Dieldrin-^{14}C Balance in Rats, Sheep and Chickens

by K. L. DAVISON
United States Department of Agriculture
Agricultural Research Service
Metabolism and Radiation Research Laboratory
State University Station
Fargo, North Dakota 58102

When ingestion of DDT and dieldrin, and presumably other chlorinated hydrocarbon insecticides, remains constant, storage of these insecticides or their metabolites in body tissues apparently reaches a plateau (1-3). Presumably a steady state has occurred where excretion of the chemicals or their metabolites equaled ingestion. If the amount of body fat increased, a plateau for storage of these lipophilic residues might be apparent without excretion of the insecticide equaling ingestion.

Experiments were conducted with rats, sheep and chickens to determine whether or not ingestion of a constant amount of dieldrin was balanced by excretion of dieldrin and its metabolites. Carbon-14-labeled dieldrin was fed, and balance was determined on the excretion of carbon-14.

Methods

Experiment 1: Six female (115-day-old) and six male (98-day-old) Sprague-Dawley rats were housed in stainless steel metabolism cages equipped with side arms for feeding. Twenty-four days were allowed for adaptation to this environment and for determination of the amount of food needed to maintain body weight. Body weight was maintained at about 230 and 340 g for female and male rats, respectively, by feeding 12 or 15 g of a commercial rat food daily. Two rats of each sex were then fed dieldrin[1] at 0.04, 0.2 or 2.0 μg/g of diet. Dieldrin-^{14}C, 99+% purity and specific activity 189 μCi/mg, supplied all of the dieldrin for the lowest level fed and this level of dieldrin-^{14}C was included in the remaining diets. Technical dieldrin supplied the additional dieldrin for the intermediate- and high-level diets.

Feces and urine were collected daily through day 7, on days 10 and 14 and weekly thereafter. Urine, collected in glass bottles, was diluted to 100 or 250 ml with water, and 1- or 2-ml aliquots were pipetted into 15 ml of dioxane cocktail and assayed for carbon-14 by liquid scintillation spectrometry. Samples of 100 to 200 mg of ground, freeze-dried feces were combusted in

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[1] In this manuscript, the word dieldrin refers to the amount of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene.

Bulletin of Environmental Contamination & Toxicology,
Vol. 10, No. 1

Schöniger flasks or with a Packard Oxidizer. The resultant carbon dioxide was trapped with ethanolamine and assayed for carbon-14 in a toluene cocktail.

After the dieldrin-^{14}C had been fed for 273 days, the rats were killed, ground, freeze-dried and assayed for carbon-14 as described for feces.

Experiment 2: Four yearling wether sheep, weighing about 45 kg, were placed in metabolism cages (4). They were fed 800 g of pelleted alfalfa hay (later reduced to 400 g to control body weight) and 200 g of ground grain mixture per day in two equal portions, morning and evening. After 2 days, dieldrin was fed in the grain portion of the diet to supply 2.4 μg or 2.0 mg/sheep per day; two sheep were fed each level. Dieldrin-^{14}C was equal in both diets and was the only source of dieldrin for sheep fed the lower level.

Excretions were collected in stainless steel containers at the same time intervals given for rats. Urine was emptied daily into polyethylene carboys when collection intervals were more than 1 day. The urine was weighed, and 0.2- or 0.5-ml aliquots were assayed for carbon-14. Feces were emptied daily into polyethylene-lined pails. At the end of each collection period, the entire collection was weighed, mixed thoroughly and sampled. After 287 days, the sheep were killed and their carcasses, including skin, bone, and GI tract and its contents, were ground and sampled. Carcasses and feces were assayed for carbon-14 as described for experiment 1.

Experiment 3: Yearling White Leghorn hens and roosters, six of each, were placed in individual wire cages and fed 60 and 70 g/day, respectively, of a commercial feed. These amounts of feed maintained body weight at about 1300 g for hens and 2000 g for roosters. After 14 days of adaptation to this diet and environment, three chickens of each sex were fed dieldrin at 0.04 or 2.0 μg/g of diet for 22 weeks. From the 23rd week to the 37th week, dieldrin was fed at 0.04 or 20.0 μg/g of diet. Excretions were collected on stainless steel trays suspended under the cages on the 1st, 4th and 7th days, and at weekly intervals thereafter. Eggs collected during the first 22 weeks were processed with the feces. Eggs collected after the 22nd week were processed separately from the feces. Surviving birds were killed during the 37th week. Feces and eggs were weighed, mixed and sampled. Feces, eggs and carcasses were processed and assayed for carbon-14 as described in experiment 1.

Diet Preparation: Technical dieldrin was dissolved in ethanol and radioactive dieldrin was dissolved in hexane. When diets were mixed for rats or chickens, measured amounts of the ethanol and hexane solutions were diluted to 400 ml with acetone. The entire acetone solution was then added to 1 or 2 kg of feed to prepare a premix. The premix was stirred for 15 minutes. This premix was then mixed for 15 min with enough feed to last about 12 weeks. The mixing procedure was the same for sheep, except that technical dieldrin was weighed and dissolved in acetone each time that feed was mixed. Radioactivity and dieldrin were determined when the feeds were fed.

Statistical: The data were analyzed by analysis of variance, when appropriate, with significance assessed at a probability of 5%.

Results

Experiment 1: Excretion of carbon-14 was greater ($P<.01$) in both feces and urine of male rats than of female rats to the 5th or 6th week (Figure 1). Maximal excretion of carbon-14 occurred by the 6th week for both sexes, and this excretion approximated the daily consumption of dieldrin-^{14}C. Excretion of carbon-14 remained nearly equal to that of consumption from the 6th week to the end of the experiment.

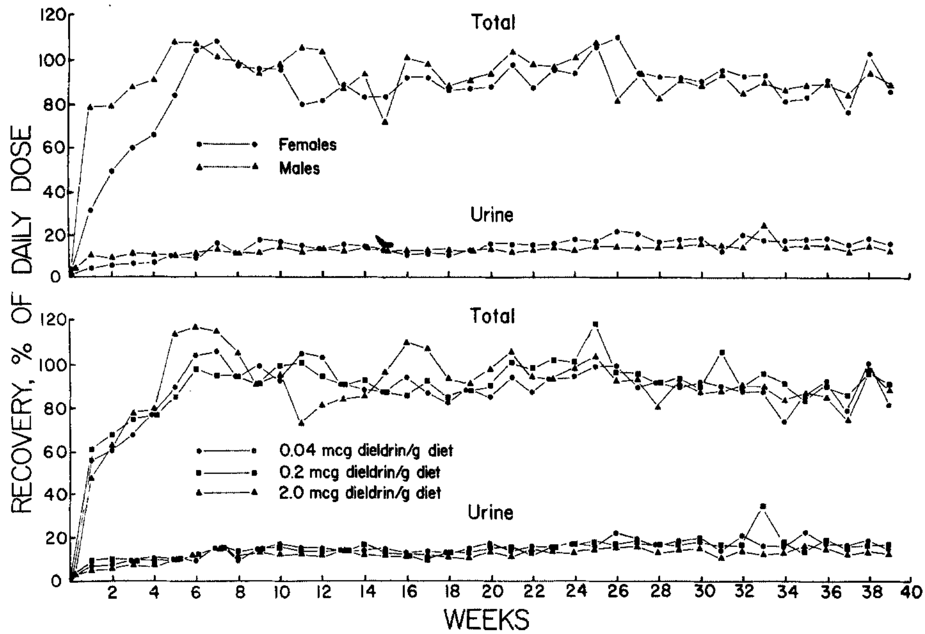

Figure 1. Recovery of carbon-14 in the excretions of male and female rats fed dieldrin-^{14}C continuously for 39 weeks.

A summary of the recovery of carbon-14 in the carcasses, urines and feces for the entire experiment is given in Table 1. Carcasses of female rats contained a higher proportion of the total dose than did those of male rats. The amount of carbon-14 excreted in urine of male and female rats was similar, but female rats excreted a smaller proportion of the dose of carbon-14 in feces than did male rats. Recovery of carbon-14 was not measurably different between the sexes, and in no case did the level of dietary dieldrin measurably affect this recovery.

Experiment 2: The excretion of carbon-14 by sheep is graphed in Figure 2 and tabulated in Table 1. Excretion of carbon-14 in the urine of the two sheep fed 2 μg of dieldrin per day increased

TABLE 1
RECOVERY OF ^{14}C IN THE CARCASSES, URINE AND FECES OF RATS AND SHEEP FED DIELDRIN-^{14}C FOR 39 AND 41 WEEKS, RESPECTIVELY

Species, Sex and Dietary Dieldrin	Recovery, % of Dose			
	Carcass	Urine	Feces	Total
Rats				
Male				
0.04 μg/g diet	2.4[a]	13.3	76.9[a]	92.6
0.2 μg/g diet	2.0	12.3	81.4	95.7
2.0 μg/g diet	1.8	10.4	82.7	94.9
Female				
0.04 μg/g diet	6.8	13.5	71.6	91.9
0.2 μg/g diet	7.2	14.3	72.4	93.9
2.0 μg/g diet	6.6	11.9	76.1	94.6
Standard error of means	±0.9	±1.0	±1.8	
Sheep				
2.4 μg/day	18.0	48.3	25.0	91.3
2.0 mg/day	20.2	33.3	26.9	80.4
Standard error of means	±3.6	±2.6	±0.3	

[a] Recovery was significantly higher in carcasses of female rats than in carcasses of male rats and significantly lower in feces of female rats than in feces of male rats, $P<0.01$.

to 88% by the 4th day, but then it declined to about 40 to 50% of the daily intake. Excretion of carbon-14 in the urine of sheep fed 2 mg dieldrin/day increased to 43% on the 6th day, and then remained at 30 to 40% of the daily intake to the 41st week. However, excretion of carbon-14 in the urine did not differ significantly ($P>.05$) between levels of dieldrin fed. Excretion of carbon-14 in the feces increased to about 30% of the daily intake (by the 8th or 9th week) for sheep fed both levels of dieldrin and generally stayed near this level to the end of the experiment. Eighteen to 20 percent of the total amount of dieldrin-^{14}C fed to the sheep was detected in the carcasses at the end of the experiment.

Experiment 3: Elimination of carbon-14 by chickens is shown in Figure 3. Except for relatively high excretion of carbon-14 the first day, excretion by males fed the lower level of dieldrin increased to the 23rd week when excretion equaled 100% of daily intake. Excretion then decreased to about 70% of daily intake by the 27th week and remained there until the end of the experiment. The increase of dieldrin from 2.0 μg/g to 20.0 μg/g in the high-level diet prepared between the 22nd and 23rd weeks was

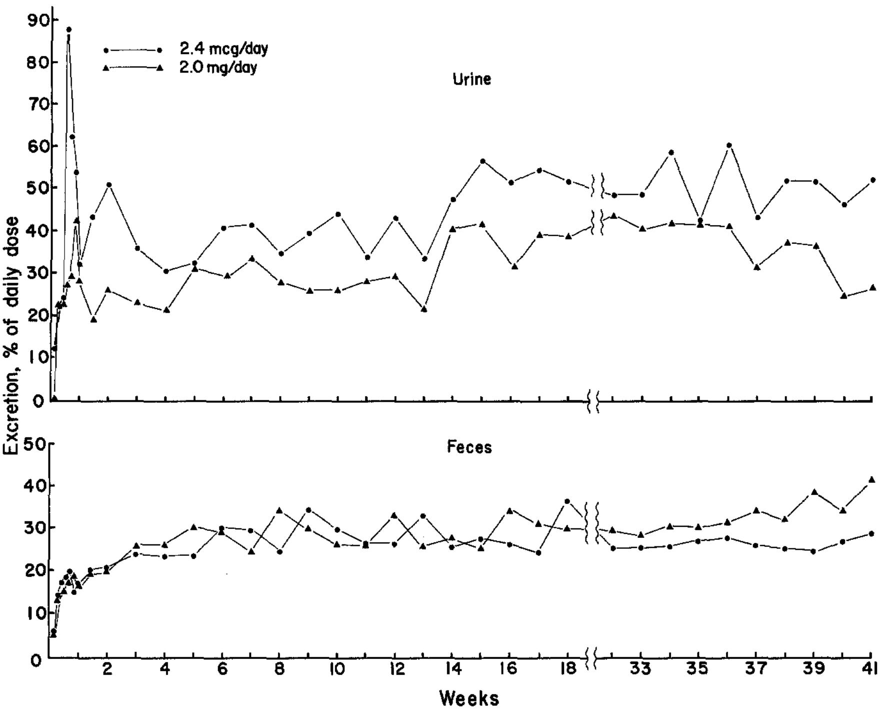

Figure 2. Recovery of carbon-14 in the excretions of sheep fed dieldrin-^{14}C continuously for 41 weeks.

unintentional. Regardless of this error, excretion by roosters fed the higher level of dieldrin increased to 100% by the 26th week. One rooster died the 35th week and another died the 36th week after exhibiting tremors and seizures.

The elimination of carbon-14 by hens varied considerably more than that by the roosters, and an analysis of variance for this experiment was not acceptable because of nonhomogeneity of variance. Elimination of carbon-14 by hens fed either level of dieldrin appeared similar, including the time interval when 20 μg dieldrin/g of diet was fed. Two hens fed the higher level of dieldrin died. One death occurred the 30th week and another occurred the 34th week. The surviving hen had tremors and seizures.

The hens laid eggs occasionally during the first 22 weeks. Egg production increased to 3 or 4 eggs/hen per week by the 22nd week, so beginning the 23rd week, the eggs were collected and analyzed separately from the excretions. The recovery of carbon-14 in excretions, eggs and carcasses is shown in Table 2. Intermittent egg-laying, combined with the relatively large amount of carbon-14 eliminated in the eggs, probably accounted for the greater variation in weekly elimination of carbon-14 by hens as shown in Figure 3.

It appeared that the rate of excretion of carbon-14 during the first 22 weeks of the experiment was lower for roosters fed

2 μg dieldrin/g of diet than for roosters fed the lower level of dieldrin (Figure 3). Elimination of carbon-14 was similar for females fed the two levels of dieldrin. Also, total recovery of the carbon-14 (Table 2) was generally low, so this experiment was duplicated with 12 additional chickens fed dieldrin-^{14}C and dieldrin for a period of 28 days.

The data from the 28-day experiment are shown in Table 3. The elimination of carbon-14 by roosters or hens fed either level of dieldrin was similar to the elimination of carbon-14 during this time interval in the previous experiment. It was obvious

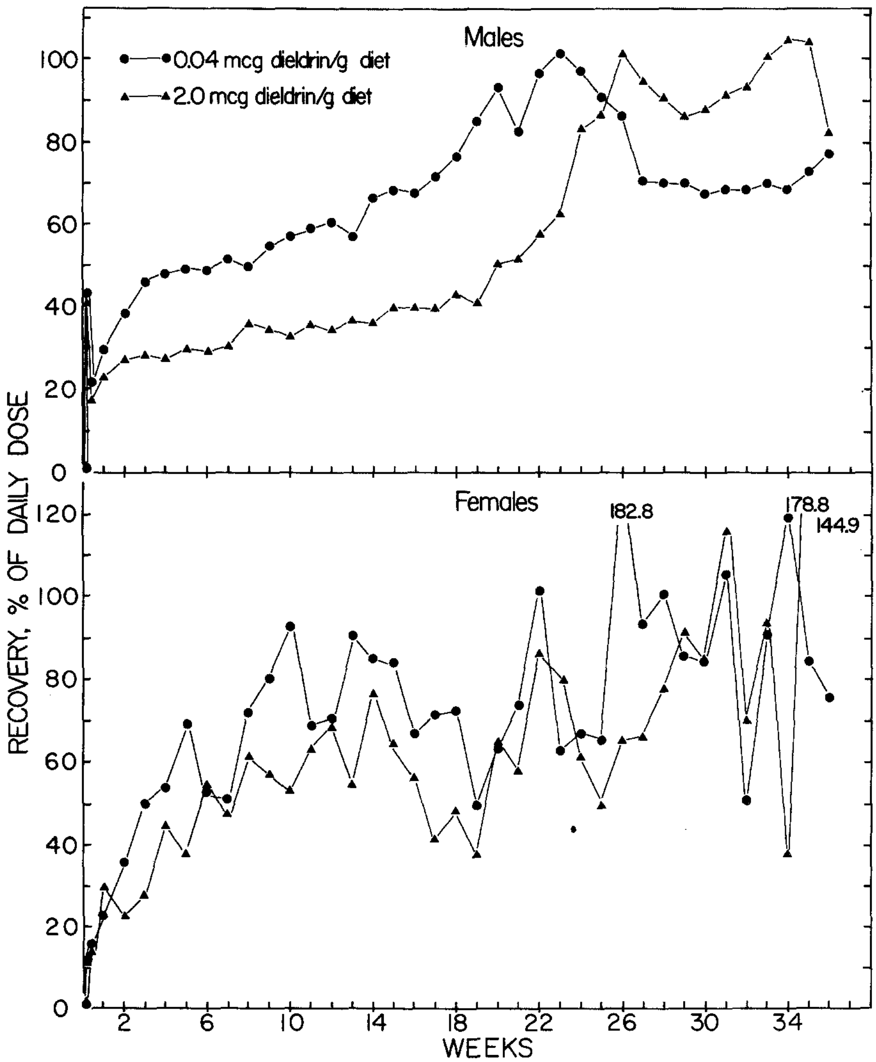

Figure 3. Recovery of carbon-14 in the excretions and eggs of chickens fed dieldrin-^{14}C continuously for 36 weeks. At the 22nd week, the higher level of dieldrin was increased from 2.0 μg/g of diet to 20.0 μg/g of diet.

that roosters fed the higher level of dieldrin excreted a smaller proportion of the ingested dieldrin during this 28-day experiment. Total recovery of carbon-14 for this shorter experiment was greater than total recovery for the previous experiment.

Discussion

Dieldrin apparently is not metabolized by animals to carbon dioxide or other volatile compounds that would be exhaled (5), so radioactive carbon contained in the ingested dieldrin most likely would either remain in the carcasses or be excreted in the urine or feces. Excretion of carbon-14 in the feces and urine of rats balanced ingestion of carbon-14 in dieldrin by the 6th week. The time required to achieve this balance was not affected by the amount of dieldrin consumed or by the sex of rats. Body weights of the rats, measured biweekly, remained virtually constant throughout the experiment. It was concluded that ingestion, storage and excretion of dieldrin by the rats had reached a steady state by the 6th week and that a steady state was maintained until the experiment was terminated.

No conclusions were made as to whether or not a steady state occurred in the sheep, because at no time did excretion of carbon-14 in urine and feces balance ingestion, and because total recovery of carbon-14 in the carcasses and excretions was relatively low. At the beginning of the experiment, body weight increased slightly in the sheep, but this increase was corrected the 22nd week by reducing the amount of pelleted hay fed to 400 g per day. The relatively poor recovery of radioactivity is attributed to some feed wastage by the sheep--they were not as fastidious in eating as the rats were--and to possibly underestimating the amount of radioactivity excreted, particularly that excreted in the urine. It is possible that the plastic carboys adsorbed some of the radioactivity from the urine (6).

TABLE 3
RECOVERY OF ^{14}C IN EXCRETIONS, EGGS AND CARCASSES OF CHICKENS FED DIELDRIN-^{14}C FOR 28 DAYS

Sex and Dietary Dieldrin	Recovery, % of Dose			
	Excretions	Eggs	Carcass	Total
Females				
0.04 µg/g diet	22.2±0.8[a]	5.9±0.5	62.1±2.6	90.2±1.7
2.0 µg/g diet	20.0±2.2	9.6±5.5	67.0±5.5	96.6±1.0
Males				
0.04 µg/g diet	32.5±2.5		66.5±7.3	99.0±7.2
2.0 µg/g diet	23.5±2.7		70.3±4.5	93.8±1.9

[a] Mean and standard error.

TABLE 2

RECOVERY OF ^{14}C IN EXCRETIONS, EGGS AND CARCASSES OF CHICKENS FED DIELDRIN-^{14}C FOR 37 WEEKS

Sex and Dietary Dieldrin	To 22nd Week[a]	23rd to 37th Week[b]			
	Excretions	Excretions	Eggs	Carcass[c]	Total[c]
	%	%	%	%	%
Females					
0.04 μg/g diet	62.6±9.3[e]	56.4± 2.7	31.2±12.3	13.7±4.2	81.4±7.6
2.0 μg/g diet[d]	49.1±2.6	61.1± 4.6	27.2±10.2	28.2±6.7	86.8±3.1
Males					
0.04 μg/g diet	59.2±2.5	76.9± 4.3		27.1±3.5	93.5±2.0
2.0 μg/g diet[d]	36.0±0.5	91.1±11.3		32.3±4.9	89.4±0.4

a Recovery is based on the amount fed by the end of 22 weeks. Eggs laid during this time period were combined with the excretions.
b Recovery is based on the amount fed from the 23rd to the 37th week, or to death.
c Recovery in carcasses and total are based on the amount fed for the entire experiment.
d Increased to 20 μg/g of diet beginning the 23rd week.
e Mean and standard error.

Roosters fed the lower amount of dieldrin balanced ingestion of carbon-14 with excretion by the 22nd week, and roosters fed the higher amount of dieldrin balanced ingestion with excretion by the 26th week. Hens also appeared to balance excretion of carbon-14 from dieldrin with ingestion by about 25 weeks, and eggs provided a major, but highly variable, route for elimination of the carbon-14. The amount of dieldrin fed did not noticeably affect the rate or route of excretion of carbon-14 by hens.

It was concluded that chickens were capable of balancing elimination of carbon-14 with ingestion of dieldrin-^{14}C. This conclusion was made after considering the relatively large variations in recovery of carbon-14 in the excretions and eggs, and the fact that recovery in the 28-day experiment was higher than recovery in the longer experiment. The length of time required to attain balance was longer for chickens than for rats, and was affected by the amount of dieldrin fed to roosters but was not affected by the amount of dieldrin fed to hens or rats.

Summary

Experiments were conducted with rats, sheep and chickens to determine whether or not excretion of carbon-14 from dieldrin-^{14}C would balance ingestion of dieldrin-^{14}C when ingestion remained constant. The data suggest that rats of either sex attained balance by 6 weeks regardless of the amount of dieldrin fed; that roosters attained balance by 22 or 26 weeks, depending on the amount of dieldrin fed; and that hens apparently attained balance by 25 weeks regardless of the amount of dieldrin fed. The data obtained with sheep were inconclusive.

Acknowledgments

I thank Nancy Brown, Alice Nolin and Kenneth Mize for their assistance with these experiments.

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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