmw each (Table V and Figure 4) in the hulls.

The hexane fraction from the hull extracts contained some [^{14}C]oxadiazon (Table V). The [^{14}C]oxadiazon with plant extract moved slower on TLC plates than standard [^{14}C]oxadiazon as verified by overspotting on TLC plates and GLC analysis (Figure 7). The methoxy derivative (IV) was not detected by TLC or GLC.

Peanut plants absorbed relatively low amounts of [^{14}C]oxadiazon from the soil during the 131 days of growth to maturity. Approximately 0.74% of the applied oxadiazon equivalent was located in the plants at maturity. This amount was distributed throughout the plant material with the largest concentration in or on the peanut hulls. The nuts contained the lowest concentration of oxadiazon equivalent of the plant parts.

Oxadiazon metabolism was apparently slow in the peanut hull, with approximately 0.3 ppmw remaining as oxadiazon. The major pathway of metabolism involved oxidation and dealkylation to phenol and alcohol derivatives, which accounted for less than 0.16 ppmw of the residue. Other unidentified material extracted accounted for 0.3 ppmw of the residue and 0.23 ppmw of the residue was not extracted.

LITERATURE CITED

Ambrosi, D., Desmoras, J., *Proc. Eur. Weed Res. Counc.* **4**, 163 (1973).
Ambrosi, D., Desmoras, J., *SUCRP Dis. Ph. No.* 17, 548 (1974).
Ambrosi, D., Kearney, P. C., Macchina, J. A., *J. Agric. Food Chem.* **25**, 868 (1977).
Boesch, R., Metivier, J., French Patent 1 397 774, 1965.
Burgaud, L., Deloraine, J., Desmoras, J., Guillot, M., Petrinko, P., Riottot, M., *Proc. Eur. Weed Res. Counc.* **1**, 219 (1969).
Carringer, R. D., Weber, J. B., Monaco, T. J., *J. Agric. Food Chem.* **23**, 568 (1975).
Crafts, A. S., Yamaguchi, S., *Calif. Exp. Sta. Man.*, 35 (1964).
Craine, E. M., *Res. Rep. No. EMC* (Hess & Clark, Ashland, OH) **75**, 123 (1975).
Craine, E. M., *Res. Rep. No. EMC* (Hess & Clark, Ashland, OH) **76**, 41 (1976).
Hirata, H., Ishizuka, K., *Agric. Biol. Chem.* **39**, 1447 (1975).
Ishizuka, K., Hirata, H., Fukunaga, I., *Agric. Biol. Chem.* **39**, 1431 (1975).
Ishizuka, K., Hosaka, H., Takase, I., Tan, K. E., Hirata, H., *Proc. FAO, WHO, IAEA Symp.*, 363 (1974).
Rapkin, E., Riech, A., *Am. Lab. (Fairfield, Conn.)* **4** (10), 35 (1972).

Received for review November 29, 1979. Accepted March 6, 1980. Contribution No. 366.

Metabolic Fate of *cis*-Photochlordane in the Rat. 1. Excretion, Tissue Distribution, and Preliminary Characterization of Metabolites

Muhammad Feroz and M. A. Quddus Khan*

Male rats treated orally, or intraperitoneally, with 3.12 mg of *cis*-photochlordane/rat cumulatively excreted, respectively, about 86 and 88% of the dose in 3 weeks. The half-life of the compound was less than 1 day in orally treated and about 7 days in intraperitoneally treated rats. Highest concentration of the residues at the end of the 3 weeks was found in fat. Intraperitoneally treated rats showed higher residual radioactivity in all tissues. Analyses of the organic extracts of feces and urine from the treated rats showed at least 22 compounds in the former and 15 compounds in the latter. These metabolites were isolated, purified, and chromatographically characterized.

Chlordane has been a widely used insecticide for agricultural, industrial, and household purposes. The technical product is a mixture of several compounds (Cochrane and Greenhalgh, 1976) of which *cis*-chlordane is an important constituent. *cis*-Chlordane is more persistent in the environment (Sanborn et al., 1976) and more toxic to fish than other related components of technical chlordane (Podowski et al., 1979). Exposure of *cis*-chlordane to UV irradiation or sunlight (Benson et al., 1971) results in the formation of the photoisomer, *cis*-photochlordane, which has higher acute toxicity than the parent compound to several vertebrate species (Podowski et al., 1979). Whereas the fate of photoisomers of other cyclodienes has been studied in various organisms [photodieldrin in the rat (Dailey et al., 1970, 1972; Reddy and Khan, 1974), mouse, housefly, (Reddy and Khan, 1974), rabbit (Reddy and Khan, 1975, 1977a), and rhesus monkey (Nohynek et al., 1979); photoisodrin in mouse and houseflies (Reddy and

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

0021-8561/80/1428-0740$01.00/0

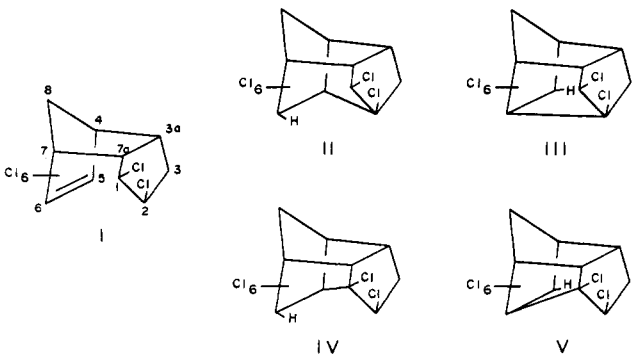

Figure 1. Structural formulae of *cis*-chlordane (I) and some of its possible photoisomers (II involves C_2–C_5 bridge; III, C_2–C_6; IV, C_1–C_5; V, C_1–C_6). Among these, II and IV have been shown to be produced.

Khan, 1977b); photoheptachlor in rabbit (Feroz and Khan, 1979b)], information on *cis*-photochlordane, a possible terminal residue of chlordane, is totally lacking. This report describes the results of excretion, storage, and biotransformations of *cis*-photochlordane in the rat.

MATERIALS AND METHODS

Chemicals. *cis*-Photochlordane (PC), ^{14}C-labeled and unlabeled, was prepared from *cis*-chlordane (1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane). The method of synthesis and the sources of the materials are described elsewhere (Podowski et al., 1979). Specific activity of [^{14}C]PC (uniformly labeled hexachloro ring) was 10.9 mCi/mmol which was adjusted to 3.91 mCi/mmol by the addition of unlabeled compound before dosing the rats.

PC arises from *cis*-chlordane (Figure 1, I) by intramolecular rearrangement which offers several possibilities. Benson et al. (1971) proposed two alternate configurations of the molecule (Figure 1, II and III) but it was not possible to decide which of the two isomeric forms was produced in their experiments. Knox et al. (1973) suggested C_2 to C_5 bridging (Figure 1, II) in the molecule. More recently, Lahaniatis et al. (1976) have indicated that another isomer (Figure 1, IV) involving cross-linking between C_1 and C_5 is also produced. Both ^{14}C-labeled and unlabeled compounds used in this study were homogeneous as tested by gas–liquid chromatography (GLC) (using four different columns) and identical with a reference standard provided by Velsicol Chemical Corp., Chicago, IL. The IR spectrum of the unlabeled compound was comparable to the one published by Onuska and Comba (1975). Despite apparent homogeneity, existence of a mixture of isomeric forms in PC cannot be ruled out.

Treatment of Rats. Five-month-old male albino rats (Charles River) were used. They were treated as follows:

(i) For information on elimination, tissue distribution, and quantitation of metabolic products, four rats were treated with [^{14}C]PC (3.12 mg in 0.75 mL of corn oil/rat). Two of the rats (body weight 368.5 and 408.7 g) received the compound orally and the other two (body weight 376.4 and 423.4 g) intraperitoneally. The dose approximated 7.91 mg/kg (75.4 μCi/kg) and was about one-fifth of the LD_{50} level for the strain (Podowski et al., 1979). The treated animals were individually held in stainless steel metabolic cages and were provided with Purina rat pellet and water ad libitum. None of the rats showed toxicity symptoms.

(ii) For collection of larger amounts of metabolites, 70 rats (7 groups of 10 rats each) were orally treated with 5 mg of unlabeled PC in 0.75 mL of corn oil/rat. The groups were held in rabbit metabolic cages.

Collection of Excreta. Feces and urine from the [^{14}C]PC treated rats were collected daily for 3 weeks when the study was concluded. The urine from each rat was made to 25 mL with distilled water, and a 0.25-mL sample was counted for estimation of renal excretion of radioactivity. The remainder from each treatment was separately pooled and frozen until analyzed. Daily collections of feces were kept separate, desiccated over calcium sulfate for 3 days at room temperature, and then extracted as detailed below. Metabolic cages were washed with methanol and water every 3 days. The washes were pooled and radioassayed at the end of the study.

Excreta from the rats treated orally with unlabeled compound was collected daily for 3 days only as studies with labeled compound had indicated that the bulk of the compound was voided during the first 3 days after treatment (see the Results section). These rats were then disposed of.

Analytical Techniques. Isolation and purification of metabolites were performed by thin-layer chromatography (TLC) and column chromatography. TLC was carried out on silica gel G plates, using the following solvents systems: (i) heptane (pure), (ii) cyclohexane–chloroform (4:1), (iii) hexane–methylene chloride (4:1), (iv) hexane–methyl ethyl ketone (4:1), (v) hexane–ethyl acetate (3:1), (vi) diethyl ether (pure), (vii) ethyl acetate (pure), (viii) benzene–ethyl acetate (1:1), (ix) chloroform–methanol (7:3), (x) propanol–ethyl acetate–water (3:2:1).

Column chromatography was performed with Sephadex LH-20 (bead size, 25–100 μm; Sigma Chemical Co., St. Louis, MO). The gel was swollen overnight in methanol–chloroform (9:1) before packing into a 2-cm i.d. column.

GLC analyses were carried out by using a Series 7300 Packard gas chromatograph (Packard Instruments, Downers Grove, IL), equipped with tritium electron-capture detectors and two 2-mm i.d. glass columns. One of the columns was 3.5 ft long and packed with 3% SE-30 on 80–100 mesh Gas-Chrom Q with a nitrogen flow rate of 40 mL/min. The other column was 5 ft long and packed with 3% QF-1 on 80–100 Chromosorb W-HP. The nitrogen flow rate through this column was 30 mL/min. Operation temperatures were as follows: inlet 220 °C, column 190 °C, and detector 220 °C. Purified metabolites were tested for the presence or absence of hydroxyl groups by treatment with Tri-Sil Z (Pierce Chemical Co., Rockford, IL) as described previously (Feroz and Khan, 1979a).

All quantitations were made by liquid scintillation counting. Techniques used in radioassay, autoradiography, and tissue sampling and processing have already been described (Feroz and Khan, 1979a,b,c).

Estimation of Residual Radioactivity in Carcass. Residual radioactivity in carcass was measured by separating skin from muscles, bones, and visceral parts. The latter were frozen and powdered with dry ice. After evaporation of dry ice, a portion (5–6 g) of the resulting paste from each carcass was dried by lyophilization. The skin was cut into small pieces (about 0.5 cm^2), and its samples were dried similarly. Proportional aliquots of the dried skin and other parts were taken, mixed, and oxidized in a Model 306 sample oxidizer (Packard Instruments), followed by liquid scintillation counting.

Extraction and Isolation of Metabolites. (*i*) *Fecal.* Dried feces (daily, separately) from [^{14}C]PC treated rats were pulverized to a powder and extracted three times with acetone (40 + 20 + 20 mL) and four times with methanol (50 + 25 + 25 + 25 mL). Radioactivity in the acetone and methanol extracts was measured. The two extracts were

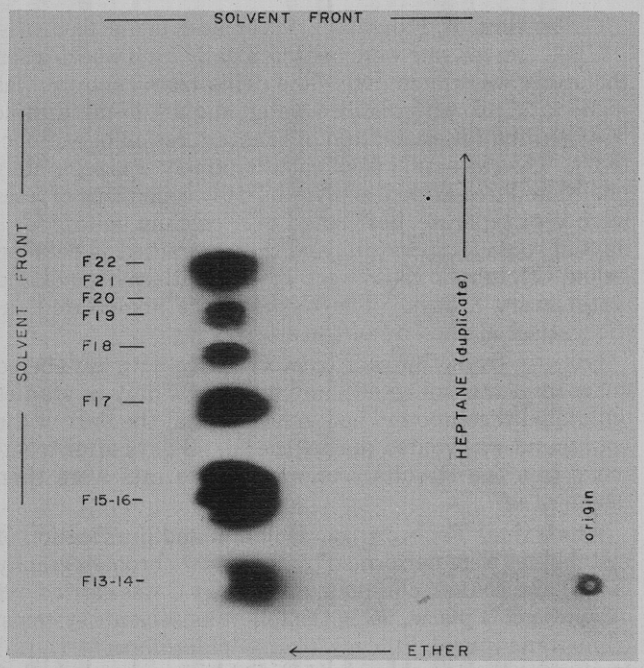

Figure 2. An autoradiogram showing separation of the nonpolar fecal metabolites of *cis*-[^{14}C]photochlordane in rats. Silica gel G-F254 plate was two-dimensionally developed with pure diethyl ether and pure heptane (duplicate). Metabolite designations indicated on left side of the spots.

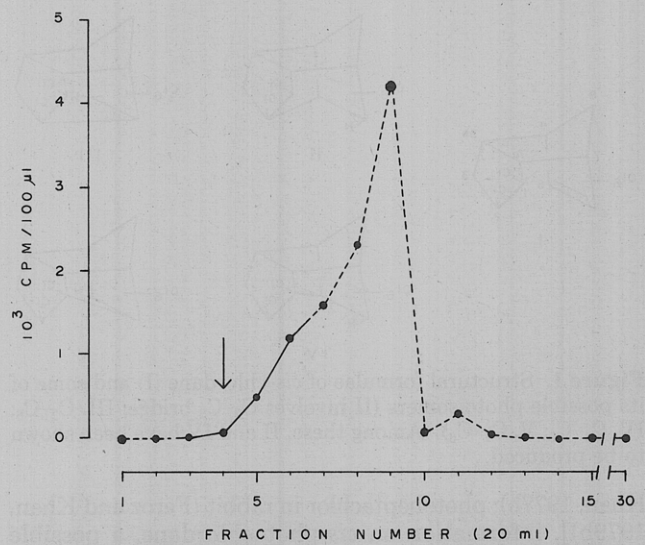

Figure 3. Purification of polar fecal metabolites of *cis*-[^{14}C]-photochlordane from rats, on Sephadex LH-20 column developed with methanol–chloroform (9:1). Solid line from fractions 3–6 shows elution of fecal pigments with maximum amount in fraction 4 (arrow). The elution pattern of radioactivity indicates multiplicity of metabolic products.

then pooled. The extracted feces were lumped and unextractables determined by oxidizing small samples (200–300 mg) as outlined for carcass.

Pooled organic extracts were dried over anhydrous sodium sulfate and flash evaporated to near dryness. The residue was dissolved in acetonitrile which was partitioned three times against hexane (volume ratios 1.6:1, respectively; with greater than 90% recoveries in acetonitrile phase). The acetonitrile phase was concentrated, chromatographed on 2-mm silica gel G Uniplates (Analtech, Newark, DE), developed (duplicate) with heptane (solvent system i), and autoradiographed for location of the metabolites. In this system, nonpolar compounds were free from the bulk of the biological debris. The remaining impurities present with these metabolites were removed by repeated TLC (i.e., chromatography, scraping, elution, and rechromatography in the same or different solvent system) on 0.25-mm silica gel G-F254 plates (Brinkmann Instruments, Des Plaines, IL) with nonpolar solvents more effectively using solvent systems i–iii. A representative autoradiogram is shown in Figure 2.

The polar metabolites were associated with fecal pigments. They were chromatographed (in portions) on a 2 × 30 cm Sephadex LH-20 column developed with methanol–chloroform (9:1) mixture at a slow flow rate (0.2–0.3 mL/min), and eluate was collected in 20-mL fractions until the recoveries were quantitative. A major portion of the pigments was removed in the step (Figure 3). The fractions containing radioactivity were pooled, concentrated, and cleaned by repeated TLC with solvent systems iv–x, on 2-mm preparatory silica gel plates. Pure diethyl ether and ethyl acetate (solvent systems vi and vii) were particularly useful for the removal of remaining fecal pigments although resolution of metabolic products from one another was poor in these solvent systems. Final purification of these metabolites also was carried out on 0.25-mm silica gel G-F254 plates.

Metabolic products in feces of the rats treated with unlabeled PC were isolated and purified by a procedure similar to that detailed above except for two modifications: (a) nonpolar metabolites were visualized by spraying one edge of the developed chromatoplates with acetonic silver nitrate, followed by exposure to UV light and (b) polar components were spiked with corresponding ^{14}C compounds to follow them in the cleanup process because other methods were not practical due to excessive amounts of interfering pigments.

(*ii*) *Urinary*. To determine a suitable procedure for extraction of urinary metabolites, urine from the [^{14}C]PC treated rats was used. Duplicate 20-mL samples of urine (pH 9.1) were extracted two times with an equal volume of ethyl acetate as follows: (a) urine without any additive, (b) urine saturated with sodium chloride, and (c) urine acidified to pH 2 with hydrochloric acid. This procedure yielded 37.2–39.08%, 53.4–55.1%, and 63.3–67.0% of the radioactivity, respectively, under the three sets of conditions both in orally and intraperitoneally treated rats. Since extraction was maximal under acidic conditions, this procedure was employed in all subsequent extractions of the urine. Unextractable metabolites were not followed further. The cleanup and isolation of metabolites were similar to those outlined for fecal metabolites, i.e., hexane–acetonitrile partitioning, column chromatography on Sephadex LH20, repeated TLC, and location of metabolites by autoradiography ([^{14}C]PC) and silver nitrate spraying (unlabeled PC). Figure 4 is a representative TLC separation of urinary metabolites.

RESULTS AND DISCUSSION

Excretion. The results of disposition of the [^{14}C]PC over the period of 3 weeks of study are shown in Figure 5. Total elimination was about 86 and 88% in the orally and intraperitoneally treated rats, respectively. Overall recoveries accounted for about 87% of the administered radioactivity in orally treated and 94% in intraperitoneally treated rats. The balance was most likely lost in handling the excreta. The bulk of the radioactivity in orally treated rats was voided during the first day after treatment which was probably due to unabsorbed compound (Figure 5). In intraperitoneally treated animals the rate of elimination was slower. The biological half-life of the compound, under these conditions, lies around 1 and 7 days, respectively, with oral and intraperitoneal treatments. The fecal route

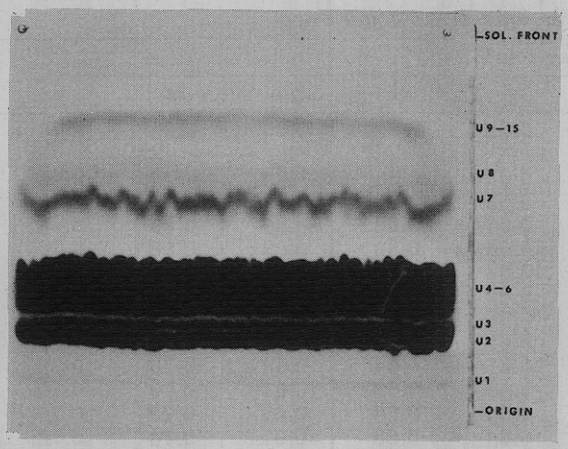

Figure 4. An autoradiogram of metabolic products in urinary extract of *cis*-[^{14}C]photochlordane treated rats. Metabolic designations shown on the right side. The plate (silica gel G-F254) was developed with chloroform–methanol (7:3).

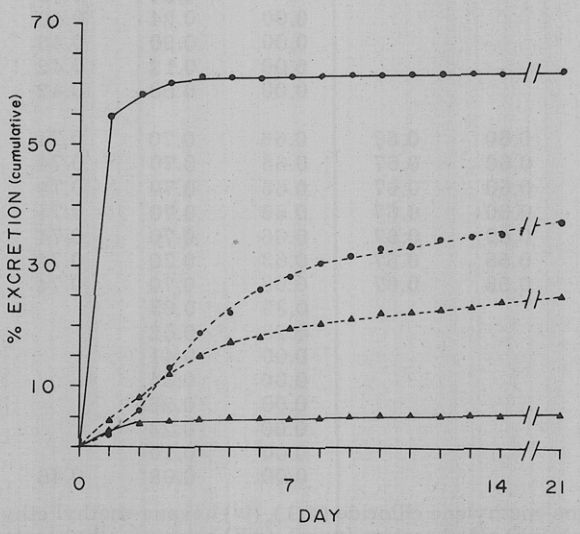

Figure 5. Excretion (percent of dose) of radiocarbon by rats administered *cis*-[^{14}C]photochlordane (3.12 mg/rat). The points shown are averages from two rats treated orally (solid lines) or intraperitoneally (broken lines). The values for feces (circles) indicate the amounts extractable with organic solvents and those for urine (triangles) give total amounts. At the end of the study, fecal unextractables amounted to 17.63 and 22.10%, cage washes 0.69 and 3.21%, and residue in carcass 0.75 and 5.65% of the dose, respectively, in orally and intraperitoneally treated rats.

of excretion was more important than the urinary route with both treatments. The difference was more pronounced in orally treated than in intraperitoneally treated rats, which, as already pointed out, could be due to the elimination of the unabsorbed compound.

Tissue Residues. Residual radioactivity in various tissues, 3 weeks after treatment of rats given a single oral or intraperitoneal dose of [^{14}C]PC, is shown in Table I. Like other chlorinated insecticides, concentration of residues was highest in adipose tissue. Muscle, brain, stomach, and heart possessed very low levels. Other tissues had varying amounts of radiocarbon, which included liver, kidneys, duodenum, and duodenal contents; the organs concerned with metabolism/elimination of the compound. Intraperitoneally treated rats showed higher residue of radioactivity than orally treated rats because they apparently retained more of the dosage by avoiding gut excretion.

Table I. Residue Levels of Radioactivity in Various Tissues of Rats Treated with *cis*-[^{14}C]Photochlordane[a]

	oral[b]		intraperitoneal[b]	
tissue	wt, g	concn,[c] ng/g or mL	wt, g	concn,[c] ng/g or mL
fat (visceral)		758.4		3743.4
liver	13.375	80.7	16.409	313.8
kidneys	2.592	213.1	3.278	716.5
muscles		7.8		24.1
skin		50.2		106.8
brain	1.985	5.2	2.057	18.1
spinal cord		Tr		7.9
testes	2.955	6.0	3.044	26.0
urinary bladder	0.134	120.7	0.099	422.0
adrenals	0.073	171.0	0.059	625.6
duodenum	2.489	74.7	1.917	115.2
duodenal contents		80.4		693.7
stomach	2.005	24.8	2.192	45.8
stomach contents				0.0
spleen	0.780	109.8	0.559	449.4
heart	1.030	13.3	1.155	61.9
lungs	1.519	103.7	2.176	169.4
blood		44.3		

[a] Three weeks after a single dose (3.12 mg/rat). [b] Average of two animals. [c] Equivalents of *cis*-photochlordane

Table II. Relative Amounts of Various Metabolites in Organic Extracts of Feces and Urine of Rats Treated with *cis*-Photochlordane

	% of recov radioactivity[a]	
metabolite	oral	intraperitoneal
feces		
F 21-22	17.29	0.47
F 19-20	2.19	0.16
F 18	3.59	0.31
F 17	52.28	1.56
F 15-16	8.33	5.60
F 13-14	2.56	1.71
F 12	4.86	29.76
F 7-11	6.53	48.19
F 1-6	2.33	12.20
urine		
U 9-15	8.63	3.43
U 8	15.61	6.19
U 7	14.86	6.40
U 4-6	27.62	48.04
U 3	7.30	14.87
U 2	24.13	20.46
U 1	1.81	0.57

[a] For extractable amounts from feces, see Figure 5; for those from urine, see text.

Metabolism. Table II presents relative amounts of biotransformation products of [^{14}C]PC in rats. It will be seen that the overall metabolic picture was complex in that a variety of conversion products of the compound was present in urinary and fecal extracts. [In studies like the present, a compound can be affected both by endogenous (biotransformations, intestinal microorganisms, enterohepatic cycle) and exogenous (storage, extraction procedure) factors. Whereas the former are parts of the overall metabolic activity of a biological system, the latter can produce artifacts from a xenobiotic. To assess the stability of PC under the storage conditions and extraction procedure followed in this work, known amounts of [^{14}C]PC in acetone (50 μg in 200 μL of acetone) were deposited on

Table III. R_f Values of Metabolites of *cis*-Photochlordane Excreted by Rats in Feces and Urine

	solvent system[a]									
compound	i[b]	ii	iii	iv	v	vi	vii	viii	ix	x
PC[c]	0.33	0.32	0.27	0.43	0.55	0.61	0.65	0.69	0.69	0.72
feces										
F22	0.59	0.42	0.39	0.55	0.57	0.63	0.68	0.69	0.72	0.75
F21	0.56	0.41	0.39	0.55	0.57	0.63	0.68	0.69	0.70	0.75
F20	0.51	0.38	0.37	0.55	0.57	0.63	0.68	0.69	0.70	0.75
F19	0.49	0.38	0.35	0.55	0.57	0.63	0.68	0.69	0.70	0.75
F18	0.42	0.36	0.32	0.55	0.57	0.63	0.68	0.69	0.70	0.75
F17	0.31	0.34	0.27	0.45	0.54	0.62	0.68	0.69	0.70	0.75
F16[d]	0.21	0.25	0.19	0.45	0.51	0.61	0.68	0.65	0.70	0.75
F15[d]	0.20	0.25	0.19	0.40	0.49	0.61	0.68	0.66	0.70	0.75
F14	0.02	0.00	0.00	0.37	0.44	0.61	0.68	0.64	0.70	0.75
F13	0.02	0.00	0.00	0.33	0.38	0.59	0.68	0.64	0.70	0.75
F12	0.00	0.00	0.00	0.24	0.29	0.54	0.68	0.61	0.70	0.75
F11[e]	0.00	0.00	0.00	0.20	0.23	0.54	0.68	0.61	0.70	0.75
F10	0.00	0.00	0.00	0.18	0.23	0.54	0.68	0.61	0.70	0.75
F9	0.00	0.00	0.00	0.18	0.21	0.50	0.68	0.60	0.70	0.74
F8[f]	0.00	0.00	0.00	0.11	0.16	0.50		0.52	0.60	0.74
F7	0.00	0.00	0.00	0.06	0.10	0.40		0.46	0.54	0.74
F6	0.00	0.00	0.00	0.00					0.40	0.59
F5	0.00	0.00	0.00	0.00					0.33	0.59
F4	0.00	0.00	0.00	0.00				0.00	0.24	0.49
F3	0.00	0.00	0.00	0.00				0.00	0.20	0.43
F2	0.00	0.00	0.00	0.00				0.00	0.12	0.42
F1	0.00	0.00	0.00	0.00				0.00	0.08	0.42
urine										
U15	0.61	0.43	0.36	0.55	0.58	0.60	0.67	0.66	0.70	0.74
U14	0.59	0.43	0.36	0.53	0.53	0.60	0.67	0.66	0.70	0.74
U13	0.50	0.40	0.32	0.53	0.53	0.60	0.67	0.66	0.70	0.74
U12	0.44	0.39	0.32	0.53	0.53	0.60	0.67	0.66	0.70	0.74
U11	0.33	0.33	0.25	0.45	0.53	0.60	0.67	0.66	0.70	0.74
U10	0.22	0.27	0.20	0.43	0.49	0.58	0.57	0.63	0.70	0.74
U9	0.17	0.27	0.20	0.41	0.49	0.58	0.67	0.63	0.70	0.74
U8	0.00	0.00	0.00		0.10			0.39	0.63	
U7	0.00	0.00	0.00		0.00			0.28	0.52	
U6	0.00	0.00	0.00	0.00	0.00			0.00	0.41	
U5	0.00	0.00	0.00	0.00	0.00			0.00	0.34	
U4	0.00	0.00	0.00	0.00	0.00			0.00	0.26	
U3	0.00	0.00	0.00	0.00	0.00			0.00	0.23	
U2	0.00	0.00	0.00	0.00	0.00			0.00	0.20	
U1	0.00	0.00	0.00	0.00	0.00			0.00	0.08	0.46

[a] (i) Heptane (pure), (ii) cyclohexane-chloroform (4:1), (iii) hexane-methylene chloride (4:1), (iv) hexane-methyl ethyl ketone (4:1), (v) hexane-ethyl acetate (3:1), (vi) diethyl ether (pure), (vii) ethyl acetate (pure), (viii) benzene-ethyl acetate (1:1), (ix) chloroform-methanol (7:3), (x) propanol-ethyl acetate-water (3:2:1); silica gel G-F254 plates. [b] Duplicate development, 16 cm each. [c] Reference standard, *cis*-photochlordane. [d] Compounds labile (see text). [e] Single spot in all systems, but GLC analysis (Table IV) revealed two components. [f] Complex of at least four components, values given for the major compound.

freshly defecated (24-h collection from one rat) rat fecal pellets. The feces were then subjected to simulated conditions and analyzed. Average recovery of radioactivity from two such replicates was 94.6% and no product other than [^{14}C]PC was detected (TLC and autoradiography). However, similar tests on the stability of other compounds described in the study were not possible due to the lack of reference standards.] The number of metabolic products voided through the feces was greater than that via urine. Comparing the routes of administration of PC, the urinary metabolic pattern was almost similar qualitatively as well as quantitatively in orally and intraperitoneally treated animals. In the fecal metabolic profile, similarity between orally and intraperitoneally treated rats was evident in qualitative but not in quantitative terms. The fecal metabolites F15-22 were more abundant (all nonpolar, Tables III and IV) in orally treated than in intraperitoneally treated rats and the reverse was found in other fecal metabolites.

Fecal Metabolites. TLC characteristics of the major fecal metabolic products (F) of PC in rats are listed in Table III. The metabolite F17 was unchanged PC but the structure of other derivatives is not known except some indication of their general nature, as seen from their GLC behavior (Table IV). The products F18-22 (possibly F16 and F15 also; see below) showed the same retention times both prior to and after treatment with trimethylsilyl derivatizing reagent, suggesting that they lacked hydroxyl groups. However, all of them exhibited shorter retention times than the parent compound, PC. Among other factors affecting retention times in GLC analyses, one is chlorine content of the molecule. The molecules with higher chlorine content tend to have longer retention times; a criterion widely applied in classification of polychlorinated biphenyls by GLC. On the assumption that this is the case, it appears that these compounds may be various products of reductive dechlorination of the parent molecule. It is supported by the observation that they were more abundant in the orally treated than in the intraperitoneally treated rats (Table II). Possibly, they arise by the action of anaerobic intestinal microflora capable of carrying out reductive processes more efficiently. Furthermore, exposure of pure [^{14}C]PC in acetone to laboratory light at ambient temperature for 8 weeks produced compounds with TLC and GLC characteristics identical with the metabolic products under discussion, and such photolytic dechlorinations of chlorinated hydrocarbons are not uncommon (Onuska and Comba, 1975; Parlar and Korte, 1977).

Table IV. Gas-Liquid Chromatographic Characteristics of *cis*-Photochlordane and Its Major Metabolic Products Extractable from Feces and Urine of Treated Rats

	retention time, min[a]			
	SE-30		QF-1	
compound	U[b]	D[b]	U[b]	D[b]
PC[c]	6.30	6.30	1.97	1.97
feces				
F22	1.95	1.95	0.59	0.59
F21	2.13	2.13	0.69	0.69
F20	2.11	2.11	0.73	0.73
F19	2.22	2.22	0.83	0.83
F18	3.54	3.54	1.18	1.18
F17	6.30	6.30	1.97	1.97
F16[d]	5.02	2.09	2.05	0.69
F15[d]	5.07	2.28	2.10	0.79
F14	2.76	3.41	1.28	0.85
F13	2.87	3.66	1.14	0.98
F12	7.38		3.39	
F11[e]	3.74 (4.33)	3.81 (4.25)	1.44 (1.81)	1.02 (1.14)
F10	4.49	7.97	1.87	2.60
F9	4.13	5.57	2.16	1.97
F8[f]	6.50	5.51	2.26	0.79
F7	4.45	6.02	2.95	3.11
F6	ND[g]		ND[g]	
F4	ND[g]		ND[g]	
urine				
U15	1.95	1.95	0.59	0.59
U14	2.13	2.13	0.69	0.69
U13	2.22	2.22	0.83	0.83
U12	3.54	3.54	1.18	1.18
U11	6.30	6.30	1.97	1.97
U10	5.02	2.09	2.05	0.69
U9	5.07	2.28	2.10	0.79
U8	3.15		1.87	
U7	ND[g]		ND[g]	
U4	ND[g]		ND[g]	
U3	ND[g]		ND[g]	

[a] Conditions stated in text. [b] U = without Tri-Sil Z treatment, D = Tri-Sil Z treated. [c] Reference standard, *cis*-photochlordane. [d] Compounds labile (see text). [e] Complex of two compounds, values in parentheses for the other component. [f] At least four components. Values for the major compound. [g] Not detectable.

Behavior of the conversion products F15 and F16 was puzzling. The two PC derivatives were mobile in nonpolar solvent systems (Table III, solvent systems i, ii, and iii), a character suggesting lack of hydroxyl groups in the molecules. However, in GLC analyses, retention times of the untreated and Tri-Sil Z treated compounds were different (Table IV), indicating their hydroxylated nature. Besides, both the compounds were labile at room temperature (about 10% degradation in 10 weeks) and produced molecular species having high R_f values (0.5–0.6) in TLC solvent system i (Table III). These degradation products exhibited the same retention times as those by Tri-Sil Z treated compounds, F15 and F16. It, therefore, appears that the retention times of the metabolites F15 and F16 after treatment with Tri-Sil Z are not real but due to the products arising from their degradation catalyzed by Tri-Sil Z by an unknown mechanism.

The products F7-14 appeared to be hydroxylated derivatives of PC (Table IV) as they behaved differently in GLC analyses, as a result of their conversion to trimethylsilyl ethers. Extremely low mobility of the metabolites F1-6 on TLC chromatoplates is indicative of their being conjugates or polyhydroxylated derivatives of PC.

It is noteworthy that some of the metabolites occurred in pairs of compounds exhibiting very close chromatographic characteristics. Pairs of the products F22 and F21, F20 and F19, F16 and F15, and F14 and F13 had not only close R_f values in all TLC solvent systems (Table III) but also nearly similar retention times on both the columns (Table IV) with and without Tri-Sil Z treatment. (F11, though behaved as a single compound in TLC systems, showed two overlapping components in GLC analyses.) Moreover, ratios of the compounds in each pair were almost constant (one member of the pair was three–five times more abundant than the other). Probably, it indicates the presence of two PC isomers undergoing parallel metabolism and giving rise to the complex metabolic pattern of the compound.

Urinary Metabolites. From among the compounds detected in urine extracts (U), U11 was unchanged PC (Tables III and IV) and corresponded to the fecal compound, F17. The products U15, U14, U13, U12, U10, and U9 were identical with F22, F21, F19, F18, F16, and F15, respectively. These biotransformations and intact PC (all nonpolar or relatively nonpolar) comprised only 8.6% of the extractable radioactivity in orally treated and 3.4% in intraperitoneally treated rats (Table II). All other urinary metabolites, U1 to U8, were polar in nature and appeared to be conjugates/polyhydroxy compounds.

ACKNOWLEDGMENT

Technical assistance by Diem-phuong Ngo and Tina Tan is appreciated.

LITERATURE CITED

Benson, W. R.; Lombardo, P.; Egry, I. J.; Ross, R. D., Jr.; Barron, R. P.; Mastbrook, D. W.; Hanson, E. A. *J. Agric. Food Chem.* **1971**, *19*, 857.

Cochrane, W. P.; Greenhalgh, R. *J. Assoc. Off. Anal. Chem.* **1976**, *59*, 696.

Dailey, R. E.; Klein, A. K.; Brouwer, E.; Link, J. D.; Braunberg, R. C. *J. Agric. Food Chem.* **1972**, *20*, 371.

Dailey, R. E.; Walton, M. S.; Beck, V.; Leavens, C. L.; Klein, A. K. *J. Agric. Food Chem.* **1970**, *18*, 443.

Feroz, M.; Khan, M. A. Q. *J. Agric. Food Chem.* **1979a**, *27*, 95.

Feroz, M.; Khan, M. A. Q. *J. Agric. Food Chem.* **1979b**, *27*, 108.

Feroz, M.; Khan, M. A. Q. *Arch. Environ. Contam. Toxicol.* **1979c**, *8*, 519.

Knox, J. R.; Khalifa, S.; Ivie, G. W.; Casida, J. E. *Tetrahedron* **1973**, *29*, 3869.

Lahaniatis, E. S.; Parlar, H.; Gab, S.; Korte, F. *Synthesis* **1976**, 1, 47.

Nohynek, G. J.; Mueller, W. F.; Coulston, F.; Korte, F. *Ecotoxicol. Environ. Saf.* **1979**, *3*, 1.

Onuska, F. I.; Comba, M. E. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 6.

Parlar, H.; Korte, F. *Chemosphere* **1977**, *6*, 665.

Podowski, A. A.; Banerjee, B. C.; Feroz, M.; Dudek, M. A.; Willey, R. L.; Khan, M. A. Q. *Arch. Environ. Contam. Toxicol.* **1979**, *8*, 509.

Reddy, G.; Khan, M. A. Q. *J. Agric. Food. Chem.* **1974**, *22*, 910.

Reddy, G.; Khan, M. A. Q. *J. Agric. Food Chem.* **1975**, *23*, 861.

Reddy, G.; Khan, M. A. Q. *J. Agric. Food Chem.* **1977a**, *25*, 25.

Reddy, G.; Khan, M. A. Q. *Gen. Pharmac.* **1977b**, *8*, 285.

Sanborn, J. R.; Metcalf, R. L.; Bruce, W. N.; Lu, P. Y. *Environ. Entomol.* **1976**, *5*, 533.

Received for review April 16, 1979. Accepted February 6, 1980. This work was supported by USPHS Grant ES-01479 from National Institute of Environmental Health Sciences.