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Metabolism, Excretion, and Tissue Distribution of [^{14}C]Photodieldrin in Nonhuman Primates following Oral Administration and Intravenous Injection

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Metabolism, excretion, and body distribution of [^{14}C]photodieldrin were studied in the rhesus monkey (*Macaca mulatta*) after single intravenous and repeated oral administration. After iv administration about 45 and 34% of the dose were excreted by the male and the female, respectively, during 21 days following dosing. Approximately two-thirds of the excreted radioactivity was detected in the urine. The body distribution showed high concentrations of photodieldrin and/or metabolites in adipose tissue, liver, bile, bone marrow, mesenteric lymph nodes, and adrenal cortex. Analysis of feces and urine showed the presence of several metabolites. The major metabolite was identified by mass spectrometry, infrared spectrometry, and gas chromatographical comparison as *trans*-photoaldrindiol which appears in the urine as glucuronic acid conjugate and as free metabolite. A second metabolite, occurring in feces and urine, appears to be a monohydroxy substituted derivative of photodieldrin.

During recent years, attention has been focused on photochemical rearrangement products of cyclodiene insecticides because of the concern that some of them may be more hazardous than the parent compounds. For example, photodieldrin, the photoisomer of dieldrin, has been shown to be more acutely toxic to mammals and birds than dieldrin (Brown *et al.*, 1967). Residues of aldrin and dieldrin in soil, water, and on plant surfaces can be converted in the presence of sunlight or microorganisms to photodieldrin (Dailey *et al.*, 1970; Rosen and Sutherland, 1967; Matsumura, 1974) which is considered a "terminal residue" (Reddy and Khan, 1974). Therefore investigations of its metabolic fate in mammals is of significant importance. Although several studies on the metabolism of dieldrin in various biological systems have been published (Brooks, 1972), little is known about the metabolism of photodieldrin in mammals. Reddy and Khan recently have shown that in the rabbit photodieldrin is converted to several metabolites including *trans*-photoaldrin diol and photodieldrin ketone (Reddy and Khan, 1975). However, it is documented that rabbits follow a different pattern in storage, excretion, and metabolism of dieldrin than do other mammals, such as rats and mice and nonhuman primates (Müller *et al.*, 1975a). We therefore chose to investigate further the

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metabolic pathway of photodieldrin in rhesus monkeys (*Macaca mulatta*) and to identify the metabolites.

MATERIALS AND METHODS

Chemicals. [^{14}C]Photodieldrin (3.4 Ci/mol), generously supplied by Shell Development Company, Modesto, California, was purified by means of tlc in benzene ethyl acetate (9:1, 7:3) solvent systems. The final product was essentially free of impurities as tested by autoradiography, radio tlc, and electron-capture gas chromatography. [^{14}C]Photodieldrin was mixed with nonradioactive photodieldrin to obtain the desired concentration for administration.

Chromatography. Thin-layer and column chromatography were employed for the separation of metabolites. The first steps of analysis of urine and feces extracts were carried out on handmade preparative 20 × 20-cm tlc plates coated with 2-mm silica gel G. (E. Merck, Darmstadt, Germany) and developed in the solvent system benzene ethyl acetate (3:1 and 9:1) and subsequently subjected to analysis of the zones of radioactivity by means of tlc radioscanners or by exposure of X-ray no-screen film (Eastman Kodak Co.) for 10 to 20 days. Then the located radioactive zones were scraped and extracted with acetone for further purification by tlc on silical gel F-254-precoated (0.25 mm) glass plates (E. Merck), column chromatography (900 × 50 mm silica gel), and high-pressure liquid chromatography.

Urine analysis. For determination of the total ^{14}C radioactivity, a 0.5-ml aliquot of the urine was added to 15 ml of scintillation solution and counted for 10 min. The urine was acidified with 1 N HCl to pH 1 and extracted in 800-ml batches with 300 ml of diethyl ether each in permanent-flow liquid/liquid extractors. The ether phase was dried over anhydrous sodium sulfate, the solvent evaporated in a rotary evaporator, and the residue applied to preparative tlc plates (2 mm) using benzene/ethyl acetate (3:1) for chromatographic separation of metabolites. The efficiency of the ether extraction was determined to be 70–95%. The zones on the tlc plates containing radioactivity were scraped and separately extracted with acetone and acetone/methanol (3:1). These extracts were subjected to further purification steps employing analytical acetic acid (9:1), ethyl acetate/formic acid/water (19:1:1), hexane/acetone (7:3), chloroform (pure), benzene (pure), ethyl acetate (pure), with subsequent high-pressure liquid chromatography (solvent:chloroform) and analysis by gas chromatography and mass spectrometry.

Feces analysis. Feces were collected every 24 hr. After weighing, the feces were homogenized with a little water. Several aliquots of about 250 mg each were combusted in a Packard sample oxidizer Model 306 and the overall radioactivity was determined by subsequent scintillation counting of $^{14}CO_2$. The remainders of the fecal homogenate were mixed with an equal amount of magnesium sulfate and extracted for 24 hr with methanol/acetone (1:1) in a soxhlet solid extractor. The efficiency of the feces extraction was determined to be 80–95%. The solvent was evaporated and the residue applied to preparative tlc for separation of the metabolites using the solvent system benzene/ethyl acetate (9:1). The additional steps of the purification and identification of the metabolites are as described with the urine analysis.

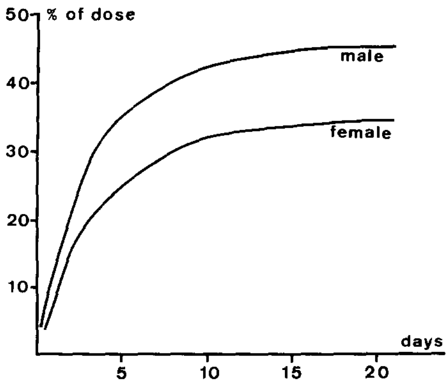

FIG. 1. Excretion of radioactivity during 21 days after intravenous administration of 2 mg/kg [^{14}C] photodieldrin to rhesus monkeys.

Tissue analysis. After dissection, several samples of about 200 mg of each organ or tissue were combusted in the sample oxidizer and the amount of $^{14}CO_2$ was determined by subsequent scintillation counting. The remainders of each organ were homogenized in methanol (w/v 1/1) and extracted for 24 hr with methanol/acetone (1:1) in a soxhlet extractor; the extracts were then analyzed for metabolites as described for urine and feces extracts.

Instrumentation. ^{14}C counting was performed with a liquid scintillation counter (Tri-Carb 2425, Packard Instrument Co.). Samples of feces and tissues were combusted in a Tri-Carb 306 sample oxidizer (Packard Instrument Co.) for subsequent scintillation counting of the $^{14}CO_2$ formed.

For direct counting of radioactivity in urine or in extracts, a scintillation cocktail was utilized which was made up from 100 ml of methanol, 100g of scintillation grade naphthalene (Eastman Kodak), 8g of Permablend III (Packard Instrument Co.), and scintillation grade *p*-dioxane (Eastman Kodak) to the 1 liter mark. In the sample oxidizer, Carbosorb and Permafluor V (Packard Instrument Co.) were used as CO_2 absorber and scintillation cocktail, respectively. To correct for quench during scintillation counting, external standardization was used.

High-performance liquid chromatography was carried out on a Waters Associates Model 201 system with a Beckman 25 scanning uv-visible spectrophotometer as detector, using a Waters μPorasil column and chloroform (Distilled in Glass, Burdick & Jackson) as solvent.

Radioactive zones on tlc plates were localized and quantitated using tlc-radioscanner LB 2722 (Berthold). Packard Model 417 gas chromatographs equipped with ^{63}Ni electron-capture detectors were used for gas–liquid chromatography [glc parameters: 6-ft × 2-mm-i.d. glass columns filled with 3% OV-1 on Chromosorb WHP (Applied Sciences)]. The same column filling was used in the 5-ft × 2-mm glass column of a Finnigan 3000 D gc–ms combination. A single-stage glass jet separator served to remove most of the carrier gas (ca. 20 ml/min helium) at the inlet of the mass spectrometer. Spectra were recorded after 70-eV electron-impact ionization.

TABLE 1

R_f VALUES OF PHOTODIELDRIN AND ITS METABOLITES IN DIFFERENT THIN-LAYER CHROMATOGRAPHY SYSTEMS (Silica Gel F-254)

Metabolite	Solvent system[a] 1	2	3
U1	0.0	0.0	0.11
U2	0.14	0.07	0.48
U3	0.55	0.45	0.95
F1	0.0	0.0	0.11
F2	0.56	0.45	0.95
Photodieldrin	0.78	0.68	0.98

[a] 1, benzene/ethyl acetate (3:1); 2, *n*-hexane/acetone (7:3); 3, chloroform/acetic acid (9:1).

Infrared spectra were recorded on a Beckman 4230 ir spectrophotometer, using a microcell and a Beckman-RIIC mirror-type beam condenser.

Animals. Two studies were completed using one male and one female juvenile rhesus monkey (1.5–2 kg) each. The animals were fed Purina Monkey Chow and water *ad libitum* and kept in metabolism cages. After an acclimatization period of 2 weeks one male animal and one female received one single dose of 4.5 mg [^{14}C]photodieldrin (2 mg/kg body wt) injected intravenously as 2 ml ethanol/1,3-propylene glycol solution. The other two animals were treated with daily doses of 2 mg [^{14}C]photodieldrin (0.8 mg/kg body wt) administered in 2 ml acetone on a porous candy after evaporation of the solvent. Urine and feces of all animals were collected separately ever 24 hr. The injected animals were sacrificed after 21 days; organs and tissues were removed and stored at −20°C for further analysis.

TABLE 2

EXCRETION OF THE DIFFERENT METABOLITES IN THE MALE AND FEMALE RHESUS MONKEY

Metabolite	Percentage of total excreted radioactivity Male	Female
U1	28	31
U2	24	20
U3	12	14
F1	23	24
F2	12	10
Minor	0.5	0.5

TABLE 3

BODY DISTRIBUTION OF RADIOACTIVITY 21 DAYS AFTER A SINGLE, INTRAVENOUS ADMINISTRATION OF 2 mg [^{14}C]PHOTODIELDRIN/kg body wt

Tissue	^{14}C Concentration (ppm)[a]	
	Male	Female
Adipose tissue	0.6–0.8	0.6–0.9
Bone marrow	0.4	0.5
Liver	1.7	1.2
Bile	1.9	1.6
Brain[b]	0.05	0.04
Adrenal cortex	0.5	0.4
Adrenal medulla	0.1	0.1
Kidney	0.1	0.1
Heart	0.05	0.03
Mesenteric lymph nodes[c]	0.5–0.8	0.4–0.7

[a] Photodieldrin and/or metabolites.

[b] Cerebrum.

[c] Mesenteric lymph nodes in close proximity to cisterna chyli.

RESULTS

Intravenous Administration of Photodieldrin

After a short period of high excretion during the first 7 days after dosing (male 39%, female 27.3% of the given dose), the excretion rate decreased rapidly and reached a nearly constant value of 0.2% of the administered dose. Approximately 50–70% of the excreted radioactivity was found in the urine, regardless of sex and time following administration. Figure 1 shows the excretion of ^{14}C radioactivity during 21 days after intravenous administration of 2 mg/kg of photodieldrin.

The total excretion of radioactivity during 21 days after administration of [^{14}C] photodieldrin amounted to 45% of the administered dose for the male monkey and 34% for the female. The radioactive material found in the excretion consisted of three major metabolites in the urine and two in the feces. No unchanged photodieldrin could be detected. The R_f values of photodieldrin and its metabolites are listed in Table 1. In addition, several minor metabolites were observed amounting to $<0.5\%$ of the total excretion which will be objects of further research.

There were slight differences in the percentage of the total excreted radioactivity in metabolites between male and female animals.

Both animals were sacrificed 21 days after dosing and the distribution of the residual radioactivity was determined. The highest concentrations of ^{14}C-labeled compounds were detected in the liver, adipose tissue, mesenteric lymph nodes, and adrenals. Except for the liver, bile, and kidney, where the extracted radioactive compounds showed tlc properties identical to those of urine metabolite 1 (U1) and feces metabolite 1 (F1), material corresponding to only photodieldrin was found in the tissues (Table 3).

TABLE 4

TISSUE CONCENTRATIONS OF RADIOACTIVITY IN A MALE RHESUS MONKEY AFTER 12 SINGLE ORAL DOSES OF 0.8 mg/g [^{14}C]PHOTODIELDRIN/kg body wt

Tissue	Concentration[a] (ppm)	Tissue	Concentration[a] (ppm)
Bile	73.1	Thymus	27.2
Adipose tissue	33.7–35.1	Pancreas	12.4
Mesenteric lymph nodes	20.6	Kidney	5.5
Adrenal cortex	8.0	Muscle	1.3
Adrenal medulla	6.4	Testes	2.9
Heart	0.65	Cerebrum	2.5
Liver	14.4	Cerebellum	1.7
Bone marrow	10.9	Meninges	1.0
Lung	2.4	Blood	1.2
		Spleen	1.4

[a] Photodieldrin and/or metabolites.

Oral Administration of Photodieldrin

After 12 daily doses of 0.8 mg/kg body wt [^{14}C]photodieldrin symptoms of acute poisoning became increasingly evident in the male animal. The symptoms appeared as soon as 20 min after administration, continued approximately 1 hr, and included hyperexcitation, irritability, poor coordination, tremor, and muscle twitches. Therefore, the animal was sacrificed and autopsied and body distribution of the radioactivity was examined. The necropsy findings failed to detect any lesions typical of acute dieldrin poisoning (Treon, 1965), but high concentrations of radioactivity were detected in the bile, adipose tissue, bone marrow, mesenteric lymph nodes, adrenals, pancreas, thymus, and brain of the animal (Table 4).

As the female animal displayed no similar symptoms, the study was continued. Between Day 70 and Day 76 the plateau level of storage was reached, resulting in an equilibrium between daily intake and excretion. Therefore the daily dosing was discontinued and further excretion of radioactivity was monitored until Day 175. When dosing was discontinued the animal had accumulated approximately 50% of the administered photodieldrin, of which another 30.1% was excreted in the period Days 76–175. During the period of daily administration of the compound, between 20 and 50% of the excreted radioactivity was detected in the urine. However, the major part of the fecal excretion was found to consist of photodieldrin which indicates that part of the dose passed the gastrointestinal tract without being absorbed. After discontinuing dosing, approximately 60% of the excreted radioactivity appeared in the urine, therefore displaying an excretion pattern similar to that occurring after intravenous administration. Three different metabolites appeared in the urine while two were found in the feces. Photodieldrin appeared exclusively in the feces. The tlc properties of the discovered metabolites were identical to those detected after intravenous administration; their relative ratio in the daily excretion is shown in Table 5.

TABLE 5

RELATIVE RATIO OF THE DIFFERENT METABOLITES DURING AND AFTER DAILY ADMINISTRATION OF 0.8 mg [^{14}C]PHOTODIELDRIN/kg body wt

	Ratio of the metabolites in the daily excretion (%)					
Period	U1	U2	U3	F1	F2	Photo-dieldrin
Days 1–76	12	13	10	3	2	60
Days 76–176	40	15	10	10	20	5

Identification of the Structure of the Excreted Metabolites

The urinary metabolite U1, when treated with 0.01 N hydrochloric acid for 24 hr or incubated 24 hr with β-glucuronidase (Ketodase, Warner–Chilcott) in 0.2 M acetate buffer, led to a product which had tlc properties identical to those of urine metabolite U2 in all solvent systems and which, after methylation, showed gas chromatographic retention times identical to those of the methylated product of U2. Therefore, it can be assumed that U1 is a glucuronic acid conjugate of U2.

A mass spectrum which was obtained from urinary metabolite U2 shows the molecular peak at *m/e* 396. The intensity ratio of the chlorine isotope peaks indicates the presence of six chlorine atoms in the molecule.

Fragments appearing at *m/e* 378 ($M^+ - H_2O$) and 343 ($M^+ - Cl{-}H_2O$), and 307 ($M^+ - Cl{-}HCl{-}H_2O$) show the presence of a hydroxy group in the molecule. An infrared spectrum obtained by the metabolite shows a sharp absorption band at 3580 cm^{-1} and a broad absorption band between 3200 and 3500 cm^{-1}, the characteristic absorptions of free and inter/intramolecular bonded hydroxy groups in a diol. The epoxy absorption at 950 cm^{-1} is missing which supports the opening of the epoxy ring of the photodieldrin molecule. After methylation with diazomethane under BF_3 catalysis (Neeman, 1962) the metabolite was compared by means of gas chromatography to authentic samples of *cis*-photoaldrin-diol[2] and *trans*-photoaldrin-diol[2] which had been subjected to the identical methylation process. Methylation of the *cis*-diol resulted in two peaks with retention times of 11.3 and 13.6 min at a column temperature of 240°C and 32 ml/min N_2. After methylation both the *trans*-diol and metabolite U2 showed two peaks at retention times of 9.5 and 11.1 min. Therefore we assume that the urinary metabolite U2 has the structure of *trans*-photoaldrin-diol.

The mass spectrum obtained by urine metabolite U3 shows the molecule peak with a chlorine cluster typical for six chlorine atoms in the molecule at *m/e* 394. A molecular weight of 394 indicates the incorporation of one oxygen atom in the photodieldrin molecule. Loss of water in the mass fragmentation resulting in peaks at *m/e* 340, according to $M^+ - HCl{-}H_2O$, suggest the presence of a hydroxy group in the molecule. However, attempts to methylate the compound using diazomethane and boron trifluoride as catalyst resulted in a compound of unknown structure

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[2] Obtained from the GSF Institut für Ökologische Chemie Birlinghoven, West Germany.

which showed a mass spectrum with a molecular peak at *m/e* 414. Matthews and co-workers (Matthews and Matsumura, 1971; Matthews *et al.*, 1969) reported the presence of an unknown extremely sensitive photodieldrin metabolite in rat urine for which they postulated a chlorohydrine structure and which could be the precursor or an intermediate of the photodieldrin metabolite pentachloroketone. In turn, we assume that the urine metabolite U3 could be a monohydroxy derivate of photodieldrin. However, lack of NMR and ir data do not permit further conclusions.

The feces metabolite F1 showed tlc properties similar to those of U1 and similar chemical reactions. The feces metabolite F2 corresponded well in its tlc properties to U3. Therefore identical structures might be suggested. However, it was not possible within the scope of this study to isolate sufficient pure amounts of these metabolites to obtain mass spectrometry or infrared spectrometry data.

DISCUSSION

Despite the close structural similarity of dieldrin and photodieldrin, the pathways and the rate of excretion and metabolic conversion in mammals follow a remarkably different pattern. The biological half-life of photodieldrin in rhesus monkeys is considerably shorter than that of dieldrin (Müller *et al.*, 1975b), which indicates a faster rate of excretion and metabolization. In addition to this, the urinary pathway of excretion seems to play a greater role in the excretion of photodieldrin by the primate organism which is in accordance with results obtained in rats (Dailey *et al.*, 1972). However, the main pathway of metabolic conversion of photodieldrin by the primate organism is the enzymatic hydration of the epoxy ring rather than direct oxidation, resulting in the formation of the corresponding *trans*-diol. The 4,5-*trans*-dihydroxydihydroaldrin is a well-known metabolite of dieldrin in rabbits (Korte and Arent, 1965), mice, rats, rhesus monkeys, and chimpanzees (Müller *et al.*, 1975a) but, except for the rabbit, it seems to be a minor metabolite when compared to the formation of the 12-hydroxydieldrin, the major metabolite in monkeys, chimpanzees, and rats. However, we found evidence that the hydration of the epoxy ring is the preferred metabolism pathway of photodieldrin in rhesus monkeys. Direct oxidation, resulting in formation of a monohydroxy derivative, also seems to occur.

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