Excretion, Distribution, and Tissue Storage of a ^{14}C-Labeled Photoconversion Product of ^{14}C-Dieldrin

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Subacute levels (5 μg per day) of ^{14}C-labeled dieldrin or photodieldrin were administered to young adult rats of both sexes for as long as 12 weeks. Photodieldrin was given both orally and intraperitoneally; dieldrin was given by stomach tube only. Urinary, fecal, and total excretion patterns as well as tissue distribution and storage of ^{14}C-labeled residues were determined. From 59.2 to 64.7% of the ^{14}C-activity administered as photodieldrin was excreted in the urine and feces of male rats and 45.9 to 49.0% was found in the excreta of females. A total of 63.6% of the ^{14}C-activity from dieldrin was excreted by male rats and only 37.0% by females. Analysis of various tissues showed that from 3 to 10 times more ^{14}C-activity was retained by females as compared to males after photodieldrin, regardless of the route of administration. A similar sex difference in tissue levels was found after administration of dieldrin. Adipose tissue was the principal storage site of both dieldrin and photodieldrin in female rats and of dieldrin in male rats. Extremely high levels of ^{14}C-activity were detected in the kidneys of male rats receiving photodieldrin.

Dieldrin (1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a - octahydro - 1,4 - *endo*, *exo* - 5,8 - dimethanonaphthalene), when present as deposits on grass and subjected to sunlight, undergoes photodecomposition (Roburn, 1963). Acute toxicity studies by Rosen and Sutherland (1967) and by Brown *et al.* (1967) have suggested that the photoconversion product of dieldrin (photodieldrin) is more toxic to certain mammalian species than the parent compound. Because of these findings, it became of interest to study excretion and storage patterns after administration of subacute levels of photodieldrin to rats of both sexes and to compare these patterns with those found after administration of dieldrin under the same conditions. Since the desired dose levels were low, it was advantageous to use radiolabeled compounds.

METHODS AND MATERIALS

Reagents and Apparatus. ^{14}C-photodieldrin was prepared by ultraviolet irradiation of ^{14}C-dieldrin (Nuclear-Chicago Corp.) using a method recently described by Benson (1969). Both compounds were judged to be essentially free of interfering substances by gas-liquid and thin-layer chromatographic techniques. The specific activity of the ^{14}C-dieldrin used was 5.3 mCi per mmole, and the ^{14}C-photodieldrin was diluted with pure unlabeled photodieldrin to a specific activity of 4.3 mCi per mmole in order to provide enough material to complete the studies as planned. Both compounds were dissolved in acetone and stored at −20° C in the dark. At the time of administration, the acetone was evaporated and the compounds were redissolved in corn oil.

All radioactivity measurements were made with a Packard Model 3375 liquid scintillation spectrometer, using a scintillation phosphor prepared by dissolving 4.0 g of 2,5-diphenyloxazole (PPO, Packard Instrument Co.) and 0.100 g of 1,4-*bis*-[2-(4-methyl-5-phenyloxolyl)] benzene (DMPOPOP, Packard Instrument Co.) in a mixture of 700 ml of toluene and 300 ml of methanol.

Procedure. Eighteen Osborne-Mendel rats of each sex, weighing approximately 100 g at the beginning of the study, were housed individually in metabolism cages and fed Purina rat pellet chow and water *ad libitum*. The animals were divided into the following six groups of six rats each according to sex, compound administered, and route of administration: (1) male, ^{14}C-photodieldrin, intraperitoneal; (2) male, ^{14}C-photodieldrin, oral; (3) male, ^{14}C-dieldrin, oral; (4) female, ^{14}C-photodieldrin, intraperitoneal; (5) female, ^{14}C-photodieldrin, oral; and (6) female, ^{14}C-dieldrin, oral. Each rat received a dose of 5 μg of either ^{14}C-photodieldrin or ^{14}C-dieldrin dissolved in 0.25 ml of corn oil each day, 5 days a week. ^{14}C-dieldrin was administered for 9 weeks and ^{14}C-photodieldrin for 12 weeks.

Quantitative collections of urine and feces were made every day and were pooled in weekly periods for each of the six groups. The weekly urine pools were diluted to 1000 ml with distilled water for convenience in aliquot sampling and calculation, and were then stored at −20° C until processed for analysis. Fecal pools were air dried, ground with mortar and pestle, pulverized to a fine powder in a Sorvall Omnimixer homogenizer, and weighed. These were also stored at −20° C.

Analysis of Urine. To determine the total ^{14}C-activity in each urine pool, a 0.2-ml aliquot was added to 15 ml of scintillation phosphor in a counting vial. Counts were corrected for quenching effects by referring to a quench correlation curve plotted previously.

In order to gain some insight as to the nature of the ^{14}C-labeled compound(s) being excreted into the urine, 100-ml aliquots of urine were continuously extracted for 48 hr with ethyl ether. The ethyl ether extract was counted and the percentage of the total ^{14}C-activity in the urine extractable by ethyl ether was calculated.

Analysis of Feces. Ten-gram portions of the dry powdered fecal samples were refluxed for 2 hr with 9 ml of 50% potassium hydroxide and 45 ml of 95% ethyl alcohol. Aliquots of this digest were counted to determine total ^{14}C-activity in the feces. This procedure yielded a 95% recovery of known amounts of standard ^{14}C-photodieldrin dissolved in acetone and applied to dried feces prior to digestion. Furthermore, ^{14}C-activity which had been incorporated into the feces physiologically by administration of ^{14}C-photodieldrin was completely recovered by alkaline digestion when compared with a combustion technique described by Watts *et al.* (1969). These workers reported a recovery greater than

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Table I. Cumulative Excretion of ^{14}C-Activity; Per Cent of Administered Dose[a]

Week	Excreted in	Photodieldrin, i.p.		Photodieldrin, oral		Dieldrin, oral	
		Males	Females	Males	Females	Males	Females
1	Urine	24.7	3.9	16.5	5.4	2.1	0.7
	Feces	30.2	21.8	27.8	21.6	51.7	26.4
2	Urine	24.3	5.4	20.7	7.6	3.0	1.0
	Feces	30.4	25.3	29.5	25.5	51.6	29.5
3	Urine	29.0	6.4	21.8	8.5	3.5	1.1
	Feces	32.4	28.2	30.8	30.2	52.6	32.0
4	Urine	29.6	6.5	22.7	8.6	3.6	1.1
	Feces	32.0	30.2	30.2	31.6	53.8	30.2
5	Urine	31.3	6.3	24.4	8.8	3.8	1.2
	Feces	32.6	30.7	30.5	34.0	56.9	31.6
6	Urine	32.0	6.4	25.2	9.1	3.8	1.2
	Feces	32.0	33.2	31.0	34.4	57.7	33.2
7	Urine	32.5	7.1	26.1	9.4	4.0	1.3
	Feces	32.3	34.0	31.3	35.0	59.2	34.7
8	Urine	32.9	7.6	26.9	9.6	4.2	1.4
	Feces	32.1	34.1	31.8	33.9	59.3	35.5
9	Urine	33.4	8.1	26.9	10.2	4.2	1.4
	Feces	31.4	34.5	31.8	35.4	59.4	35.6
10	Urine	33.7	8.8	27.2	10.5		
	Feces	31.4	35.2	31.9	36.4		
11	Urine	33.8	9.4	27.3	10.8		
	Feces	30.9	36.4	31.9	38.0		
12	Urine	33.8	9.4	27.3	10.9		
	Feces	30.9	36.5	31.9	38.1		

[a] ^{14}C-photodieldrin was administered 5 days a week for 12 weeks; ^{14}C-dieldrin was administered 5 days a week for 9 weeks.

Table II. Tissue Levels (ppb) of ^{14}C-Activity after Administration of ^{14}C-Photodieldrin or Dieldrin[a]

Tissue	Average wet weight of organ (grams)		Photodieldrin, i.p.		Photodieldrin, oral		Dieldrin, oral	
	Males	Females	Males	Females	Males	Females	Males	Females
Brain	1.8	1.7	8.0	92.1	7.7	85.4	7.9	40.1
Heart	1.1	0.7	7.0	99.6	10.5	103	9.8	55.3
Skeletal muscle	...	...	8.7	134	9.8	147	15.0	131
Spleen	0.6	0.5	15.3	122	16.8	88.5	8.6	32.7
Kidney	2.6	1.5	2110	152	1950	162	264	84.2
Liver	12.3	7.6	81.4	222	96.0	237	46.6	95.1
Lung	1.7	1.0	93.3	311	48.2	280	51.5	156
Adrenal	0.047	0.053	43.9	407	27.5	341	82.3	368
Fat	...	...	246	2080	208	1990	553	2590

[a] ^{14}C-Photodieldrin was administered 5 days a week for 12 weeks; ^{14}C-dieldrin was administered 5 days a week for 9 weeks. Calculations of values as ppb were based on the specific activity of the administered compound.

95% of ^{14}C-activity by combustion of fresh bean leaves to which known amounts of ^{14}C-benzoic acid standard had been applied.

Analysis of Tissues. At the conclusion of the dosing schedule, the animals were sacrificed by decapitation 24 hr after the last dose of toxicant had been administered. Tissues were removed, washed in saline, blotted dry, weighed, and stored at −20° C.

At the time of analysis, the various tissues were pooled by groups, weighed, and homogenized in a Virtis "45" homogenizer. Total ^{14}C-activity in each tissue pool was obtained by combustion of 1.5- to 2.0-g aliquots using the combustion technique of Watts *et al.* (1969).

RESULTS AND DISCUSSION

None of the animals displayed any signs of toxic manifestations during the course of the experiment and, at autopsy, no pathological conditions could be observed macroscopically.

The data in Table I show that male rats excreted a greater percentage of administered ^{14}C-activity in their urine than females. Urinary excretion is considerably more important in the elimination of photodieldrin and/or its metabolites than of dieldrin and/or its metabolites, regardless of sex. The sex difference for dieldrin metabolite excretion has been reported previously by Kunze and Laug (1953) and Datta *et al.* (1965). Since a much larger percentage of the administered ^{14}C-activity is excreted into the urine of both sexes after ^{14}C-photodieldrin than after ^{14}C-dieldrin, it can be assumed that the two compounds are handled somewhat differently.

Of the ^{14}C-activity excreted into the urine from ^{14}C-photodieldrin administration, an average of 85.7% (range 73.3 to 94.2%) was extracted with ethyl ether from the urine of males and only 48.9% (range 39.1 to 58.3%) from the urine of females. Similarly, after ^{14}C-dieldrin administration, 69.9% (range 62.7 to 78.5%) was recovered from urine of males and

only 37.3% (range 23.4 to 52.0%) from urine of females. This would indicate that female rats excrete more hydrophilic metabolites and/or conjugates than males, and that a greater proportion of these water-soluble compounds are excreted after dieldrin administration than after photodieldrin, even though the total amount is much smaller.

A constant 30 to 32% of the administered dose of ^{14}C-photodieldrin was excreted as ^{14}C-labeled compounds in the feces of male rats after the first week and throughout the course of the experiment (Table I). In contrast, 21% of the administered dose of ^{14}C-photodieldrin was excreted as radiolabeled compounds into the feces of female rats the first week; this excretion rate steadily increased throughout the 12-week period and reached approximately 36 to 38% of the administered dose. Furthermore, there was no indication that a plateau in the excretion rate of female rats had been reached at the conclusion of the experiment.

Fecal excretion was by far the major route of elimination of ^{14}C-activity after ^{14}C-dieldrin administration to either sex, with the males excreting almost twice as much as the females.

The total of the urinary and fecal excretion of radiolabeled compounds by male rats was considerably higher than that of females after administration of either ^{14}C-photodieldrin or ^{14}C-dieldrin. After the eighth week of ^{14}C-photodieldrin administration, the males had reached a plateau in total excretion of ^{14}C-labeled compounds; however, the total excretion by the female rats continued to increase slowly throughout the 12-week period. This might indicate that the saturation level in tissues had been approached in females but was not yet reached in males. Therefore the females would be expected to have higher storage levels in their tissues than males, and this was indeed the case (Table II). The levels of radioactivity in the tissues of the females after ^{14}C-photodieldrin was given were, in most cases, from 3 to 10 times higher than those of males, regardless of the route of administration. The exception to this pattern was the kidneys of male rats; the levels of ^{14}C-activity were extremely high in this organ. The reason for these high storage levels is not known, but from calculations involving urine volume and urinary isotope concentrations, such levels cannot be accounted for by retained urine.

^{14}C-activity in tissue, except for adipose tissue, appeared to be about the same or slightly lower after ^{14}C-dieldrin administration, as compared to ^{14}C-photodieldrin, perhaps because of the shorter period of dieldrin administration. It is unlikely, however, that the shorter length of time would account for the lower ^{14}C-activity in kidneys of males given dieldrin. It should be pointed out that ^{14}C-activity found in the kidneys of males after ^{14}C-dieldrin administration was about three times higher than that found in the lungs, the vital organ with the next highest activity. This confirms the findings of Kunze and Laug (1953) who measured the levels of a possible metabolite of dieldrin in male rat kidney by a bioassay technique based on toxicity to flies. The apparent storage of ^{14}C-labeled compounds in the kidneys of males after oral ^{14}C-photodieldrin administration was even greater, about 50 times that of the lungs. A careful evaluation of the significance of these high levels is warranted.

The striking sex difference in tissue storage and rate of excretion of ^{14}C-activity found in this study has been reported previously by Korte (1965) after ^{14}C-aldrin administration. Thus it is possible that this phenomenon is in some way related to the findings of Wong and Terriere (1965) that *in vitro* epoxidizing activity of liver microsomes of male rats was much higher than that of females. In addition to microsomal epoxidation, a sex-linked detoxification mechanism might well be operative *in vivo*.

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