# Metabolism, Excretion, and Tissue Distribution of [14C]Photodieldrin in Male Rabbits, following Single Oral and Intraperitoneal Administration

Gunda Reddy and M. A. Q. Khan*

Metabolism, excretion, and tissue distribution of [14C]photodieldrin were studied in male rabbits following single oral and intraperitoneal treatments. About 55 to 60% of the administered dose was eliminated in the urine and 1 to 3% was excreted in the feces, in 9 days, in oral or intraperitoneal treated rabbits. Less than 1% radioactivity of urine and feces was extractable in the organic phase showing that most of it was water soluble or conjugated in nature. All 23 tissue samples obtained at autopsy, 9 days after the treatment, showed a minimum of 0.001% of the given dose. Higher concentrations of 14C residues were found in fat followed by liver and then other tissues. Relatively higher concentrations of residues were present in tissues of intraperitoneally treated animals. Analysis of the organic extract of the urine, feces, and liver by TLC showed the presence of seven metabolites along with photodieldrin. Kidney extracts showed, along with photodieldrin, only two of the metabolites detected in urine, feces, and liver. Quantitative analyses of water-soluble and conjugated radioactive photodieldrin or its metabolites, present in the urine, feces, liver, and kidney, were performed after their enzyme and acid hydrolysis.

Chlorinated hydrocarbon insecticides undergo photolytic reactions to form several products. The photoconversion products of most of these insecticides become more toxic than their corresponding parent compounds. For example, photodieldrin and photoaldrin, the photoisomers of dieldrin and aldrin, respectively, are about 2 to 12 times more toxic than the corresponding parent compound to food-chain organisms (see Khan et al., 1974). The acute toxicity studies to mammals and to some birds also show that photodieldrin is more toxic than dieldrin (Brown et al., 1967; F.A.O., 1968; Basson, 1971).

Photodieldrin, the metabolic product of aldrin, dieldrin, or photoaldrin, is considered as the "terminal residue" (Egan, 1969). It is considerably persistent in the environment (Suzuki et al., 1974; Reddy and Khan, 1975a). Since the residues of aldrin and dieldrin in soil, water, or on plant surfaces can be converted, in the presence of sunlight as well as by microorganisms and algae, to photodieldrin (Robinson et al., 1966; Rosen et al., 1966; Harrison et al., 1967; Lichtenstein et al., 1970; Matsumura et al., 1970; Ivie and Casida, 1971a,b; Patil et al., 1972; Klein et al., 1973; Kohli et al., 1973), the latter is considered as an environmental contaminant. Therefore, studies of its metabolism in various animals are of significant importance. Limited studies of the *in vivo* metabolism and distribution of photodieldrin in rats (Dailey et al., 1970; Klein et al., 1970; Dailey et al., 1972) and *in vitro* metabolism by mixed-function oxidase of various animals (Reddy and Khan, 1974) revealed that there was a species and sex difference in its metabolism. As photolysis of aldrin and dieldrin occurs on plant surfaces the effect of photodieldrin on herbivorous animals is of ecological significance. The herbivorous mammal, rabbit, was therefore chosen to study the metabolism of photodieldrin. Experiments were conducted to determine the metabolic fate of [14C]photodieldrin in rabbits when administered orally and intraperitoneally in a single dose. In addition, various tissues, urine, and feces were analyzed for the amount and nature of the radioactivity.

## MATERIALS AND METHODS

**Chemicals.** [14C]Photodieldrin was prepared from [14C]dieldrin (Amersham-Searle, sp act. 75 mCi/mmol) as described by Rosen and Carey (1968). The final purified product was essentially free of interfering compounds as tested by electron capture gas chromatography and by thin-layer chromatography (TLC) followed by X-ray autoradiography. [14C]Photodieldrin was mixed with nonradioactive photodieldrin to obtain the desired concentration for administering to the animals.

**Animals.** Male rabbits (Scientific Small Animals), 8 to 9 months old (weighing ca. 2.5 to 3 kg), were fed on rabbit chow and water *ad libitum* and maintained at 12 to 15 hr of daylight. Two rabbits were injected intraperitoneally or treated orally by feeding needles with [14C]photodieldrin, 25 to 29 µCi (20 to 30 mg/kg) in 2 ml of corn oil. After the treatments, the rabbits were placed in metabolism cages, one rabbit per cage. Urine and feces were collected separately for every 24 hr. After 9 days, the animals were killed and different tissues were dissected and frozen until analysis.

**Chromatography.** TLC was employed for the separation of metabolites. Samples were spotted on 0.25 mm thick silica gel F-254 precoated glass plates (E. Merck, Darmstadt, Germany), developed in the solvent system, benzene–ethyl acetate (3:1), and then exposed to X-ray no-screen film (Kodak Eastman Co.) for 10 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.

**Radioassay.** Radioactive measurements were performed on a liquid scintillation spectrometer (Packard Instrument, Model 3390 equipped with a Model 544 absolute activity analyzer). The counting efficiency of the instrument with the absolute activity analyzer was 99%. Samples of organic extracts (10 to 100 µl), aqueous phases (100 µl), solid feces (100 mg), or solubilized tissues (100 mg) were counted in 10 ml of a liquid scintillation fluid. The composition of the liquid scintillation fluid was similar to that described by Matthews and Matsumura (1969).

**Urine Analysis.** To determine the total 14C radioactivity excreted in each urine sample collected, a 0.1-ml aliquot of the urine was added to 10 ml of scintillation solution and counted. In order to study the organosoluble radioactivity (photodieldrin and its metabolites), about 50 ml of the urine was extracted, three to four times, with 50 ml of diethyl ether. All ether extracts were pooled, dried over anhydrous sodium sulfate, and then evaporated on a flash evaporator. The residue was redissolved in acetone and analyzed by liquid scintillation for total 14C radioactivity in

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

the organic phase. The acetone solutions of the urine extracts of all 9 days were pooled, concentrated, and applied on a number of TLC plates for chromatographic separation of metabolites. The plates were developed in benzene–ethyl acetate (3:1) and exposed to X-ray film for 10 to 20 days.

The efficiency of the ether extraction was checked by extracting known amounts of [14C]photodieldrin added to 50 ml of normal urine and was found to be 75 to 80%. Constant shaking of urine with ether for 24 to 48 hr did not increase the efficiency of extraction. There were no detectable levels of breakdown products in the ether extract of urine, following the 24 to 48 hr shaking, when analyzed by TLC autoradiography. This indicated that the added photodieldrin was stable in the urine at room temperature.

After the ether extraction, 0.1 ml of the urine (aqueous phase) was radioassayed to measure water-soluble [14C]photodieldrin and its metabolite(s). The aqueous phases of the urine samples for all 9 days were pooled and lyophilized. The residue was extracted 3 to 4 times with about 100 ml of methanol. All methanol extracts were pooled and evaporated on a flash evaporator. The methanol extract was analyzed for water-soluble and conjugated metabolites of photodieldrin. The radioassay of salts and other solids which were insoluble in methanol showed that they contain about 2% of the radioactivity in the urine, i.e. not extracted with methanol.

To learn more about the nature of the residues present in the aqueous phase, the enzymic and acid hydrolyses were carried out as described by Dorough et al. (1974). The whole methanol extract was first dissolved in about 120 ml of citrate–phosphate buffer (pH 5.0) and then transferred to 25-ml erlenmeyer flasks (6 ml/flask). The flasks were then incubated at 37°. A β-glucuronidase (Type B-3, bovine liver, Sigma Chemical Co.) solution (300 units/0.1 ml) was added at intervals of 10 min for 1 hr and incubations were continued for an additional 2 hr. All incubation mixtures were pooled and then extracted 3 times with ethyl acetate. The ethyl acetate extract was radioassayed for enzyme-released radioactivity. The water layer from the enzyme incubation mixtures was adjusted to 1 N HCl and heated at 90° in a water bath for 1 hr. After cooling, it was neutralized with NaOH and then extracted with ethyl acetate. Aliquots of the ethyl acetate extract were counted for acid-released radioactivity. The water layer (0.1 ml) was counted to determine the water-soluble radioactivity.

**Feces Analysis.** Feces were collected every 24 hr. They were weighed and stored in a freezer until analyzed. The extraction of [14C]photodieldrin or its metabolite(s) excreted in the feces was carried out according to the methods of Dorough et al. (1974). About 10 g of feces was extracted three times with about 50 ml of acetonitrile and about 30 ml of water. The combined filtrate was concentrated until only water was left, which was then extracted three times with about 100 ml of ethyl acetate. The ethyl acetate was evaporated and the residue redissolved in acetone. The aliquots of the latter were counted to measure the organosoluble 14C radioactivity. The ethyl acetate extracts (organic phase) of fecal samples collected at different days (1 to 9) were pooled and subjected to TLC analysis. Using the above procedure, the recovery of the known amount of [14C]photodieldrin added to 5 to 10 g of normal feces was found to be 70 to 75%.

In order to measure water-soluble or conjugated radioactivity present in the aqueous phase of fecal extracts, a 0.1 ml aliquot of it was counted in 10 ml of liquid scintillation solution. Then individual water extracts of fecal samples collected on different days were pooled and adjusted to 1 N HCl. After heating at 90° for 1 hr and then neutralizing with NaOH, extraction was carried out with ethyl acetate. The ethyl acetate extract was evaporated to dryness and the residue was taken in acetone. An aliquot of the latter and of the water phase left after ethyl acetate extraction

were counted for acid-released and water-soluble radioactivity, respectively.

The extracted fecal solids (100 mg) were counted directly in toluene based cocktail (Matthews and Matsumura, 1969) as described by Karapally et al. (1973) for rabbit feces. The radioactivity recovered from the solid feces using the above method was in agreement (within ±10%) with the method of Dailey et al. (1970) and with Soluene-350 digestion when tested. In order to determine the acid-released and water-soluble and bound radioactivity in the extracted feces, the fecal solids (9 days' samples pooled) were placed in 1 N HCl at 90° for 1 hr, then cooled, neutralized with NaOH, and filtered. The filtrate was extracted with ethyl acetate. Radioassays were performed on ethyl acetate extracts, water, and on solid feces for acid-released, water-soluble, and bound radioactivity, respectively.

**Tissue Analysis.** All animals were sacrificed after 9 days of the treatment. The tissues were dissected out and frozen until further analysis. Blood samples were collected with a syringe containing 1% sodium citrate solution. Bile was collected from the gall bladder. The assay of total 14C radioactivity was performed by placing a tissue (ca. 100 mg wet weight) or aliquot of blood (0.1 ml) or bile (10 to 50 μl) in 1 ml of Soluene-350 (Packard) and dissolved at 50° for 2 to 4 hr. After tissues had been dissolved, 10 ml of toluene scintillation solution was added to the vials and radioactivity counted.

The radioactive residues in the liver and kidney were extracted from the tissues, as described earlier for feces, with acetonitrile and water (Dorough et al., 1974). The extracts were concentrated until only water remained which was then reextracted with ethyl acetate. An aliquot of the latter was analyzed by counting for total extracted organosoluble radioactivity as well as by TLC for the presence of metabolites. The water layer was mixed with the solids, and the mixture adjusted to 1 N HCl and heated for 1 hr at 90°. After cooling, the mixture was neutralized with NaOH, filtered, and extracted with ethyl acetate. The ethyl acetate extract was radioassayed for the acid-released radioactivity. The water phase (0.1 ml) or tissue solids (100 mg) were counted in liquid scintillation solution.

## RESULTS

All treated animals were found to be normal without any symptoms of toxic manifestation. However, it was observed that treated animals excreted comparatively less amounts of urine and feces than the untreated rabbits. In 9 days, the control and treated rabbits excreted (average of 2 animals) 1.3 and 0.6 l. of urine and 270 and 80 g of feces, respectively. It was an unusual coincidence that all treated animals did not excrete urine on days 3 and 5. However, this is not uncommon in normal animals.

**Urine.** The major pathway of excretion of 14C radioactivity (photodieldrin and its metabolites) was through the urine. A total of about 55 to 60% of the administered dose (intraperitoneal or oral) was excreted in 9 days after the treatment (Figure 1). The percent excretion gradually increased with time, reaching the maximum after 4 to 6 days, and then decreased slowly. Analyses of ether-extractable (organic phase) 14C radioactivity by liquid scintillation counting showed that less than 1% of the injected dose was excreted as organosoluble materials as compared with 54% of the total radioactivity remaining in the aqueous phase (Table I). This indicated that most of the [14C]photodieldrin or its metabolites were either hydrophilic or conjugated in nature. There was not much difference, in the pattern or in the amount of the total percent excretion of the administered dose, between orally and intraperitoneally treated rabbits.

Ether extracts of urine of all 9 days were pooled and analyzed by TLC followed by autoradiography. A number of metabolites along with free photodieldrin were detected
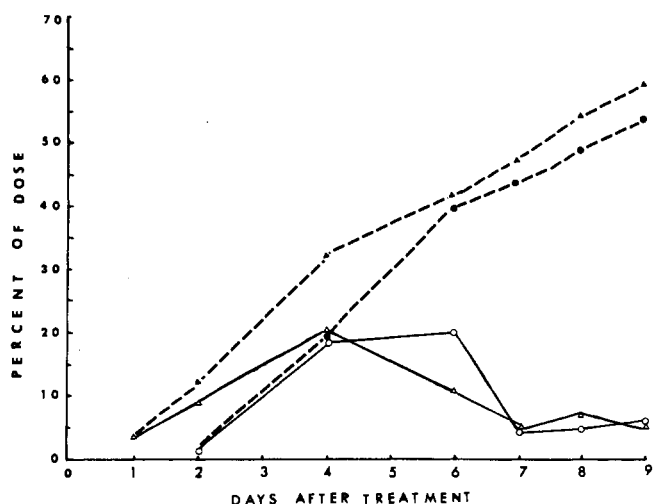
**Figure 1.** Radioactivity, percent of the administered dose, excreted in the urine by male rabbits following single oral (O—O, on different days; ●- - -●, cumulative) or intraperitoneal (△—△, on different days; ▲- - -▲, cumulative) treatments.

**Table I. Excretion of [14]C Radioactivity in the Urine of the Rabbits Treated with [14C]Photodieldrin**[a]

| | Percent of the administered dose | | | | | |
|---|---|---|---|---|---|---|
| Days after treatment | Intraperitoneal | | | Oral | | |
| | Org. phase | Aq phase | Total | Org. phase | Aq phase | Total |
| 1 | 0.040 | 3.79 | 3.830 | | | |
| 2 | 0.102 | 10.53 | 10.632 | 0.024 | 0.77 | 0.794 |
| 4 | 0.250 | 21.21 | 21.460 | 0.082 | 18.26 | 18.342 |
| 6 | 0.110 | 6.92 | 7.030 | 0.050 | 19.35 | 19.400 |
| 7 | 0.060 | 4.55 | 4.610 | 0.031 | 2.64 | 2.671 |
| 8 | 0.011 | 3.27 | 3.281 | 0.014 | 4.29 | 4.304 |
| 9 | 0.048 | 4.03 | 4.078 | 0.013 | 3.00 | 3.013 |
| Total | 0.621 | 54.30 | 54.921 | 0.214 | 48.31 | 48.524 |

[a] Average values of two animals.

(Figure 2). These metabolic products and photodieldrin were designated as A, B, C, D, E, F, H (metabolites), and G (photodieldrin), whose $R_f$ values were 0.00, 0.06, 0.11, 0.22, 0.37, 0.45, 0.62, and 0.52, respectively, in a benzene–ethyl acetate (3:1) solvent system. Two of the metabolites designated as A and F are tentatively identified as *trans*-photoaldrin diol and photodieldrin ketone, respectively (Reddy and Khan, 1975b).

As seen in Table I most of the ^{14}C radioactivity in the urine was in the aqueous phase showing the hydrophilic nature of the products. Since these water-soluble products could be the conjugated metabolites, the lyophilized aqueous phase was subjected to enzymic ($\beta$-glucuronidase) and acid hydrolysis. The results are presented in Table II. It is interesting to note that most of the ^{14}C-labeled radiocarbon was water soluble in nature, when compared with the bound radiocarbon (enzyme- and acid-hydrolyzed products). It was also found that the amount of the water-soluble radiocarbon was higher in the urine of the orally treated

rabbits. The low total recovery of radioactivity in the urine aqueous phase (Table II) of intraperitoneal treated animals might be due to the radioactivity bound to salts and other solids insoluble in methanol.

**Feces.** The results of the excretion of photodieldrin in feces are presented in Table III. The excretion of radioactivity in feces was very low. The total percent excretion in feces was higher in the orally treated rabbits than the intraperitoneally treated ones. The total of about 3% radioactivity (1.9% in organic, 1.1% in aqueous, and 0.3% bound) was excreted in 9 days in orally treated rabbits as compared to about 1% (0.4% in organic, 0.4% in aqueous, and 0.3% in solids) in intraperitoneally treated rabbits. There was not much difference in the percent radioactivity excreted in organic and aqueous phases. The levels of radioactivity bound to fecal solids were also very low. This indicated that the major pathway of photodieldrin excretion was via urine. Analysis of pooled organic extracts of feces by TLC followed by autoradiography showed the presence of seven metabolites along with photodieldrin (Figure 2).
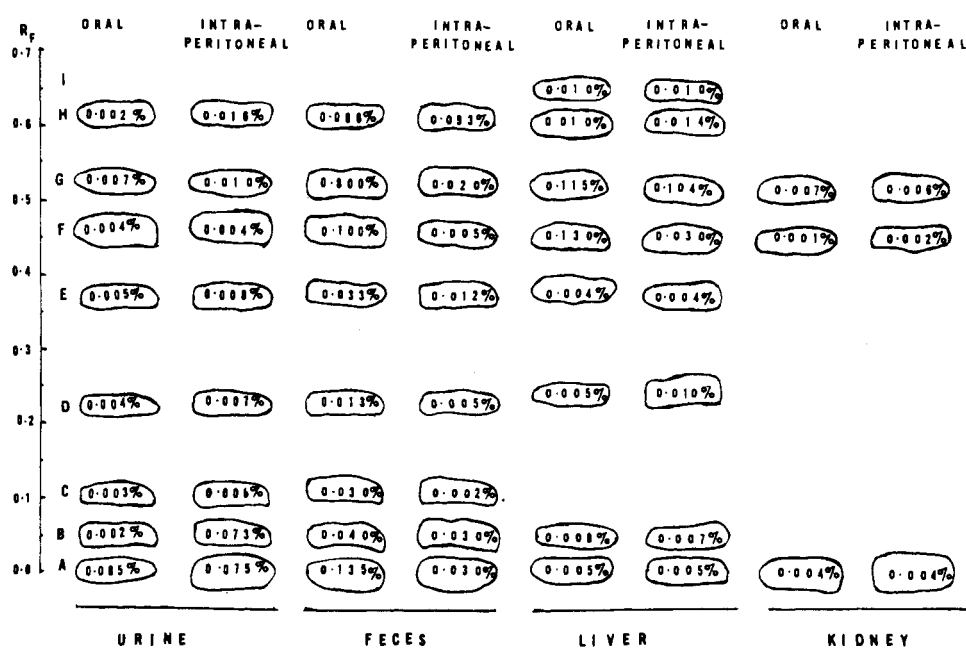


**Figure 2.** Thin-layer chromatographic presentation of [14C]photodieldrin and its metabolites in urine, feces, liver, and kidney. Spots A, B, C, D, E, F, H, and I are unidentified metabolites. Spot G represents photodieldrin. Organic phases of various fractions were applied on TLC plate and developed in the solvent benzene–ethyl acetate (3:1) and exposed to X-ray film for 10 to 20 days.

## Table II. Nature of $^{14}$C Radioactivity in Aqueous Phase of Urine[a]

| Nature of radioact. | % of administered dose | |
|---|---|---|
| | ip | Oral |
| Enzyme-released A | 13.1 | 10.0 |
| Acid-released B | 2.0 | 3.0 |
| Water-solubles C | 18.0 | 31.0 |
| Total | 33.10 | 44.0 |

[a] Aqueous phase of urine (9 days) after ether extraction was lyophilized and then extracted with methanol: A, radioactivity extracted with ethyl acetate from the aqueous phase after $\beta$-glucuronidase hydrolysis; B, radioactivity extracted with ethyl acetate from the aqueous phase of the urine after enzyme hydrolysis and treatment with 1 N HCl; C, radioactivity remaining in the water phase after extraction of enzyme (A) and acid (B) released radioactivity.

They were designated as A, B, C, D, E, F, H as metabolites, and G as photodieldrin, whose $R_f$ values were similar to those observed in ether extractable urine metabolites. The results, presented in Table IV, show that the aqueous phase and solids of feces on acid hydrolysis released low levels of radioactivity. Most of the radioactivity was present as water solubles in both fractions, which was relatively higher in orally treated rabbits. Some radioactivity (0.01 to 0.08%), after acid hydrolysis and extraction, was still bound with fecal solids.

**Tissues.** Analyses of tissue samples showed that $^{14}$C radioactive residues were still present in various tissues (9 days after treatment). The highest concentration of $^{14}$C residues was found in the fat followed by liver, gall bladder, bile, nerves, and kidney and other tissues (Table V). The $^{14}$C residues were relatively higher in the tissues of animals treated intraperitoneally than those fed orally. Although we did not follow the sequence of [$^{14}$C]photodieldrin distribution and elimination from various tissues during the 9-day period, after 9 days all tissues studied contained a minimum of 0.001% of the given dose.

In addition to the estimation of radiocarbon residues in various tissues, we analyzed the nature of $^{14}$C radioactive residues in the liver and kidney. The results, presented in Table VI, show that most of the radioactivity was present as free lipophilic substance(s) in the organic phase. Both tissues showed the presence of conjugated and water-soluble radioactivity. Since liver is the site of detoxication and

kidney the main route of elimination, we analyzed the nature of residues present in the organic extract of both tissues. The ethyl acetate extracts of kidney and liver were spotted on TLC plates and analyzed by autoradiography for detectable levels of metabolites. There were two metabolites present along with photodieldrin in the kidney extract (Figure 2). They were designated as A, F (metabolites), and G (photodieldrin) whose $R_f$ values were 0.00, 0.47, and 0.52, respectively, in the benzene–ethyl acetate (3:1) solvent system. The same number of metabolites was noticed in the kidney extract of orally and intraperitoneally treated animals.

The ethyl acetate extracts of the liver contained considerable lipids which interfered with TLC separation of metabolites. Therefore, [$^{14}$C]photodieldrin and its metabolites were isolated from lipids with acetonitrile and hexane (Jones and Riddick, 1952) prior to TLC analyses. The analysis of the organic extracts of the liver by TLC showed the presence of seven metabolites along with photodieldrin (Figure 2). They were designated as A, B, D, E, F, H, I (metabolites), and G (photodieldrin), whose $R_f$ values were 0.00, 0.06, 0.37, 0.46, 0.61, 0.65, and 0.52, respectively, in benzene–ethyl acetate (3:1) solvent system. It can be seen from the figure that about 0.1% of the administered dose was still present as photodieldrin in the liver after 9 days. No qualitative differences in the formation of metabolites were observed in the organic extract (free) of the livers of orally and intraperitoneally treated rabbits.

## DISCUSSION

The major pathway of excretion of [$^{14}$C]photodieldrin or its metabolites in rabbits was through urine. About 55% of the injected dose was eliminated through urine in 9 days; most of it was hydrophilic or conjugated. Apparently, there was not much difference in the rate of excretion of photodieldrin whether administered orally or intraperitoneally. However, there was considerable difference in water-soluble radioactivity, being higher in the urine of orally treated rabbits. Rabbits on intravascular injections with [$^{14}$C]photodieldrin have been reported to excrete 14.6% of the administered dose in the urine and 1.6% in the feces, in 96 hr, as hydrophilic metabolites (Klein et al., 1969). Barnett and Dorough (1974), working with [$^{14}$C]chlordane, suggested that conjugative metabolism is more efficient in rabbits. This is also true with other insecticides such as dieldrin (Korte and Arent, 1965) and [$^{14}$C]lindane (Karapally et al., 1973). In our studies 1–3% of the injected dose was eliminated through feces in 9 days. However, the excretion in feces of orally treated rabbits was higher when compared with intraperitoneally treated animals. The pattern of ex-

## Table III. Excretion of $^{14}$C Radioactivity in the Feces of Rabbits Treated with [$^{14}$C]Photodieldrin[a]

| Days after treatment | Percent of the administered dose | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Intraperitoneal | | | | Oral | | | |
| | Org. | Aq | Solids | Total | Org. | Aq | Solids | Total |
| 1 | 0.006 | 0.011 | 0.001 | 0.018 | 0.790 | 0.131 | 0.088 | 1.009 |
| 2 | 0.038 | 0.015 | 0.002 | 0.055 | 0.083 | 0.119 | 0.078 | 0.280 |
| 3 | 0.059 | 0.193 | 0.001 | 0.253 | 0.388 | 0.105 | 0.014 | 0.507 |
| 4 | 0.023 | 0.023 | 0.001 | 0.047 | 0.235 | 0.035 | 0.082 | 0.352 |
| 5 | 0.028 | 0.040 | 0.003 | 0.071 | 0.080 | 0.260 | 0.030 | 0.370 |
| 6 | 0.048 | 0.075 | 0.011 | 0.134 | 0.118 | 0.236 | 0.039 | 0.393 |
| 7 | 0.030 | 0.017 | 0.002 | 0.049 | 0.100 | 0.095 | 0.010 | 0.205 |
| 8 | 0.026 | 0.024 | 0.003 | 0.053 | 0.080 | 0.080 | 0.010 | 0.170 |
| 9 | 0.196 | 0.045 | 0.010 | 0.251 | 0.065 | 0.060 | 0.010 | 0.135 |
| Total | 0.454 | 0.443 | 0.034 | 0.931 | 1.939 | 1.121 | 0.361 | 3.421 |

[a] Average values of two animals.

## Table IV. Nature of the Radioactive Residues in the Aqueous Phase, and Fecal Solids, after Ethyl Acetate Extraction

| Nature of residue[a] | Percent of the administered dose | | | |
|---|---|---|---|---|
| | Aq phase | | Fecal solids | |
| | ip | Oral | ip | Oral |
| Acid-released A | 0.020 | 0.400 | 0.004 | 0.080 |
| Water-soluble B | 0.130 | 0.450 | 0.021 | 0.150 |
| Solids C | | | 0.010 | 0.080 |

[a] A, radioactivity extracted from the feces (9 days) of aqueous phase or fecal solid, after acid hydrolysis; B, radioactivity remaining in the water phase after extraction of acid-released radioactivity; C, bound radioactivity of the fecal solids after acid treatment.

## Table V. Distribution of ¹⁴C Radioactivity after 9 Days in Various Tissues[a]

| Tissues | Percent of the administered dose | | | |
|---|---|---|---|---|
| | Intraperitoneal | | Oral | |
| | % ¹⁴C act.[b] | Levels, ppm[c] | % ¹⁴C act.[b] | Levels, ppm[c] |
| Liver | 0.050 | 500 | 0.040 | 400 |
| Kidney | 0.009 | 90 | 0.007 | 70 |
| Testis | 0.002 | 20 | 0.001 | 10 |
| Heart | 0.004 | 40 | 0.002 | 20 |
| Lungs | 0.006 | 60 | 0.004 | 40 |
| Brain | 0.002 | 20 | 0.001 | 10 |
| Nerves | 0.030 | 300 | 0.004 | 40 |
| Spleen | 0.002 | 20 | 0.001 | 10 |
| Gall bladder | 0.021 | 210 | 0.020 | 200 |
| Bile[d] | 0.015 | 150 | 0.010 | 100 |
| Blood[d] | 0.003 | 30 | 0.002 | 20 |
| Prostate gland | 0.003 | 30 | 0.004 | 40 |
| Abdominal fat | 0.065 | 650 | 0.041 | 410 |
| Subcutaneous fat | 0.062 | 620 | 0.050 | 500 |
| Front leg muscle | 0.002 | 20 | 0.002 | 20 |
| Back thigh muscle | 0.002 | 20 | 0.002 | 20 |
| Skin | 0.003 | 30 | 0.002 | 20 |
| Stomach | 0.003 | 30 | 0.003 | 30 |
| Stomach content | 0.005 | 50 | 0.003 | 30 |
| Cecum | 0.007 | 70 | 0.006 | 60 |
| Cecum content | 0.006 | 60 | 0.005 | 50 |
| Intestine | 0.005 | 50 | 0.002 | 20 |
| Eye lens | 0.001 | 10 | 0.001 | 10 |

[a] Average values of two experiments, each with two replicates. [b] Per gram of wet weight tissue. [c] Calculated as parts per million based on the specific activity of the administered dose. [d] Per milliliter.

## Table VI. Nature of [¹⁴C]Photodieldrin Residues in the Tissues of Rabbits 9 days after Treatment

| Tissue and nature of residue[a] | Percent of the administered dose | |
|---|---|---|
| | ip | Oral |
| (1) Liver | | |
| Free | 1.043 | 0.660 |
| Acid released | 0.057 | 0.038 |
| Water soluble | 0.037 | 0.002 |
| Solids | 0.110 | 0.266 |
| Total | 1.247 | 0.966 |
| (2) Kidney | | |
| Free | 0.047 | 0.044 |
| Acid released | 0.088 | 0.015 |
| Water soluble | 0.006 | 0.004 |
| Solids | 0.002 | 0.001 |
| Total | 0.143 | 0.064 |

[a] Free, ¹⁴C radioactivity extracted with ethyl acetate; acid released, radioactivity extracted with ethyl acetate from the aqueous phase and extracted tissues after treatment with 1 N HCl; water soluble, radioactivity remaining in water phase after acid treatment and ethyl acetate extraction; solids, radioactivity unextractable from the solids after acid treatment and ethyl acetate extraction.

Photodieldrin, although persistent in the environment for a considerable length of time (Reddy and Khan, 1975a), is degraded in higher animals. According to our studies, the organic extracts of urine and feces of the rabbit show the presence of seven metabolites. Korte and Arent (1965) reported six metabolites in the urine of rabbits treated with dieldrin, the major metabolite of which was 6,7-*trans*-dihydroxydihydroaldrin. However, we tentatively identified two of the metabolites obtained in the urine as *trans*-photoaldrin diol and photodieldrin ketone (Reddy and Khan, 1975b).

All tissues of rabbits analyzed showed considerable amounts of ¹⁴C-labeled residues after 9 days of treatment. The greater concentration was in the fat and liver. The residues in the tissues of intraperitoneally treated rabbits seem to be higher than those of the orally treated animals. Dailey et al. (1970) administered photodieldrin, both orally and intraperitoneally, to rats and found the principal storage of the compound in fat tissue. They also showed extremely high levels of ¹⁴C activity in the kidney of male rats. The biological half-life of photodieldrin in the fat of male and female rats was considerably less than that of dieldrin (Brown et al., 1967). Walton et al. (1971) also reported that the concentration of photodieldrin in the fat of male and female rats is less than that of dieldrin. Recently, Hayes (1974) showed that after a single oral administration of dieldrin in rats, the highest concentration of dieldrin in the brain was reached in 4 hr, which then started declining slowly. The concentration of dieldrin in muscle remained essentially steady during the interval from 4 to 48 hr. The concentration of dieldrin in the fat which was higher than that in the brain at 4 hr continued to increase during the first 24 hr.

McCully et al. (1968) administered *p,p'*-DDT, orally, intraperitoneally, and intramuscularly, to rats, sheep, chicken, rabbits, and guinea pigs. They found, except in chicken, a greater concentration of total residues in the fat and livers following intraperitoneal injection than oral or intramuscular administration. They are of the opinion that the functional cecum of the rabbit might be providing a suitable environment for DDT metabolism. A similar type of mechanism may be involved in the conversion and elimina-

cretion of photodieldrin through feces in rabbits is contrary to that of rats. Dailey et al. (1972) administered [¹⁴C]photodieldrin orally to rats, 5 days a week, and found the percent excretion of the compound to be higher in feces than in urine. Equal amounts of excretion in feces of orally and intraperitoneally treated rats were also observed at the end of a 12-week period (Dailey et al., 1970). Higher levels of excretion of orally administered dieldrin through feces were also observed in rats (Matthews et al., 1971), sheep (Hedde et al., 1970), and cows (Wilson and Cook, 1972).

tion of photodieldrin and its metabolites in orally treated animals thereby causing low levels of $^{14}C$-labeled residues in tissues.

Analyses of organic extracts of liver and kidney show a number of metabolites. Most of the metabolic products found in liver extract are also observed in the urine and feces extracts with the exception of the two metabolites designated C and I. It is assumed that metabolite C might have formed or accumulated in the urine and feces during the process of excretion and elimination. Only two metabolic products are detected in the kidney.

Our attempts in identifying the above metabolites will help to understand the detoxication mechanisms involved in the metabolism of photodieldrin in rabbits.

## ACKNOWLEDGMENT

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# Effect of Hexachlorophene on Reproduction in Rats

Gerald L. Kennedy, Jr.,* Sandra H. Smith, Moreno L. Keplinger, and Joseph C. Calandra

Administration of hexachlorophene to three successive generations of albino rats at dietary levels of 12.5, 25, and 50 ppm failed to produce any changes with respect to mating, fertility, length of gestation, and number of deliveries. Numbers of progeny produced, survival of the young, and weight of the animals at weaning were not altered by exposure to the chemical. All progeny obtained from test and control groups were free of gross external abnormalities and displayed no unusual behavior during the experiment. Histopathologic evaluation of parental animals from each of the three generations and from weanlings of the final (F3b) generation failed to reveal any lesions which could be attributed to the ingestion of hexachlorophene.

Hexachlorophene, 2,2'-methylenebis(3,4,6-trichlorophenol), has been widely used as an antibacterial agent in a wide variety of products. The toxicity of the material has been recently reviewed (Kimbrough, 1974). Since the material is a polychlorinated polycyclic compound, concern has been expressed over the possible long-term effects of repeated, low-level exposures to the chemical.

Thorpe (1967) reported that the oral administration of hexachlorophene (HCP) to male rats as well as to sheep produced degeneration of spermatogenic cells. Gaines and Kimbrough (1971) reported reduced survival in F1 generation offspring fed 100 ppm. Studies with hamsters (Alleva, 1973) have indicated that HCP did not interfere with reproduction in that species. The present study assesses the influence of feeding HCP on reproduction in rats fed over three consecutive generations.