possibility for the formation of metabolites in which both the aromatic and the aliphatic parts of the molecule have been oxidized, e.g., to phenolic *p,p'*-DDA metabolites.

A closer investigation on the metabolism of other para,para'-disubstituted DDT compounds such as *p,p'*-DDT and *p,p'*-DDD will probably give conclusive evidence for the formation of phenolic metabolites also from these compounds as suggested previously (see references above).

It would be of interest to determine whether the above metabolic process or the metabolites are responsible for any biological effects caused by *p,p'*-DDE. In the case of *p,p'*-DDT and *o,p'*-DDT it has been questioned if phenolic metabolites are the ultimate cause of the estrogenic activity of these compounds (Welch et al., 1969; Feil et al., 1973). Likewise, it was proposed that a metabolite of *o,p'*-DDD containing a hydroxy group in a 4 position of the phenyl rings may be responsible for the adrenolytic activity of this compound (Reif et al., 1974). Investigations on the biological activity of the major metabolite II of *p,p'*-DDE are under way in this laboratory.

ACKNOWLEDGMENT

Part of this work was performed at the Environmental Toxicology Unit, Wallenberglaboratoriet, Stockholm, and the author is indebted to Bo Jansson and Ann-Cathrine Ambjörs for valuable technical assistance.

LITERATURE CITED

Agosin, M., Morello, A., Scaramelli, N., *J. Econ. Entomol.* **57**, 974 (1964).
Agosin, M., Scaramelli, N., Gil, L., Leteler, M. E., *Comp. Biochem. Physiol.* **29**, 785 (1969).
Aue, W. A., Hastings, C. R., Kapila, S., *J. Chromatogr.* **77**, 299 (1973).
Buu-Hoi, N. P., Xuong, N. D., Lavit, D., *J. Chem. Soc.*, 1034 (1954).
Daly, J. W., Jerina, D. M., Witkop, B., *Experientia* **28**, 1129 (1972).
Dinamarca, M. L., Agosin, M., Neghme, A., *Exp. Parasitol.* **12**, 61 (1962).
Feil, V. J., Lamoureux, C. H., Styrvoky, E., Zaylskie, R. G., Thacker, E. J., Holman, G. M., *J. Agric. Food Chem.* **21**, 1072 (1973).
Feil, V. J., Lamoureux, C. H., Zaylskie, R. G., *J. Agric. Food Chem.* **23**, 382 (1975).
Fishbein, L., *J. Chromatogr.* **98**, 177 (1974).
Goto, M., Hattori, M., Sugiura, K., *Chemosphere*, 177 (1975).
Harris, E. E., Frankforter, G. B., *J. Am. Chem. Soc.* **48**, 3144 (1926).
Hodgson, H. H., Beard, H. G., *J. Chem. Soc.*, 147 (1926).
Hodgson, H. H., Jenkinson, T. A., *J. Chem. Soc.*, 1740 (1927).
Hutzinger, O., Jamieson, W. D., Safe, S., Paulmann, L., Ammon, R., *Nature (London)* **252**, 698 (1974).
Jansson, B., Jensen, S., Olsson, M., Renberg, L., Sundström, G., Vaz, R., *Ambio* **4**, 93 (1975).
Jensen, S., Sundström, G., *Ambio* **3**, 70 (1974a).
Jensen, S., Sundström, G., *Nature (London)* **251**, 219 (1974b).
Jensen, J. A., Cueto, C., Dale, W. E., Rothe, C. E., Pearce, G. W., Mattson, A. M., *J. Agric. Food Chem.* **5**, 919 (1957).
Kohli, J., Jones, D., Safe, S., *Can. J. Biochem.* **54**, 203 (1976).
McOmie, J. F. W., Watts, M. L., *Chem. Ind. (London)*, 1658 (1963).
Montagne, P. J., *Recl. Trav. Chim. Pays-Bas* **39**, 339 (1920).
Morello, A., *Can. J. Biochem.* **43**, 1289 (1965).
Oppenoorth, F. J., Houx, N. W. H., *Entomol. Exp. Appl.* **11**, 81 (1968).
Reif, V. D., Sinsheimer, J. E., *Drug Metab. Dispos.* **3**, 15 (1975).
Reif, V. D., Sinsheimer, J. E., Ward, J. C., Schteingart, D. E., *J. Pharm. Sci.* **63**, 1730 (1974).
Ruzo, L., Jones, D., Safe, S., Hutzinger, O., *J. Agric. Food Chem.* **24**, 581 (1976a).
Ruzo, L. O., Safe, S., Hutzinger, O., *J. Agric. Food Chem.* **24**, 291 (1976b).
Safe, S., Hutzinger, O., Jones, D., *J. Agric. Food Chem.* **23**, 851 (1975).
Safe, S., Jones, D., Hutzinger, O., *J. Chem. Soc., Perkin Trans. 1*, 357 (1976).
Sanchez, E., *Can. J. Biochem.* **45**, 1809 (1967).
Schall, C., Dralle, C., *Chem. Ber.* **17**, 2528 (1884).
Schuntner, C. A., Schnitzerling, H. J., *J. Chromatogr.* **21**, 483 (1966).
Seyferth, D., Heeren, J. K., Singh, G., Grim, S. O., Hughes, W. B., *J. Organomet. Chem.* **5**, 267 (1960).
Sundström, G., Hutzinger, O., Safe, S., Ruzo, L., Jones, D., in "Sublethal Effects of Toxic Chemicals on Aquatic Animals", Koeman, J. H., Strik, J. J. T. W. A., Ed., Elsevier, Amsterdam, 1975a, p 177.
Sundström, G., Jansson, B., Jensen, S., *Nature (London)* **255**, 627 (1975b).
Welch, R. M., Levin, W., Conney, A. H., *Toxicol. Appl. Pharmacol.* **14**, 358 (1969).

Received for review January 20, 1976. Accepted August 19, 1976. A travel grant from Wallenbergsstiftelsens Jubileumsfond is gratefully acknowledged.

Metabolism of Hexachlorophene in the Rabbit. Excretion, Tissue Distribution, and Characterization of the Urinary Glucuronide Conjugate

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Rabbits were given intraperitoneal doses of 10 or 15 mg/kg [^{14}C]hexachlorophene. About 48–52% of the administered radioactivity was excreted in the feces and 21–25% in the urine over a 3.5–4-day period. Unchanged hexachlorophene (HCP) was a major constituent of feces (78%) and urine (29%) of rabbits receiving a 15 mg/kg dose of [^{14}C]HCP. The major urinary metabolite (56%), HCP glucuronide, was isolated and characterized.

Hexachlorophene (HCP) was used extensively in many commercial products as a germicide until the recent finding of central nervous system disorder (Kimbrough, 1974), optic nerve atrophy (Udall and Malone, 1970), hyperthermia (Nakaue et al., 1973), and teratogenicity (Kimmel et al., 1974). Early investigations concerning the metabolism of HCP suggested that orally administered HCP was slowly and incompletely absorbed (Wit and Van Genderen, 1962). Subsequent studies showed, however, that an oral dose of HCP was rapidly and almost completely absorbed (Buhler et al., 1977), and that the bis-

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phenol was metabolized in a number of species with the formation of a glucuronide conjugate (St. John, Jr., and Lisk, 1972; Black et al., 1974; Gandolfi and Buhler, 1974; Buhler et al., 1977). This conjugate, however, has not previously been isolated and identified in urine or feces.

In the present study we have investigated the distribution, excretion, and metabolism of HCP in the rabbit and have isolated and characterized the major urinary conjugate metabolite. A preliminary report of these studies has appeared earlier (Gandolfi et al., 1972).

MATERIALS AND METHODS

Hexachlorophene [HCP; 2,2′-methylenebis(3,4,6-trichlorophenol)] was recrystallized from isopropyl alcohol–water (mp 165–165.5 °C). Chromatographically pure [*methylene*-^{14}C]hexachlorophene (1.13–3.42 mCi/mmol) was obtained from New England Nuclear Co. (Boston, Mass.) and diluted with recrystallized HCP for these studies.

Hexachlorophene mono(methyl acetoglucuronate) [2-(methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate)-2′-hydroxy-3,3′,5,5′,6,6′-hexachlorodiphenylmethane; $C_{26}H_{22}O_{11}Cl_6$; mol wt 723.18] was synthesized as a reference standard. Methyl acetobromoglucuronide was first prepared by the procedures of Bollenback et al. (1955). Approximately 1.5 g of this material was added to an equal amount of HCP in benzene in the presence of 1 g of silver carbonate (Wotiz et al., 1959). After standing for 48 h at 25 °C, the silver salts were removed by filtration, and the filtrate was taken to dryness in vacuo. The resultant material was dissolved in 50% ethanol and allowed to precipitate in the refrigerator over a several day period. Recrystallization from ethanol yielded 3.7% (theoretical) of pale yellow clusters of fine needles which first melted at 151 °C but after resolidification melted at 168–169 °C.

Extraction and Fractionation of Urinary and Fecal Radioactivity. Male New Zealand white rabbits (2.5–3.5 kg) received intraperitoneal doses of 10 mg/kg (sp act. 60.6 μCi/mmol) or 15 mg/kg (sp act. 248 μCi/mmol) [^{14}C]HCP dissolved in 2 ml of corn oil. Urine and feces were periodically collected and frozen for future extraction after preparation of suitable counting aliquots.

Feces were extracted three times with methanol–water (19:1), a procedure which readily removed almost 80% of the radioactivity from fecal residue. The fecal extract was taken to dryness and fractionated by extraction and enzyme hydrolysis.

Stepwise extraction and β-glucuronidase and sulfatase hydrolyses of urine and the fecal extract into conjugate–nonconjugate were performed via the method of Buhler et al. (1966). Acid hydrolysis was also employed to cleave conjugates.

Aliquots of urine or water and dried aliquots of organic solvents were counted in diotol scintillator (Buhler et al., 1966). Aliquots of feces and tissue (wet or dry) were digested with 60% perchloric acid and 30% hydrogen peroxide (1:2) by the method of Mahin and Lofberg (1966).

Respiration Studies. Radiorespirometric techniques of Wang (1967) were employed to test the possible conversion of [^{14}C]HCP to $^{14}CO_2$ or other volatile radioactive metabolites. Four rabbits (3 kg) were given an intraperitoneal injection of 10 mg/kg of [^{14}C]HCP (sp act. 70.7 μCi/mmol) in this study.

Thin-Layer and Column Chromatography. The solvent systems used for developing the thin-layer plates and the chromatographic mobility of HCP and related compounds have been described previously (Gandolfi and Buhler, 1974; Buhler et al., 1977). Glucuronides were chromatographed using butanol–acetic acid–water (4:1:1 or 4:1:5) and isopropyl alcohol–ammonia–water (8:1:1) as solvent systems. Derivatized glucuronides were chromatographed with benzene–acetone (9:1), 0.75% methanol in chloroform, and 0.50% methanol in dichloromethane.

HCP was removed from the thin-layer plates as soon as possible because it discolored to a brownish oil when exposed to light. Shaffer et al. (1971) reported a similar degradation of HCP when it was exposed to UV light.

Extracts of the urine and feces from rabbits were also separated on columns of silicic acid (BioSil A, 100–200 mesh) prior to analytical procedures. Columns were prepared and developed with either benzene–hexane–methanol–water (BHMW) (7:3:6:5) or *n*-butyl alcohol–benzene–ammonium hydroxide (BBA) (1:1:1).

Gas Chromatography. Electron-capture gas chromatography was used for the analysis of HCP (Buhler et al., 1973), HCP metabolites (Buhler et al., 1977), and ultraviolet photodegradation products of HCP. The column temperature was operated at optimum conditions for the compound under analysis.

Mass Spectra. The mass spectra of various compounds were obtained in a Varian MATS CH-7 spectrometer (Varian Aerograph, Walnut Creek, Calif.) equipped with a direct entry probe as previously described (Buhler et al., 1973). Column temperatures were operated at the following optimal conditions: bisphenols and bisphenol methyl ethers (210 °C); monophenol derivatives, benzodioxanes, and salicylic acids (130–160 °C); and monophenols and monophenol ethers (100 °C). For glucuronides of HCP, spectra were taken rapidly at several temperatures since the glucuronides, like pentachlorophenol glucuronide (Tashiro et al., 1970), decomposed rapidly under mass spectral conditions.

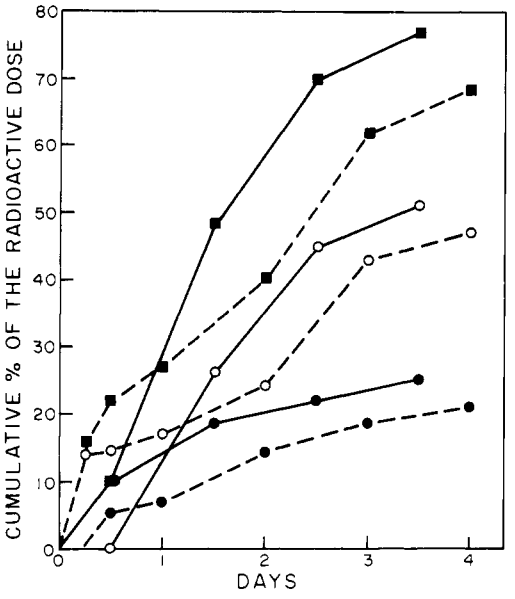

Figure 1. Excretion of radioactivity in the urine and feces of rabbits receiving (—) 10 mg/kg or (- - -) 15 mg/kg intraperitoneal administration of [^{14}C]HCP: (○) feces, (●) urine, and (■) total. The values for the 15 mg/kg dose are the mean from two (3 kg) rabbits and the 10 mg/kg dose values are the pooled samples of six (2.5–3.5 kg) rabbits.

RESULTS

Absorption and Excretion. Excretion of radioactivity with respect to time by the rabbits after a single dose of labeled HCP is shown in Figure 1. Radioactivity appeared primarily in the feces, with 51.7 and 47.7% of the dose being recovered 3.5–4 days following 10- and 15-mg/kg

Table I. Tissue Distribution of Radioactivity in Rabbits 4 Days after Intraperitoneal Administration of [^{14}C]HCP (15 mg/kg)[a]

Tissue	Sp act., dpm × 10^3/g dry wt		% of radioact. dose	
	A	B	A	B
Blood cells	0.47	0.041	0.013	0.001
Blood plasma	27.6	7.77	0.78	0.22
Brain	0.31	0.071	0.00092	0.00027
Caeca	2.36	0.54	0.017	0.0049
Caeca contents	11.5	1.56	0.52	0.096
Fat	0.38	0.27	0.075	0.053
Gall bladder and bile	32.2	1.25	0.020	0.0042
Heart	2.01	0.43	0.0052	0.0014
Large intestine	1.97	0.51	0.015	0.0046
Large intestine contents	5.23	2.17	0.13	0.040
Small intestine	1.91	0.73	0.025	0.0090
Small intestine contents	7.48	2.19	0.025	0.0090
Kidney	3.99	1.05	0.027	0.011
Liver	10.7	3.69	0.61	0.26
Lung	5.22	1.40	0.018	0.0062
Muscle	0.45	0.091	0.23	0.046
Spleen	0.69	0.19	0.00049	0.00013
Stomach	1.34	0.44	0.0083	0.0032
Stomach contents	5.84	1.65	0.14	0.026
Testes	1.90	0.93	0.058	0.0050
Total			2.80	0.83

[a] Two approximately 3-kg male rabbits. Total amount of blood, fat, and muscle estimated according to Donaldson (1924).

doses, respectively. About 24.9 and 20.8% of the radioactivity, respectively, was excreted in the urine over the same time period. Total recoveries were 77.0 and 68.5% of the dose in animals given 10 and 15 mg/kg [^{14}C]HCP.

No $^{14}CO_2$ or other volatile radioactive metabolites were detected in the respired air of rabbits dosed with 10 mg/kg [^{14}C]HCP.

Residual Radioactivity. Four days after two animals received a 15-mg/kg intraperitoneal dose of [^{14}C]HCP, the radioactivity remaining in the tissues was determined. Highest specific activities were found in the plasma, bladder contents, and gall bladder, while the brain, fat, and muscle were among the lowest (Table I). The radioactivity found in the stomach contents may reflect gastric or salivary secretion of HCP and its metabolites, or coprophagy as previously suggested for the rat (Buhler et al., 1977).

Isolation, Fractionation, and Analysis of Urine and Fecal Radioactivity. An aliquot of the pooled urine and feces collected from 0 to 4 days from the two rabbits that received 15-mg/kg intraperitoneal doses of [^{14}C]HCP was extracted and fractionated after β-glucuronidase and sulfatase hydrolysis into conjugated and nonconjugated fractions. Conjugated metabolites (62.2% of total radioactivity) predominated in the urine (Table II). A similar fractionation of radioactivity from the urine of six rabbits dosed with 10 mg/kg [^{14}C]HCP was observed (58% conjugated metabolites and 42% unconjugated).

Almost all of the radioactivity in the urine was acidic in nature, which suggests the presence of either unchanged HCP, acidic metabolites, or extractable acidic conjugates. All urine extract fractions from rabbits dosed with 15 mg/kg [^{14}C]HCP (Table II) were combined and concentrated on a rotary evaporator to a small volume. One-fifth of the pooled concentrate was applied to a silicic acid column and developed with benzene–hexane–methanol–water (7:3:5:5). Most (97%) of the applied radioactivity eluted in a single peak with the same chromatographic mobility as HCP. Mass spectral analysis of this radioactive

Table II. Distribution of Radioactivity in Urinary and Fecal Fractions of Rabbits after Intraperitoneal Administration of [^{14}C]HCP (15 mg/kg)[a]

Fraction	% of total urine radioact.[b]	% of total feces radioact.[c]
Unconjugated	29.1	78.1
Conjugated	62.2	1.7
Glucuronide	56.0	
Sulfate	5.7	
Acid hydrolyzed	1.5	
Aqueous residue (unextracted)	7.6	
	99.9	79.8

[a] Pooled 0–4-day urine and fecal extracts from two approximately 3-kg rabbits were fractionated as described under Materials and Methods. [b] Containing 20.8% of the administered dose. [c] Pooled feces contained 47.7% of the administered dose. About 20.2% of the radioactivity in feces was not removed by the methanol-water extraction procedure employed.

component yielded a spectrum identical with that for authentic HCP (Buhler et al., 1973) and, in addition, only HCP dimethyl ether was detected by gas chromatography after methylation of this urinary product. A trace of "carry-over" unhydrolyzed HCP conjugate was also determined to be present.

The urinary metabolites of HCP in the rabbit after intraperitoneal injection with 15 mg/kg [^{14}C]HCP thus were found to consist of HCP glucuronide (11.7% of the dose), unconjugated HCP (6.0% of the dose), and unextracted material (1.6% of the dose). No other acidic metabolites of HCP were found upon column chromatography of the combined urine extracts.

About 98% of the radioactivity in the 0–4-day fecal extract (37.2% of the dose) was present in the unconjugated fraction (Table II), primarily in the form of HCP as evidenced by gas and thin-layer chromatography. An additional amount (0.8% of the dose) appeared as conjugated material. Additional radioactivity (9.6% of the dose) not removed from the feces by methanol–water extraction was mainly present as HCP conjugate and a small amount of unchanged HCP.

Further purification of the major radioactive components of feces was accomplished by silicic acid chromatography of the pooled unconjugated and conjugated fecal extract (Table II). Over 96% of the radioactivity present in the major column peak was HCP on a basis of thin-layer and gas chromatography and mass spectroscopy. The remaining radioactivity was distributed between unhydrolyzed HCP conjugate and an unidentified oil.

Isolation and Identification of Hexachlorophene Glucuronide. Urine from six New Zealand white rabbits receiving intraperitoneal administration of alternating 10 mg/kg doses of labeled (2 doses) and unlabeled (2 doses) HCP was collected over a 3.5-day interval following each dose.

The urine collected between 0.5 and 2.5 days following each dose of HCP was pooled and separated into conjugates and nonconjugates by the method of Polakova et al. (1971). The conjugate fraction contained 58% of the urine radioactivity and consisted of almost entirely HCP glucuronide.

Conjugates were removed from the urine with Amberlite XAD-2 resin (Gandolfi and Buhler, 1974) and after separation from the resin were then concentrated to an oil. The radioactive material was applied to a large silicic acid column which yielded a single radioactive peak when eluted by organic solvents of increasing polarity. The

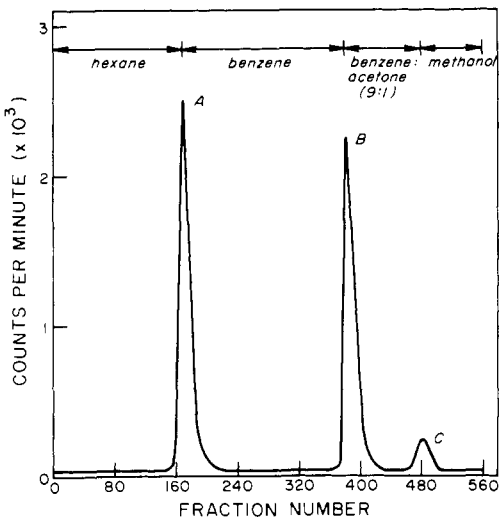

Figure 2. Silicic acid column chromatography of methylated and acetylated rabbit urine extract after column chromatography cleanup with Florisil and Alumina. Urine extract from rabbits receiving 10 mg/kg intraperitoneal doses of [^{14}C]HCP.

fractions containing the major radioactive components were pooled, concentrated, and treated with diazomethane and then with acetic anhydride–pyridine. The oily product was further purified by chromatography on a Florisil column and then, after concentrating the fractions from the single radioactive peak, on an Alumina F-120 column. Both were developed with increasingly polar solvents. The fractions from the major radioactive peak were combined, evaporated, then applied to a long silicic acid column (80 cm), and eluted with increasingly polar solvents as shown in Figure 2. Chromatography of the combined fraction A on silica gel thin-layer plates developed with benzene–acetone (9:1) showed that it contained a single radioactive component with an R_f value identical with that of synthetically prepared HCP mono(methyl tri-*O*-acetylglucuronate) after the latter was treated with diazomethane. Fraction B consisted of a single radioactive component with the same chromatographic mobility as that of synthetically prepared HCP mono(methyl tri-*O*-acetylglucuronate) following treatment of the latter with acetic anhydride–pyridine.

Apparently the HCP monoglucuronide was not completely methylated during derivitization and, as a result, some HCP monoglucuronide was later acetylated in the free phenolic position where methylation should have occurred. Incomplete methylation has also been found (Buhler and Rasmusson, 1976) when HCP is methylated by diazomethane in certain tissue extracts.

The last peak, fraction C (Figure 2), was not identified. It did not migrate from the origin upon silica gel thin-layer chromatography in any of the solvent systems used in these studies. Since treatment of this radioactive fraction with additional diazomethane or acetic anhydride–pyridine did not modify its chromatographic mobility, it is not unreacted HCP monoglucuronide. The nature of this material is not known but it might be photochemical degradation products of HCP (Shaffer et al., 1971) formed during the lengthy isolation of the glucuronide.

Mass Spectral Analysis of Hexachlorophene Glucuronide. Mass spectral analysis of synthetic HCP mono(methyl tri-*O*-acetylglucuronate) showed a six-chlorine parent pattern at *m/e* 717 and a chlorinated pattern typical of HCP (Buhler et al., 1973) at *m/e* 404. Additional fragments were *m/e* 316 (methyl acetoglucuronate) and 196 (major HCP fragment ion).

Fraction A gave a six-chlorine pattern at *m/e* 418 (HCP monomethyl ether) and a nonchlorinated ion at *m/e* 316 (methyl acetoglucuronate fragment). Mass spectral analysis of peak B yielded two six-chlorine patterns, one at *m/e* 446 (HCP monoacetyl ester) and the other at *m/e* 405 (HCP), and a nonchlorinated ion occurring at *m/e* 316 (methyl acetoglucuronate fragment). From these data, fraction A can be identified to be HCP monomethyl ether mono(methyl tri-*O*-acetylglucuronate) and fraction B as HCP monoacetyl ester mono(methyl tri-*O*-acetylglucuronate).

DISCUSSION

In the present study, rabbits excreted between 21 and 25% of the administered radioactive dose in the urine within 4 days. By contrast less than 10% of the HCP or its metabolites was recovered in the urine of other species of animals studied (St. John, Jr., and Lisk, 1972; Black et al., 1974; Buhler et al., 1977). The molecular weights of any HCP conjugates are sufficiently high that they would probably be excreted in the bile rather than by the kidney (Williams, 1971). This hypothesis has been confirmed in the rat where biliary excretion of HCP glucuronide is the major route of elimination of the bisphenol (Gandolfi and Buhler, 1974). The biliary excretion of xenobiotics, however, is relatively slow in the rabbit (Stowe and Plaa, 1968); consequently greater quantities of conjugated HCP would appear in the urine of that species.

Some residual radioactivity remained in the tissues 4 days after [^{14}C]HCP was administered to rabbits. The highest percent of the radioactive dose was found in the liver (0.44%), but appreciable amounts also were present in the intestines, bladder contents, caeca contents, stomach contents, fat, and muscle. The distribution of radioactivity in the rabbit was thus quite similar to that found in rats receiving oral or intraperitoneally administered [^{14}C]HCP (Buhler et al., 1977). The appearance of residual radioactivity in the intestinal tract and the slow rate of excretion in the rabbit suggest that HCP also undergoes biliary excretion and enterohepatic circulation as found in the rat (Gandolfi and Buhler, 1974).

The nature of the urinary and fecal metabolites of HCP in rabbits and rats dosed with [^{14}C]HCP was quite similar (Buhler et al., 1977). In the urine of both species, acidic metabolites of HCP predominated, occurring mainly in the form of conjugates. Although similar proportions of conjugated and nonconjugated metabolites were present in the urine of both species, the rabbit excretes a greater percentage of the dose in the urine than the rat. Apparently both animals metabolize HCP in a similar manner, differing only in the relative amounts excreted in the urine.

The major and probably only metabolite of HCP found in the rabbit is thus HCP monoglucuronide. This same metabolite has been similarly identified as a biliary excretion product in HCP-treated rats (Gandolfi and Buhler, 1974).

The monoglucuronide conjugate of HCP is probably (Mahler, 1954) strongly acidic; therefore, further conjugation of the bisphenol does not occur (Williams, 1971). Steric hindrance of the HCP phenolic groups (Haque and Buhler, 1972) could also contribute to the formation of HCP monoglucuronide rather than a diglucuronide conjugate.

The formation of HCP monoglucuronide and the elimination of the conjugate in the bile presumably represent a detoxification mechanism since glucuronide conjugates tend to be intrinsically less toxic than the

parent compounds (Williams, 1959). The aglycone, which is known to have deleterious effects on liver function (Nakaue et al., 1973; Gandolfi et al., 1974), was thus modified and presumably removed from its site of action.

LITERATURE CITED

Black, J. G., Sprott, W. E., Howes, D., Rutherford, T., *Toxicology* **2**, 127 (1974).
Bollenback, G. N., et al., *J. Am. Chem. Soc.* **77**, 3310 (1955).
Buhler, D. R., Dost, F. N., Rasmusson, M. E., Gandolfi, A. J., *Xenobiotica*, submitted for publication (1977).
Buhler, D. R., Harpootlian, H., Johnston, R., *Biochem. Pharmacol.* **15**, 1507 (1966).
Buhler, D. R., Rasmusson, M. E., unpublished results, 1976.
Buhler, D. R., Rasmusson, M. E., Nakaue, H. S., *Environ. Sci. Technol.* **7**, 929 (1973).
Donaldson, H. H., "The Rat", Memoirs of the Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, Pa., 1924, 469 pp.
Gandolfi, A. J., Buhler, D. R., *Xenobiotica* **4**, 693 (1974).
Gandolfi, A. J., Dost, F. N., Buhler, D. R., *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **31**, 605 (1972).
Gandolfi, A. J., Nakaue, H. S., Buhler, D. R., *Biochem. Pharmacol.* **23**, 1997 (1974).
Haque, R., Buhler, D. R., *J. Am. Chem. Soc.* **94**, 1824 (1972).
Kimbrough, R. D., *Crit. Rev. Toxicol.* **2**, 445 (1974).
Kimmel, C. A., Moore, W., Hysell, D. K., Stara, J. F., *Arch. Environ. Health* **28**, 43 (1974).
Mahin, D., Lofberg, R., *Anal. Biochem.* **16**, 500 (1966).
Mahler, W., *J. Am. Chem. Soc.* **76**, 3920 (1954).
Nakaue, H. S., Dost, F. N., Buhler, D. R., *Toxicol. Appl. Pharmacol.* **24**, 239 (1973).
Polakova, A., Gwilliam, C. G., Quamme, G. A., Williamson, D. G., Layne, D. S., *Can. J. Biochem.* **49**, 368 (1971).
St. John, L. E., Jr., Lisk, D. J., *J. Agric. Food Chem.* **20**, 389 (1972).
Shaffer, G. W., Nikawitz, E., Manowitz, M., Daeniker, H. U., *Photochem. Photobiol.* **13**, 347 (1971).
Stowe, C., Plaa, G., *Annu. Rev. Pharmacol.* **8**, 337 (1968).
Tashiro, S., Sasamoto, T., Aikawa, T., Tokunaga, S., Taniguchi, E., Eto, M., *J. Agric. Chem. Soc. Jpn.* **44**, 124 (1970).
Udall, V., Malone, J. C., *Proc. Int. Soc. Study Drug Toxicity* **11**, 244 (1970).
Wang, C. H., in "Methods of Biochemical Analysis", Glick, C. D., Ed., Wiley, New York, N.Y., 1967, pp 311–368.
Williams, R. T., "Detoxication Mechanisms", Wiley, New York, N.Y., 1959, 796 pp.
Williams, R. T., in "Fundamentals of Drug Metabolism and Drug Disposition", La Du, B. N., Mandel, H. G., and Way, E. L., Ed., Williams and Wilkens Co., Baltimore, Md., 1971, pp 187–205.
Wit, J. G., Van Genderen, H., *Acta Physiol. Pharmacol. Neerl.* **11**, 123 (1962).
Wotiz, H. H., Smakula, E., Lichtin, N. N., Leftin, J. H., *J. Am. Chem. Soc.* **81**, 1704 (1959).

Received for review April 19, 1976. Accepted September 7, 1976. This work was supported by Grants FD 00041 and ES 00210 from the Food and Drug Administration and the National Institutes of Health, U.S. Public Health Service. Manuscript issued as Technical Paper No. 4215 from Oregon Agricultural Experiment Station.

Metabolism of [^{14}C]Photodieldrin in House Flies

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Metabolism of [^{14}C]photodieldrin was studied in male and female house flies. It was found that the rate of metabolism is relatively higher in female flies. The analyses of extracts of whole flies (organic and aqueous) and their feces by TLC followed by autoradiography revealed the presence of three metabolites and photodieldrin. Two of these metabolites were identified as *trans*-photoaldrindiol and another one as a ketone derivative of photodieldrin.

Dieldrin, which is one of the most persistent chlorinated hydrocarbon insecticide chemicals, is considered an environmental contaminant (cf. Edwards, 1970). Its residues are reported to form photoconversion products (Roburn, 1963; Rosen et al., 1966), which are generally more toxic than their parent compound (Khan et al., 1974). Photodieldrin, the photoisomer of dieldrin, is considered as a "terminal residue" (Khan et al., 1974). Therefore, studies of its metabolic fate in various biological systems are of considerable importance. Several studies on the metabolism of dieldrin in mammals, insects (cf. Menzie, 1974), and microorganisms (Matsumura, 1974) have been conducted but there is only a limited number of studies of the metabolism of photodieldrin. The investigations in insects have revealed that photodieldrin is oxidatively metabolized to photodieldrin ketone (Khan et al., 1969). Klein et al. (1969) have shown that photodieldrin is converted to two metabolites in the mosquito, *Aedes aegypti*, and to three metabolites in the cabbage looper; one of the metabolites in both these species is hydrophilic in nature. However, none of these metabolites have been chemically characterized. We, therefore, carried out further investigations to learn more about the metabolic pathways of photodieldrin in house flies and to identify the metabolites.

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MATERIALS AND METHODS

Chemicals. [^{14}C]Photodieldrin (25 μCi/μmol), generously supplied by Shell Chemical Co., was further purified by TLC in benzene–ethyl acetate (3:1) and benzene solvent systems. The final purified product was essentially free of interfering compounds as tested by electron-capture gas chromatography and autoradiography.

Instrumentation. Thin-layer chromatography (TLC) was employed for the separation of metabolites. Samples were applied on 0.25-mm thick silica gel F-254 precoated glass plates (E. Merck, Darmstadt, West Germany) and developed in solvent system and then exposed to X-Ray No-Screen film (Eastman-Kodak Co.) for 15 to 20 days. After developing the film, the darkened areas were noted and R_f values corresponding to them were recorded for radioactivity measurements. The areas of silica gel corresponding to the spots were scraped and extracted with acetone; aliquots of the latter were counted in liquid scintillation solution (Reddy and Khan, 1975).

Analyses of gas–liquid chromatography and liquid scintillation counting were performed as described pre-