Dieldrin Metabolism, Excretion, and Storage in Male and Female Rats

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The metabolism, excretion, and storage of a dose of dieldrin such as might result from an environmental exposure was studied by feeding male and female rats 10 ppm of dieldrin-^{14}C in 3 g of their regular diet. The feces and urine were collected for 9 days, the rats were sacrificed, and the major organs and tissues were analyzed for radioactivity. The sexes differed in the rate in which they excreted the radioactivity in the feces, in the nature of the metabolites excreted in the urine, and in the amount of dieldrin stored in the tissues. The radioactivity extracted from the tissues of the female rats was primarily dieldrin, whereas that extracted from the tissues of the males consisted of varying amounts of Klein's metabolite, depending upon the tissue. The major metabolite of dieldrin is excreted in the feces and has been shown to be rapidly conjugated with glucuronic acid *in vitro* by microsomal enzymes from the livers of both sexes.

The study of dieldrin metabolism in mammals has been a particularly long and perplexing problem for the insecticide toxicologists involved. Kunze and Lang (1953) first reported finding a possible metabolite of dieldrin in the kidneys and urine of male rats. Later, Korte and Arent (1965) were the first to isolate and identify a metabolite of dieldrin, 6,7-*trans*-dihydroxydihydroaldrin, from the urine of rabbits. More recently, Richardson *et al.* (1968) and Matthews and Matsumura (1969) isolated and tentatively identified a metabolite of dieldrin from the feces and a second metabolite from the urine of male rats. Most recently Hedde *et al.* (1970) have reported isolating six metabolites of dieldrin, two of which are water-soluble conjugates, from the urine of castrated sheep. Matthews and Matsumura (1969) have also shown dieldrin metabolism to occur in isolated rat liver microsome preparations, the major metabolites being those excreted in the urine and feces and a glucuronide conjugate of unknown structure.

Each of the studies of dieldrin metabolism to date has utilized relatively high doses of the insecticide, often administered daily over a period of several weeks. Also, most of the studies of dieldrin metabolism have involved only a single sex and little or no attention has been given to the distribution of dieldrin and/or its metabolites in the organs and tissues of the animals studied. Since the metabolism, excretion and distribution of relatively large doses of this insecticide may be significantly different from those which occur with normal exposure to relatively low environmental doses, we were prompted to study these parameters in male and female rats which received a single dose of 10 ppm of dieldrin-^{14}C in 3 g of their regular diet.

EXPERIMENTAL

Dieldrin-^{14}C, specific activity 72.4 mCi/mmol, 96% pure, was obtained from Amersham-Searle and further purified by thin-layer chromatography (tlc). The rats used in the experiment were randomly bred albinos obtained from Charles River Breeding Laboratories, Wilmington, Mass.; they were held in individual metabolism units, model E110, Maryland Plastics, Inc., and fed D&G Rat and Mouse Diet, distributed by the Price Wilholte Co., Frederick, Md. All solvents used in extractions and tlc were of reagent grade. A Büchi Rota-vapor-R, Rinco Instrument Co., Inc., was used to concentrate the solvents used for extraction prior to chromatographic purification or analysis on 250-μ precoated silica gel G plates obtained from Analtech, Inc. Radioactivity on the tlc plates was detected by exposure to Kodak No-Screen medical X-ray film and quantitated by liquid scintillation counting in 25-ml counting vials containing 10 ml of Bray's scintillation counting solvent mixture (Bray, 1960) in a Beckman LS-250 liquid scintillation system, Beckman Instruments, Inc.

The following biochemical compounds were obtained from Sigma Chemical Co., St. Louis, Mo.: uridine diphosphoglucuronic acid (UDPGA); β-glucuronidase (bovine liver, 3,000,000 Fishman units per gram); β-glucuronidase (bacterial, 50,000 units per gram); and Trizma base (99.9%).

Administration of Dieldrin-^{14}C. Three male (avg wt 279 g) and three female (avg wt 196 g) rats were deprived of food for 8 hr prior to receiving a single meal, 3 g of rat and mouse diet, containing 10 ppm of dieldrin-^{14}C. After the rats had consumed the food containing the dieldrin-^{14}C they were fed *ad libitum*. Urine and feces were collected every 8 hr for 9 days following the administration of dieldrin-^{14}C; after the last collection the rats were sacrificed and dissected.

Extraction of Purification of Radioactivity Excreted in the Feces. Before extraction, the daily samples of feces, approximately 9 g each, were air-dried under a stream of warm air for 24 hr and ground into a fine powder in a mortar and pestle. Each sample was then extracted three times for 5 min with 50 ml of boiling hexane in a Dubnoff-Metabolic Shaking Incubator, Precision Scientific. Liquid scintillation counting of the radioactivity remaining in the extracted feces indicated that this extraction procedure was 75 to 80% effective. The hexane extracts were concentrated and spotted on a number of tlc plates for chromatographic purification using a mobile phase of ether–hexane (1:1). The mobile phase was allowed to travel the length of the tlc plates, the solvents were evaporated, and the plates were exposed to X-ray films for 1 week. Following exposure and development of the X-ray films, the silica gel on the tlc plates corresponding to the blackened spots on the appropriate X-ray film were scraped from each of the tlc plates containing similar extractions. The silica gel fractions containing dieldrin-^{14}C or one of its metabolites extracted from each daily sample of feces were thoroughly mixed and carefully weighed; a weighed portion was then taken for liquid scintillation counting in order to estimate quantitatively the daily excretion of dieldrin and its metabolites. Dieldrin-^{14}C and its metabolites extracted from the feces were purified by tlc using the systems described in Table I.

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Table I. Thin-Layer Chromatography of Dieldrin and Metabolites of Dieldrin Excreted by the Rat

Mobile phase[a]	Metabolite R_f's: 6,7-*trans*-Dihydroxydihydroaldrin[b]	Polar metabolite[c]	Klein's metabolite[d]	F-1[d]	Dieldrin
Methylene chloride	0.02	0.05	0.56	0.42	0.67
Ether:hexane (1:1)	0.04	0.07	0.27	0.38	0.62
Hexane:acetone (1:1)	0.60	0.66	0.73	0.75	0.81
Ether:hexane (9:1)	0.26	0.26	0.52	0.61	0.74
Benzene:ethyl acetate (3:1)	0.14	0.18	0.63	0.64	0.78

[a] In each case the stationary phase was a precoated 250 μ thin-layer chromatographic plate of silica gel G obtained from Analtech, Inc. [b] The identity of 6,7-*trans*-dihydroxydihydroaldrin was confirmed by cochromatography with authentic material synthesized in our laboratory. [c] The identity of this compound is not known. [d] Klein's metabolite and metabolite F-1 cochromatographed with unlabeled samples generously supplied by Fumio Matsumura of the University of Wisconsin. The structures of these metabolites have been investigated further, and these results will be reported elsewhere (McKinney and Matthews, 1971).

Extraction and Purification of the Radioactivity Excreted in the Urine. Urine excreted by each of the sexes was measured and stored at 4° C until it was extracted. The total radioactivity in each urine sample was estimated by adding 0.1 ml of urine to 10 ml of counting solvent, counting in a liquid-scintillation counter and calculating the cpm in the total sample from that in the 0.1-ml aliquot. Following estimation of the total radioactivity excreted in the urine, each sample, approximately 50 ml each, was extracted three times with 50 ml of chloroform and 0.1 ml of each urine sample was counted as described above to estimate the unextracted radioactivity. The chloroform extracts were dried over anhydrous sodium sulfate (J. T. Baker Chemical Co.), concentrated, and analyzed for dieldrin-^{14}C and its metabolites by the methods described for the extractions of the fecal metabolites.

Extraction of the Organs and Tissues. The major organs, the liver, and samples of the thigh muscle and intraperitoneal fat were dissected from the three animals of each sex and pooled according to sex. The mesenteric fat was stripped from the intestines and they and the stomachs were opened and washed of their contents. Each group of organs and the livers were weighed; the organs and weighed samples of the liver, muscle, and fat were then minced with the scissors and ground with a mortar and pestle containing 10 g of sea sand (Fisher Scientific Co.) and sufficient anhydrous sodium sulfate to absorb the moisture. Each mixture was ground into a dry granular mass, extracted, and the extracts were analyzed for dieldrin-^{14}C and its metabolites using the techniques described for extraction of the feces.

PREPARATION OF MICROSOMES

Male or female rats (approximately 200 g each) were sacrificed by a blow on the head; the rats were bled and the livers immediately removed and placed in chilled 0.05 *M* Tris-HCl buffer (pH 7.4 at 37° C). After the livers were blotted and weighed they were homogenized in cold 0.05 *M* Tris-HCl buffer to make a 10% mixture (w/v). The homogenate was centrifuged at 12,000 × *g* for 20 min in an International Refrigerated centrifuge model B-20 and the resulting supernatant at 105,000 × *g* for 60 min in a Beckman L2-65B ultracentrifuge. The microsomal pellet was resuspended in half the previous volume with cold 0.154 *M* KCl and centrifuged at 105,000 × *g* for 30 min. The supernatant was decanted and the pellet resuspended in cold 0.05 *M* Tris-HCl, so that 0.5 g of wet liver corresponded to 1.0 ml of microsomal homogenate. A portion of the suspension was mixed with Triton X-100 according to the procedure of Mulder (1970). Microsomal protein was determined by the method of Lowrey *et al.* (1951).

GLUCURONYL TRANSFERASE ASSAY

A new and rapid method was used to measure the formation of glucuronide conjugates. An acetone solution of labeled apolar metabolite (approximately 4000 cpm) was added to a liquid scintillation vial and the solvent evaporated under nitrogen. To the vial was added 0.9 ml of 0.05 *M* Tris-HCl, 0.050 mg of UDPGA, and 0.4 mg of microsomal protein (from microsomal suspensions with or without Triton X-100) to yield a final volume of 1.0 ml. The contents were incubated under nitrogen for 10 min at 37° C in a metabolic shaker (Precision Scientific Company Model 16-X-11) and the reaction was stopped by the addition of 10 ml of a nonaqueous scintillation fluid prepared according to Lucier and Menzer (1968), followed by vigorous shaking of the scintillation vials. Any glucuronide formed partitions into the aqueous phase and the unconjugated substrate partitions into the nonaqueous phase. Therefore, conjugation of the substrate was measured by liquid scintillation counting of the reaction vial since radioactivity incorporated into a glucuronide conjugate would not be detected. In parallel experiments, results obtained using the rapid method were similar to those obtained by more laborious conventional techniques.

The suspected β-glucuronide was incubated with either bovine liver β-glucuronidase by the method of Knaak and Sullivan (1968) or bacterial β-glucuronidase by the method of the Sigma Chemical Co. (1958). Hydrolysis of the glucuronide was assayed by partition of radioactivity into the nonaqueous scintillation fluid by a reversal of the method used to assay glucuronyl transferase activity.

TLC OF DIELDRIN AND ITS METABOLITES

The radioactive compounds extracted from the feces, urine, organs, and tissues were purified by tlc by the use of various combinations of the mobile phases listed in Table I. Final purification was achieved by two-dimensional tlc using ether:hexane (1:1) and methylene chloride for F-1 and Klein's metabolite and hexane:acetone (1:1) and ether:hexane (9:1) for 6,7-*trans*-dihydroxydihydroaldrin and the unidentified polar metabolite. The R_f values listed in Table I were obtained after each of the compounds had undergone final purification.

RESULTS

Dieldrin Excretion In the Feces. In both male and female rats fed dieldrin-^{14}C approximately ten times as much radioactivity was excreted in the feces as in the urine. Three to four times as much radioactivity was extracted from the feces of the males as from those of the females, but in each case

Table II. Radioactivity Extracted from the Feces[a]

Day	% Total ^{14}C fed extracted from each daily sample		Compounds containing ^{14}C					
			% Dieldrin		% Polar metabolites		% Hydroxylated metabolite (F-1)	
	Male	Female	Male	Female	Male	Female	Male	Female
1	3.0	0.6	7.8	62.3	8.5	<1	83.7	37.4
2	6.1	2.1	2.6	47.9	14.9	4.0	82.3	47.9
3	3.6	1.7	1.7	15.7	18.8	3.9	79.4	80.2
4	1.8	0.9	1.4	18.8	18.3	2.9	80.2	78.2
5	2.4	0.7	1.9	24.6	5.5	3.7	92.5	71.5
6	1.7	0.8	2.0	17.7	4.8	5.3	93.1	76.8
7	2.8	0.5	2.4	34.3	3.6	1.9	93.9	63.7
8	1.5	0.5	2.6	25.7	4.4	2.8	92.8	71.3
9	1.6	0.5	1.3	18.9	7.8	3.7	90.7	77.3

[a] Approximately 25% of the radioactivity was not extracted from the feces.

most of the radioactivity was in the form of a metabolite designated F-1 by Matthews and Matsumura (1969) (Table II). The parent compound never represented a very high percent of the total radioactivity extracted from the feces of the male rats, but initially it represented better than 60% of the radioactivity extracted from the female feces and continued to represent near 20% throughout the course of the experiment. Two polar metabolites were extracted from the feces of both male and female rats. The more polar of the two cochromatographed with 6,7-*trans*-dihydroxydihydroaldrin synthesized in our laboratory. The second polar metabolite was detected in only trace amounts and has been included with *trans*-dihydroxydihydroaldrin under the heading "Polar metabolites" in Table II. These polar metabolites comprised a significant percent of the total radioactivity extracted from the feces collected from the male rats during the first 4 days, but after the fourth day and in all extractions of feces collected from the females, they were excreted in relatively small amounts. F-1 comprised from 70 to 80% of the radioactivity extracted from the fecal samples collected from the female rats by the third day and remained near this level throughout the course of the experiment.

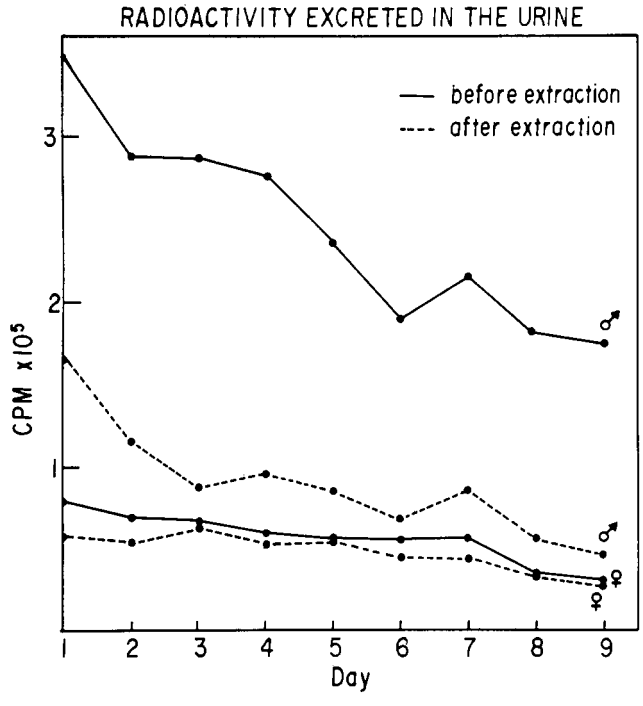

Figure 1. Radioactivity excreted in the urine by male and female rats fed dieldrin-^{14}C. Radioactivity in the urine before and after extraction with chloroform

Dieldrin Excretion In the Urine. As was observed in the fecal extractions, excretion of radioactivity in the urine was three to four times greater in male than in female rats fed dieldrin-^{14}C. Unlike the results obtained with extraction of the feces, the ratios of the major metabolites differed according to sex. Approximately 50 to 65% of the radioactivity excreted in the urine by male rats was extracted by chloroform. On the other hand, from 75 to greater than 95% of the radioactivity excreted in the urine of the female rats was not extracted into chloroform (Figure 1). The major portion of the radioactivity extracted from the urine of the male rats consisted of the dieldrin metabolite previously described as Klein's metabolite (Klein *et al.*, 1968). This metabolite constituted greater than 70% of the radioactivity extracted from the first daily sample of urine excreted by the male rats; this percent increased to 90 by the third day and to 97 or more after the sixth day of the experiment. The remainder of the radioactivity extracted from the urine of the male rats consisted primarily of dieldrin with traces of *trans*-dihydroxydihydroaldrin in the first two daily samples. The radioactivity extracted from the urine samples from the females consisted of approximately 20% dieldrin for the first two samples, but little or no dieldrin thereafter, and never consisted of more than a trace of Klein's metabolite. Most of the radioactivity extracted from the urine of the female rats was *trans*-dihydroxydihydroaldrin.

Dieldrin Extraction from the Organs and Tissues. Most of the organs and tissues from the female rats contained more radioactivity per gram than similar organs and tissues from the male rats (Table III). Exceptions to this generalization were the relatively large amounts of radioactivity extracted from the male kidneys and stomachs; the lungs and intestines of the males contained amounts of radioactivity comparable to those of the females. All other organs and the fat of the females contained three to four times the radioactivity of similar organs of the males. The form of the extracted compounds also differed with the sexes and among the organs of the males. With the exception of relatively small amounts of *trans*-dihydroxydihydroaldrin extracted from the stomachs and small intestine, the radioactivity extracted from the organs and tissues of the females was almost entirely dieldrin (Table III). On the other hand, the radioactivity extracted from the males varied from near 100% dieldrin from the brain and fat to 85% Klein's metabolite from the kidneys. *trans*-Dihydroxydihydroaldrin and the unidentified polar metabolite

Table III. Radioactivity Extracted from the Organs and Tissues

Organ or tissue	% Total ^{14}C fed extracted from each organ or tissue		Compounds containing ^{14}C					
			% Dieldrin		% Polar metabolites		% Klein's metabolites	
	Male	Female	Male	Female	Male	Female	Male	Female
Muscle	0.01/g	0.06/g	96	>99	<1	<1	3.5	<1
Fat	0.32/g	0.68/g	>99	>99	<1	<1	<1	<1
Liver	0.58	1.15	68	>99	<1	<1	31.2	<1
Lung	0.26	0.25	52	>99	<1	<1	47.1	<1
Heart	0.02	0.04	80	>99	<1	<1	20.0	<1
Kidney	1.33	0.14	13	>99	<1	<1	85.6	<1
Brain	0.03	0.07	>99	>99	<1	<1	<1	<1
Stomach	0.64[a]	0.24	18	76	22.1	24.2	24.9	<1
Small intestine	0.19	0.16	81	92	12.2	6.7	6.5	<1
Large intestine and cecum	0.19	0.17	52	>99	16.5	<1	31.0	<1
Testes	0.07		62		<1		38.0	

[a] Thirty-five percent of this radioactivity was in metabolite F-1; male stomachs were the only organs or tissues in which this compound was detected.

were extracted from the stomachs and intestines of the males, but the primary metabolite of dieldrin, F-1, was detected only in the stomachs of male rats.

IN VITRO METABOLISM OF DIELDRIN F-1-^{14}C

Dieldrin F-1-^{14}C (0.01 μg) was rapidly conjugated with glucuronic acid when incubated with male or female rat liver microsomes and UDPGA. Approximately 65% conversion of F-1 to its glucuronide occurred in 10 min with liver microsomes from either sex (Table IV). Incubations under carbon monoxide, nitrogen, or air gave essentially the same results, indicating oxidative reactions did not influence the results. Incubations minus either UDPGA or microsomes demonstrated the requirement for both glucuronyl transferase and cofactor for glucuronide formation of the F-1 metabolite (Table IV). Attempts to hydrolyze the proposed F-1 glucuronide with 1 *N* HCl or β-glucuronidase were unsuccessful.

Triton X-100, which markedly activates glucuronyl transferase activity towards *p*-nitrophenol and 1-naphthol, only slightly enhanced the rate of glucuronide conjugation of F-1. In this respect F-1 conjugation is similar to that of *O*-glucuronidation of testosterone which is also not enhanced by treatment of the microsomal fraction with detergent (Lucier and Matthews, 1971).

DISCUSSION

The results presented here indicate that the major excretion route for dieldrin and its metabolites in rats is in the feces. Metabolism and excretion of this insecticide was several times more rapid in male than in female rats and the difference probably lies in the greater ability of male rats to convert dieldrin to its more polar metabolites. The primary metabolite excreted by both sexes was the hydroxylated metabolite, F-1. This metabolite was rapidly conjugated *in vitro* by a glucuronyl transferase from the microsomal fraction of rat liver. The F-1 glucuronide was rather unique in that it was not hydrolyzed by a β-glcuronidase or 1 *N* HCl. Unfortunately, the design of this study does not allow us to speculate as to whether the F-1 extracted from the feces was excreted as a conjugate and subsequently hydrolyzed by microorganisms in the gut or excreted in the free form. It is likely, however, that the radioactivity which was not extracted from the feces represents glucuronide conjugates of F-1 and *trans*-dihydroxydihydroaldrin as well as other polar metabolites. *trans*-Dihydroxydihydroaldrin, a minor metabolite of dieldrin extracted from the feces, has also been shown to be conjugated with a glucuronic acid by rat liver enzymes (Matthews and Matsumura, 1969).

Male rats excreted three to four times as much radioactivity in the urine as did the females. The difference here appears to be the ability of the males to produce Klein's metabolite. This dieldrin metabolite which has also been shown to be produced by the mixed-function oxidases of male rat liver (Matthews and Matsumura, 1969) is also the major metabolite of photodieldrin excreted by male rats in the urine (Klein *et al.*, 1970). These results confirm the observation by Klein *et al.* (1970) that female rats excrete very little, if any, of this compound. The amount of radioactivity which was inextractable from the urine of the two sexes was much more nearly equal.

Since the male rats excreted much more radioactivity than did the females, it was expected that the organs and tissues of the females would contain a good deal more radioactivity than those of the males. This was generally the case with a few notable exceptions; the kidneys and stomachs of the males contained several times as much radioactivity as did those of the females, and the lungs and intestines contained comparable amounts. In almost every case the organs of the males contained a relatively large amount of Klein's metabolite; this metabolite was detected in only trace amounts in the female organs. The fact that Klein's metabolite was the only dieldrin metabolite detected in any organ or tissue of the males other than the stomach and intestines may indicate that it is readily produced in the males, but not readily excreted. The relatively large amount of this metabolite which was

Table IV. Conjugation of Dieldrin Metabolite F-1 with Glucuronic Acid

	Percentages F-1-^{14}C remaining after incubation with the following flask contents[a]			
	F-1	F-1 + UDPGA	F-1 + RLM	F-1 + UDPGA + RLM
Males	99.4 ± 3.3	93.5 ± 4.8	98.4 ± 5.3	34.4 ± 2.7
Females	103.0 ± 4.6	94.8 ± 3.1	98.0 ± 4.9	33.8 ± 2.0

[a] Percentages computed as an average of six incubations.

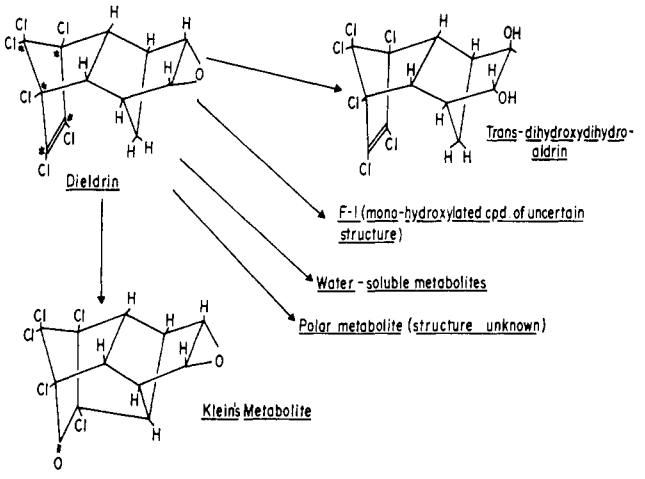

Figure 2. *Dieldrin Metabolism Excretion and Storage in Male and Female Rats* (* denotes the position of the ^{14}C). **Dieldrin, excreted in small amounts in the urine and feces of both sexes and extracted from every major organ and tissue of both sexes.** *trans*-**Dihydroxydihydroaldrin, a minor metabolite extracted from the urine, feces and gastrointestinal tract of both sexes. F-1, excreted in the feces exclusively, is the major metabolite of dieldrin excreted by both sexes. Water-soluble metabolite(s), excreted in the urine and feces of both sexes. Polar metabolite, extracted in trace amounts from the urine and feces of both sexes. Klein's metabolite, the urine and several organs of male rats contained large amounts of this compound, but it was detected in only trace amounts in female rats**

extracted from the male kidneys indicates this to be the case. Klein *et al.* (1968) reported this compound to be more toxic and faster acting against houseflies than the parent compound, dieldrin.

The presence of significant amounts of the polar metabolites in the stomach and intestines of both sexes and F-1 in the stomachs of the male rats may indicate that these compounds are produced in these organs as well as the liver, as demonstrated by Matthews and Matsumura (1969). On the other hand, it may indicate that these compounds are excreted in the bile as conjugates which are subsequently hydrolyzed by intestinal microbes and the metabolites are reabsorbed into the intestinal walls. The presence of these compounds in the stomachs could be explained if the small intestine were disconnected below the entrance of the bile duct.

The results obtained in this study indicate that the half-life of a small dose of dieldrin is approximately 1 week to 10 days in the male rat and at least three times that long in female rats. A summary of dieldrin metabolism and storage in male and female rats is presented in Figure 2.

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