Accumulation, Distribution, and Excretion of Mirex-^{14}C in Animals Exposed for Long Periods to the Insecticide in the Diet

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Mirex-^{14}C administered in the diets of laboratory rats, Japanese quail, and mosquito fish was retained at high levels in the body, and was not metabolized to any detectable extent. Maximum residue accumulation occurred in adipose tissue, and after 16 months of continuous treatment, mirex levels in the fat of rats and male birds were about 120- and 185-fold greater than dietary intake levels, respectively. Ratios of the levels of mirex in the tissues of rats and quail to those in the diet were approximately the same at feeding levels of 0.3, 3.0, and 30.0 ppm. No plateau in tissues was observed in either rats, male quail, or mosquito fish. Mirex residues in the tissues of female quail were much lower than in males, because the female birds eliminated very large mirex quantities through the egg yolk. Examination of several reproductive parameters, including egg hatch, chick growth, and survival, showed that high levels of mirex in the egg yolk, up to 200 ppm, caused no adverse effects. Dissipation rates of mirex from the body after removing the insecticide from the diet varied with the animal species. The half-life of the residues in the whole body of the fish was 130 days, while in the fat of female and male quail it was about 20 and 30 days, respectively. In the fat of female rats, the residues had declined by only 40% after being returned to a normal ration for 10 months.

Mirex (dodecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane) is a totally chlorinated organic insecticide prepared by the dimerization of hexachlorocyclopentadiene. The compound is used primarily for controlling populations of the imported fire ant (*Solenopsis spp.*) in several of the southeastern states.

Although mirex is used on a rather limited geographical basis and the amounts required for efficacy are quite low, this highly symmetrical, polycyclic chlorocarbon is very resistant to degradation by physical, chemical, and biochemical forces in the environment. Previous studies have shown mirex to be essentially unaffected by metabolic processes of higher animals and plants (Dorough and Ivie, 1974; Gibson *et al.*, 1972; Ivie *et al.*, 1974; Mehendale *et al.*, 1972), although certain microorganisms appear capable of degrading mirex at a very slow rate (Andrade and Wheeler, 1973). Mirex also degrades very slowly in sunlight (Gibson *et al.*, 1972) and release of the compound into the environment would likely result in residues persisting unchanged for extended periods.

Because mirex is a stable and highly lipophilic compound, it would be expected to interact with the biota in regions where it is used, and indeed the occurrence of mirex residues in tissues of a variety of organisms is well documented (Markin *et al.*, 1972). However, controlled experiments are lacking relative to the dynamics of mirex absorption, distribution, and excretion by animals exposed chronically to the insecticide. The current studies were initiated to consider these factors in rats, birds, and fish. Such information is necessary if the environmental impact of mirex is to be properly interpreted.

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METHODS AND MATERIALS

Treatment. *Rats.* Ninety-six female albino rats (Sprague-Dawley, 175 g) were divided into 8 groups of 12 animals each. The rats were placed in holding cages and two groups each were given feed containing 0, 0.3, 3.0, and 30.0 ppm of mirex.

The treated feed was prepared by adding acetone solutions of mirex-^{14}C to commercial laboratory meal (Ralston-Purina Co.) and mixing thoroughly in a Hobart mixer. The 0.3-ppm level was prepared using uniformly labeled mirex-^{14}C (6.34 mCi/mmol, Mallinckrodt Chemical Works, St. Louis, Mo.) without dilution with unlabeled mirex. However, in mixing feed for the 3.0- and 30.0-ppm treatment levels, the mirex-^{14}C was diluted tenfold with authentic mirex (Allied Chemical Corp., Baltimore, Md.) to a specific activity of 0.634 mCi/mmol. The treated feed contained 7740 dpm/g at the 0.3- and 3.0-ppm feeding levels, and 77,400 dpm/g at the 30.0-ppm level. The tenfold increase in radiocarbon content of the 30.0-ppm feed was chosen to provide greater sensitivity at this level. After being exposed to mirex in the diet for 6 and 12 months, selected groups of animals were removed from treatment and placed on control feed.

Quail. Newly hatched Japanese quail (*Coturnix coturnix japonica*) were obtained from a flock maintained at the Department of Animal Sciences, University of Kentucky. These birds have for several years exhibited an excellent record of egg production, fertility, hatchability, chick growth, and survival.

The newly hatched quail were divided into eight groups of 50 chicks each, and two groups each were provided a Purina Startina diet containing mirex-^{14}C (5.68 mCi/mmol, Mallinckrodt Chemical Works) at levels of 0, 0.3, 3.0, and 30.0 ppm. At 6 weeks of age, the birds were transferred to a commercial laying mash diet containing the radioactive mirex.

The treated food was prepared by mixing acetone solutions of radiolabeled mirex with the feed. Mirex-^{14}C was used without dilution with unlabeled mirex to give feed containing 0.3 ppm of mirex (6900 dpm/g). In the diets containing higher mirex concentrations, the radiocarbon content of the feed was maintained at 6900 dpm/g, but sufficient unlabeled mirex was added to adjust the total mirex levels to 3.0 and 30.0 ppm.

Small samples of each batch of treated food from both

the rat and quail studies were analyzed by direct liquid scintillation counting to ascertain that the diets contained the appropriate levels of mirex. Over the entire length of the study, the radiocarbon content of all feeds did not vary more than ±2%.

Fish. Mosquito fish, *Gambusia affinis,* were seined locally and maintained in aerated 1-gal glass bowls. The fish were fed a commercial diet for 7 days before treatment was initiated. Then 80 fish were transferred to fresh water and placed on a diet fortified with 50 ppm of mirex-^{14}C. The treated feed was prepared by adding mirex-^{14}C in acetone to the commercial fish food and grinding in a mortar and pestle until dry. Food fed to control fish was similarly treated with acetone only. After 15 days of treatment, half of the fish were removed and placed in another bowl where they were fed untreated food.

Sampling. At designated intervals, two rats were removed from each holding cage and placed in metabolism cages for 24 hr. Total feces and urine voided were quantitated and frozen until analyzed. At appropriate intervals, two rats were sacrificed and samples of brain, muscle, liver, kidney, fat, skin, and hair were removed for radioassay, extraction, and analysis.

Feces and eggs of the quail were collected for analysis, and food intake and excretion were quantitated. Periodically, two males and two females from each feeding level were sacrificed and samples of brain, muscle, liver, kidney, neck fat, skin, and feathers were removed for radioassay, extraction, and analysis.

Three fish were removed for analysis of total radiocarbon content at each of several sampling times throughout the experiment. Fish fed the mirex diet for 56 days were extracted and the nature of the radioactivity investigated.

Quail Reproduction Studies. When the birds reached 5 weeks of age, 15 females and 5 males were transferred from the control and each of the three treatment levels to cages separate from the remaining birds. Records were kept of egg production in this designated laying flock, and the eggs from these birds were used in all subsequent studies involving reproduction. The remaining birds, and the eggs obtained from them, were used for routine analyses of radioactive residues in the tissues and eggs.

Two hatchability studies were conducted to determine the effects of feeding graded levels of mirex to breeding Japanese quail on fertility, hatchability of fertile eggs, and initial chick weight. Chicks from the second hatchability study were raised to maturity to investigate the carry-over effects of mirex in the breeding diet on survival and reproductive performance of the progeny.

For the hatchability studies, 100 eggs were collected from each of the treatment groups during the 15th and 16th weeks of the mirex feeding experiment. The eggs from each group were identified and set in modified setting trays in a Jamesway incubator. At the end of the 14th day of incubation, the eggs from each group were transferred to separate, covered hatching baskets. The live chicks from each group were removed and counted on the 16th, 17th, and 18th days. At the end of the 18th day of incubation, all unhatched eggs were removed from the incubator and the eggs broken to determine fertility and to examine the dead embryos for possible defects and/or to determine the stage of development of the embryos.

In the progeny test, the chicks hatched from each experimental group were weighed and placed in separate pens in an electrically heated battery brooder. All groups were fed the same nontreated starting and growing diets until the age of 34 days. The birds were then weighed and 18 females and 6 males were selected and placed in separate breeding pens and fed a nontreated laying diet. Mortality and egg production records were obtained over a 3-month period for each pen. Eggs from each pen were set weekly to obtain fertility and hatchability data.

Radioassay. Quantitative radioassays were performed with a Packard Tri-Carb Model 3380/544 scintillation counter. Solid samples, 250–500 mg of tissue, feces, whole gambusia, etc. were combusted in a Beckman Biological Material Oxidizer (BMO) and the $^{14}CO_2$ trapped by passing the combustion gases through a 2:1 solution of 2-methoxyethanol and 2-aminoethanol. An aliquot of trapping solution, 4 ml, was pipetted into 15 ml of scintillation fluid (3a70B, Research Products International, Elk Grove Village, Ill.) and quantitated by liquid scintillation counting. The efficiency of the oxidizer was determined during each run by combustion of control samples fortified with mirex-^{14}C. All values were corrected.

Materials such as rat urine (0.5 ml), egg yolk (100 mg), egg white (100 mg), quail feces (50 mg), and tissue extracts (0.2 ml) were radioassayed by placing the material directly into 15 ml of scintillation fluid. Oxygen combustion of egg yolk, egg white, quail feces, and lyophilized rat

Table I. Mirex-^{14}C Equivalents in the Fat of Rats and Quail Fed 0.3, 3.0, and 30 ppm of Mirex-^{14}C in the Diet

	Ppm of mirex-^{14}C equivalents at indicated feeding level					
	Rats (female)			Quail (male)[a]		
Time	0.3	3.0	30	0.3	3.0	30
Animals on Treatment						
4 days				3.5	32	291
7 days	1.6	12	81	6.0	42	560
1 month	3.3	38	332	9.4	100	984
6 months	19.0	211	2047	30.4	355	3450
12 months	32.8	286	2808	47.3	450	4820
16 months	36.0	371	3730	56.4	546	5529
Removed from Treatment after 6 Months						
7 days	19.3	201	1980	31.0	304	3160
14 days	18.7	191	1907	19.5	212	2290
1 month	16.5	163	1670	14.2	139	1439
3 months	14.7	145	1490	7.4	71	702
6 months	14.3	143	1440	3.1	29	320
12 months	13.7	131	1340	4.2	25	270
Removed from Treatment after 12 Months						
1.5 months				32.7	362	3500
3 months				27.9	287	2940

[a] Data from males given here for comparison with rats since egg production greatly influence residue levels in the fat of female birds.

Table II. Radioactive Residues in the Tissues of Rats Fed 30.0 ppm of Mirex in the Diet

Time	Ppm of mirex equivalents[a]						
	Brain	Muscle	Liver	Kidney	Skin	Subcutaneous tissue[b]	Hair
			Rats on Treatment				
7 days	5.5	5.5	15.7	9.6	25.7	33.4	6.1
14 days	6.6	50.0	32.0	11.1	40.9	138.7	9.7
1 month	8.6	6.4	38.9	12.7	82.4	170.5	5.4
6 months	18.1	9.1	63.4	22.1	229.6	211.3	11.8
12 months	23.9	26.5	98.9	34.3	374.5	278.7	22.6
15 months	36.9	49.5	158.0	52.1	421.6	321.3	25.2
			Removed from Treatment after 6 Months				
7 days	15.7	8.6	61.2	18.0	231.3	204.6	6.7
14 days	14.2	8.6	58.6	16.9	220.9	189.0	7.3
1 month	12.6	7.4	55.5	14.9	216.7	198.2	6.5
3 months	11.0	6.6	42.8	12.5	176.2	40.7	5.3
6 months	10.3	6.4	32.8	10.8	184.4	67.2	5.4
12 months	9.9	6.3	25.2	10.1	133.6	55.7	4.9

[a] Ratios of the levels of residues in the tissues to those in the diet were the same for all three feeding levels, 0.3, 3.0, and 30.0 ppm. [b] Connective tissue between epidermis and muscle.

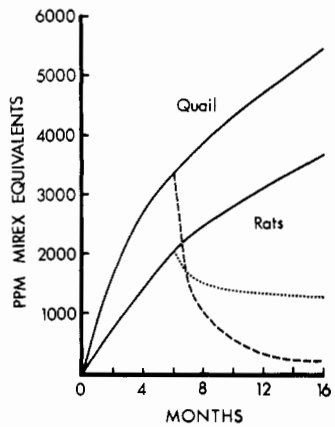

Figure 1. Radioactive residues in fat of male Japanese quail and female rats fed 30.0 ppm of mirex-^{14}C in the diet. Solid lines show residues in animals on mirex-containing diet. Broken lines show residues in animals removed from treated diet after 6 months. Ratios of the levels of residues in the fat to those in the diet were essentially identical for all three feeding levels (0.3, 3.0, and 30.0 ppm).

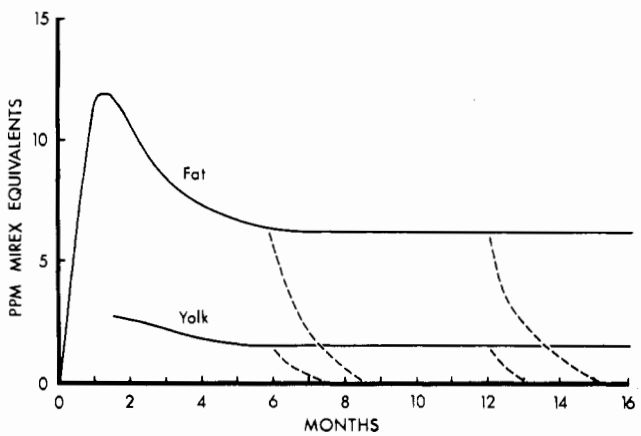

Figure 2. Radioactive residues in egg yolk and fat of female Japanese quail fed 0.3 ppm of mirex-^{14}C in the diet. Solid line shows residues in animals on mirex-containing diet. Broken line shows residues in animals removed from treated diet at 6 and 12 months. Ratios of the levels of residues in the fat and yolk to those in the diet were the same for feeding levels of 0.3, 3.0, and 30 ppm of mirex.

urine confirmed that direct scintillation counting was an efficient means of determining the radiocarbon contained therein.

Radioactivity on thin-layer chromatography (tlc) plates was located by radioautography using Kodak or du Pont medical X-ray film.

Extraction and Analysis. All materials except urine were extracted directly with acetone. Samples were homogenized in acetone using a Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.). Centrifugation yielded the acetone extract which was decanted and solid material which was then extracted three more times with acetone. Radioassay of the combined acetone extracts was accomplished by direct liquid scintillation counting while the radioactivity in the solids was determined by oxygen combustion. The urine was extracted thoroughly with ethyl acetate and both phases radioassayed.

The extracts were concentrated and a portion was applied to tlc plates (Merck silica gel F-254 chromatoplates) and developed in heptane. The remaining portion of each extract was subjected to cleanup using a Florisil–aluminum oxide column (Gibson *et al.*, 1972) prior to gas chromatography and mass spectral analysis.

Whole body analysis was accomplished by chopping the whole animals, excluding those samples removed for oxygen combustion and extraction, in a Waring Blendor. Samples of the slurry were radioassayed by oxygen combustion, and the homogenate was extracted and analyzed as described above.

Extracts were analyzed in two different gas–liquid chromatography (glc) systems equipped with electron capture detectors. The columns and operating parameters were: (1) Varian Aerograph Model 1400, 5 ft × ⅛ in. stainless steel column packed with 2% SP 2401 on 100–120 mesh Chromosorb W-HP, injector temperature 210°, column 195°, detector 215°; (2) Varian Aerograph Model 1700, 10 ft × 0.25 in. Pyrex column packed with 5% OV-101 on 100–120 mesh gas Chrom Q, injector 285°, column 200°, and detector 215°.

Mass spectra were determined on a Finnigan Series 1015C gas chromatograph–mass spectrometer with a 2-ft Pyrex column of 1% OV-17 on Chromosorb Q, column 200°, injector 230°, and mass spectrometer manifold temperature of 140°.

Fate of Mirex-^{14}C Photoproducts in Rats. Studies were conducted to determine the patterns of uptake, distribution, and excretion of mirex photoproducts when administered to rats. Mirex-^{14}C (50 μCi, 5.68 mCi/mmol) was dissolved in acetone and streaked over Eastman

Table III. ^{14}C Residues in the Tissues of Japanese Quail Fed 30 ppm of Mirex-^{14}C in the Diet

	Ppm of mirex equivalents											
	Brain		Muscle		Liver		Kidney		Skin		Feathers	
Time	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Birds on Treatment												
4 days[a]	1.6		4.9		34.3		7.1		48.8		5.9	
7 days	2.5		5.9		47.4		9.4		62.6		14.7	
14 days	2.2	2.4	5.6	5.4	16.7	23.5	12.1	12.1	137.7	180.7	15.5	8.8
1 month	3.3	3.5	8.0	12.4	30.2	30.5	21.1	13.3	233.2	130.4	25.7	30.4
6 months	4.9	1.4	50.6	10.0	55.1	55.7	58.2	18.3	600.5	92.8	36.2	26.5
12 months	7.4	1.9	72.8	7.9	114.3	25.5	64.0	10.4	1495.4	99.1	196.7	39.6
16 months	7.2	2.2	87.0	11.4	138.0	30.9	88.9	11.6	872.8	129.0	114.8	33.0
Removed from Treatment after 6 Months												
7 days	5.0	2.3	45.8	6.8	62.4	18.2	52.5	5.9	906.1	100.9	47.4	15.8
14 days	5.1	3.1	38.2	5.9	52.3	19.3	61.0	4.6	1028.3	77.9	51.7	8.8
1 month	5.2	1.7	45.2	4.5	51.2	7.1	42.4	2.5	818.2	81.1	40.9	14.3
3 months	0.4	0.5	14.4	1.4	8.6	1.5	4.9	0.6	765.3	40.7	33.5	7.3
6 months	0.2	0.2	3.5	0.5	6.5	0.5	3.5	0.2	620.1	19.5	29.4	5.6
10 months	0.1	0.1	5.0	0.1	5.4	0.1	2.9	0.1	575.8	1.4	28.4	1.9

[a] Sex of quail could not be determined at this age.

Chromatogram Sheets (Type 6061, 0.1-mm gel thickness, Eastman Kodak Co., Rochester, N. Y.) at a concentration of about 0.1 μCi/cm^2. The plates were exposed to germicidal lamps (General Electric G15T8, 5 cm lamp to subject) for 24 hr. The gel was extracted with methanol, and the extracts spotted as a band on a silica gel tlc plate. Development of the plates in heptane and subsequent autoradiography indicated that the radiocarbon consisted of 26% unchanged mirex, 35% of photoproducts that migrated above the origin (nonpolar photoproducts), and 29% of products that remained at the origin (polar photoproducts).

Two-dimensional tlc (heptane; hexane–acetone, 4:1) indicated the nonpolar photoproducts to consist of five components, 70% of which was composed of a photoproduct previously reported (Layton and Alley, 1973; Gibson *et al.*, 1972). Two-dimensional tlc of the polar mirex photoproducts (chloroform–methanol, 2:1; ether–hexane, 2:1) showed at least five components of this mixture, and about 40% of the total remained at the origin.

Two groups of three female rats (Sprague-Dawley, 200 g) each received treatment on 7 consecutive days with either the polar or nonpolar mirex-^{14}C photoproducts. The compounds (0.2 μCi in 0.1 ml of dimethyl sulfoxide/day) were administered orally by stomach tube to the lightly etherized animals. The dosage levels were equivalent to approximately 0.1 mg/kg of mirex equivalents per day. The animals were held in individual metabolism cages to allow separate collection of urine and feces. One rat from each group was sacrificed 1 day after the final treatment, and the remaining two rats from each treatment were sacrificed 7 days later. Radiocarbon in urine was analyzed by liquid scintillation counting; feces and tissue samples were analyzed by oxygen combustion.

RESULTS

Observations on Animal Health. Throughout these studies animals were observed for any indication that extended mirex treatment resulted in changes in general health status. During the entire time of the rat study, only two test animals and three control animals developed abnormalities. The test rats exhibited bleeding in the region of the external gentilia and severe abdominal swelling. Both animals were being fed 3.0 ppm of mirex-^{14}C in their diets. The first animal developed these symptoms 15 months into the study and was sacrificed. The second animal died after 16 months of treatment. The uterus of each animal was enlarged. During this same period three control animals developed large growths in the lateral abdominal area and were sacrificed. Nearly equal numbers of test and control animals died during the quail and fish studies, and there were no indications of toxicity in any of the treatment groups.

Table IV. Elimination of Mirex-^{14}C Equivalents *via* the Feces from Rats and Male Quail Fed 30 ppm of Mirex-^{14}C in the Diet

Time on treatment	Cumulative % of total radioactivity consumed[a]	
	Rats[b]	Quail[b]
1 day	24.2	15.8
7 days	30.1	20.8
1 mo.	28.2	26.2
6 mo.	27.5	27.6
12 mo.	26.4	27.9
16 mo.	26.3	25.4

[a] % = cumulative μg eliminated/cumulative μg consumed. [b] Percentages of the doses eliminated were the same in animals fed 0.3 and 3.0 ppm of mirex in the diet.

Table V. Radiocarbon Residues in the Body of *Gambusia affinis* Fed 50 ppm of Mirex in the Diet

Days	Ppm of mirex equivalents in whole body	
	On treatment	When removed from treatment after 15 days
1	1.5	24.1
3	5.4	24.5
6	11.0	25.7
9	18.1	22.1
15	25.0	23.6
21	29.7	20.0
28	35.0	19.4
42	47.4	17.8
56	52.6	16.7
130		11.6
200		7.3

Accumulation, Distribution, and Dissipation of Mirex-^{14}C Equivalents. *Rats.* Radiocarbon was rapidly absorbed and was retained by tissues of rats following consumption of diets containing mirex-^{14}C (Figure 1, Tables I and II). Residues reached very high levels in adipose tissue, and in rats maintained on the mirex diets for 16 months, mirex equivalents in the fat were approximately

Table VI. Radioactive Residues Remaining in Tissue Solids after Extraction with Acetone

Tissue	% of total ^{14}C residues in sample[a]	
	Rats	Quail
Brain	1.0	*b*
Muscle	1.0	1.0
Liver	3.0	0.3
Kidney	8.0	*b*
Fat	1.0	0.3
Skin	0.5	0.8
Feces	8.0	6.8
Whole body	0.3	0.5
Egg yolk		1.0

[a] Averages of all extractions conducted during study. No differences were noted in the extractability of residues from tissues of animals fed different levels of mirex in the diet or for different periods of time. These data apply to both male and female quail. [b] Total tissues required for radioassay.

Table VII. Radiocarbon Excretion in Urine and Feces of Rats Receiving Daily Oral Administration of Mirex-^{14}C Photoproducts for 7 Days[a]

Days	Cumulative % of total administered radiocarbon			
	Polar photoproducts		Nonpolar photoproducts	
	Urine	Feces	Urine	Feces
On treatment				
1	10.4	39.1	0.1	14.8
3	10.1	51.1	0.1	17.1
5	10.2	58.4	0.3	17.8
7	10.2	59.5	0.3	18.8
Off treatment				
2	10.8	64.0	0.3	21.1
4	10.8	65.8	0.3	21.4
7	11.0	69.6	0.3	21.6

[a] 0.1 mg/kg of mirex equivalents per day.

120-fold higher than corresponding dietary intake levels (Figure 1). Although radiocarbon residues reached considerably higher concentrations in fat, all tissues studied contained appreciable quantities of radioactivity. Skin and subcutaneous tissue, containing large quantities of fat, accumulated substantially higher residues than most of the other tissues (Table II). No plateau of residue accumulation was observed in any tissue during the feeding period.

Ratios of the level of residues in tissues to those in the diet were essentially identical for all three feeding groups. Thus, 16-month exposure of rats to 0.3 ppm of dietary mirex resulted in residues in the fat of 36-ppm mirex equivalents, whereas corresponding residues resulting from feeding 3.0 and 30.0 ppm of mirex were 10- and 100-fold higher (Table I).

Radiocarbon dissipated very slowly from the tissues following removal of rats from the mirex diets (Figure 1, Tables I and II). Analysis of tissues from animals provided mirex-treated food for 6 months, then removed from treatment for additional periods of up to 10 months, indicated that one-half or less of the residues in most tissues had been eliminated 10 months after treatment stopped.

Quail. Japanese quail exhibited a pattern similar to rats for the accumulation and retention of mirex residues following dietary exposure to the radiolabeled insecticide (Figure 1, Tables I and III). Maximum residues occurred in the fat and, on a parts per million basis, reached considerably higher levels in the fat of male quail than in that of rats exposed under comparable parameters. Feed consumption by quail, and thus mirex intake, was about 2.5-fold greater than that of rats (approximately 25 g/100 g of body weight per day for quail *vs.* 10 g/100 g of body weight per day for rats), but excretion patterns were similar for rats and quail (Table IV). Thus, the considerably higher residue levels observed in the adipose tissues of male quail were not surprising. After 16 months continuous mirex treatment, radiocarbon residues in the fat of male quail were almost 200-fold higher than dietary intake concentrations and showed little tendency toward reaching a plateau of residue accumulation (Figure 1). Other tissues analyzed (Table III) revealed much lower radiocarbon than in fat, and, as in the rat study, skin showed high radiocarbon concentrations. The quail studies were also similar to the findings with rats in that ratios of the levels of residues in the tissues to those in the diet were essentially the same for all three feeding levels at all analysis intervals.

Differences in tissue residue patterns were observed between male and female quail (Table III). Once the females began producing eggs (approximately 6 weeks of age), residues in all tissues were substantially lower than in males at all subsequent analysis intervals. In the fat, particularly, radiocarbon residues showed spectacular differences between the two sexes (Figure 2, Table I). An equilibrium between mirex intake and elimination through the eggs was rapidly established, resulting in a plateau of residue levels in all tissues (Figure 2, Table III). The male quail, which were unable to efficiently eliminate the absorbed radiocarbon, continued to accumulate residues in most of the organs throughout the study.

Female quail were considerably more efficient than males in eliminating existing body burdens of radiocarbon following cessation of mirex-^{14}C treatment (Figure 2, Tables I and III). This again was attributed to elimination of residues through the egg yolk of the female birds. Females removed from mirex-^{14}C diets after 6 or 12 months of treatment eliminated the existing residue burden at a relatively rapid rate, and after 3 months, residual radiocarbon in most tissues was reduced by at least 75% (Figure 2, Table II). Although the male birds could not eliminate mirex residues as efficiently as females, they were nevertheless capable of excreting most of the material within a few months after mirex treatment had stopped. Whereas about 40% of the mirex-^{14}C equivalents in most tissues of the rats had been excreted 10 months after treatment had stopped, in male quail the comparable period for most tissues was not more than 3 months. However, radiocarbon residues in skin and feathers of both male and female birds were considerably more persistent.

Fish. Feeding diets containing mirex-^{14}C to *Gambusia* resulted in retention of radioactive residues within the body (Table V). The levels of radiocarbon retained increased with time of exposure and, after 56-days treatment, whole body levels were approximately 50 ppm mirex equivalents. Removal of fish from the treated diets after 15-days exposure resulted in slow dissipation of body residues; 30 days were required for the residues to dissipate by 50%. After 200 days, about 25% of the original body burden was still retained by the fish.

Excretion of Residues by Rats and Quail. During the period of mirex treatment, both rats and quail excreted approximately 25% of the total ingested radiocarbon by way of the feces (Table IV). Radioassay of urine throughout the rat studies indicated negligible excretion of radioactivity *via* this route, and thus the major portion of total mirex consumed by rats and male quail was retained by the body tissues. This high retention of ingested radiocarbon is consistent with the very high tissue residues observed.

^{14}C residues in tissues of female quail reached plateaus

Table VIII. Radiocarbon Residues in Tissues of Rats Receiving Daily Oral Administration of Mirex-^{14}C Photoproducts for 7 Days[a]

	Ppm of mirex equivalents at indicated days after final treatment			
	Polar photoproducts		Nonpolar photoproducts	
Tissue	0	7	0	7
Brain	0.07	0.09	0.08	0.07
Muscle	0.06	<0.01	0.06	0.04
Liver	2.37	1.64	0.45	0.29
Kidney	0.20	0.11	0.14	0.04
Fat	0.19	0.13	4.07	7.39

[a] 0.1 mg/kg of mirex equivalents per day.

soon after egg laying began (Figure 2, Table III). Since relatively low amounts of ingested radioactivity were excreted in the feces, it was apparent that most of the consumed radiocarbon was eliminated through the egg yolk, as egg white and shell contained only trace amounts of residues. At full egg production and after tissue residues had reached plateaus, an equivalent of about 70% of the total daily dietary mirex was eliminated through the eggs.

Although quantitative data were not obtained on the excretion of radiocarbon residues in the feces of rats and quail removed from mirex treatment after 6 months, combustion of selected feces sampled indicated that mirex residues continued to be excreted in the feces of these animals for at least 10 months. These observations were consistent with the slow dissipation of mirex residues from the tissues.

Chemical Nature of Radiocarbon Residues. Although previous studies have indicated that mirex is highly resistant to metabolic attack by rats and quail following administration of single oral doses (Gibson *et al.*, 1972; Ivie *et al.*, 1974; Mehendale *et al.*, 1972), radiocarbon residues in the current study were examined to determine if prolonged exposure of animals to mirex might result in some degree of metabolism. Both the monohydro product generated by sewage sludge microorganisms (1,2,3,4,5,5,6,7,8,9,10-undecachloropentacyclo-[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane) (Andrade and Wheeler, 1973) and the very similar monohydro mirex photoproduct (1,2,3,4,5,5,6,7,9,10,10-undecachloropentacyclo-[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane (Layton and Alley, 1973; Gibson *et al.*, 1972) were considered as likely potential metabolites, and thus the possibility that these compounds might be generated by rats, quail, or fish was considered. The tlc and glc chromatographic systems employed in these studies gave satisfactory resolution of these products from mirex.

Extraction procedures were developed to allow maximum possible recovery of radiocarbon from the tissues and excreta so that a quantitative determination of the chemical nature of residues might be made. Radiocarbon recoveries from tissues and eggs of quail and of whole *Gambusia* were on the order of 99%, as were recoveries from all tissues of rats except liver and kidney (Table VI). Recoveries from rat and quail feces and rat kidney samples were about 92–93%. In every case, tlc and glc examination of the extracted radioactivity revealed only a single compound which corresponded in chromatographic behavior to mirex. Analysis of selected samples by mass spectrometry confirmed that the residue was indeed the unmetabolized parent compound. Although it is possible that a small per cent of the radiocarbon which was not extractable from these samples may represent metabolic products, this could not be verified.

Fate of Mirex-^{14}C Photoproducts in Rats. Administration of polar and nonpolar mirex-^{14}C photoproducts to rats resulted in considerably different patterns of radiocarbon excretion in urine and feces (Table VII). One week after the final treatment with the polar compound, about 80% of the total administered radioactivity had been eliminated, and most of this was through the feces. Low levels of residues were still being excreted at the time the animals were sacrificed, and thus it seems likely that the residual 20% of radiocarbon from the polar photoproduct treatment would have been excreted even more completely had the studies continued further. Only negligible levels of radioactivity appeared in urine following treatment of rats with the nonpolar photoproducts (Table VII). Elimination by way of the feces was essentially complete within a week after the final treatment, and only about 20% of the total administered radioactivity was excreted by this route. Thus, about 80% of the dose remained in the body when the study was terminated.

Table IX. Effect of Mirex in Breeding Diet on Fertility, Hatchability, and Average Chick Weight at Hatching

Mirex level of diet, ppm	Fertility, %	Hatchability of fertile eggs, %	Average chick weight, g
0	92.77[a]	77.92[a]	6.36[a]
0.3	94.19	79.63	6.52
3.0	87.79	82.78	6.47
30.0	89.20	78.98	6.49

[a] Means of each of the three treatment groups did not differ significantly from controls ($P \leq 0.05$).

Tissue analyses (Table VIII) indicated that the polar photoproducts were retained much more by the liver than by other tissues studied. One week after the final dose, however, levels in all tissues had declined considerably. Radiocarbon retained in the body following treatment with the nonpolar mirex-^{14}C photoproducts was localized primarily in the fat (Table VIII). Rats sacrificed 1 week after the final treatment showed reduced residues in all tissues except fat. Residues in the fat appeared to increase after 7 days; however, this was not conclusive since the data were obtained from so few animals.

Quail Reproduction Studies. Consumption of diets containing mirex-^{14}C for periods as long as 16 months resulted in no apparent effects on egg production in Japanese quail. Females from control and each of the three treatment groups began laying during the sixth week, and were in full production by 10 weeks of age. There were no significant differences in egg production or egg weight between control and treatment groups at any time during the study.

The data in Table IX are the combined results of the two hatchability studies. Chi-square analysis for treatment effects indicated no significant differences in fertility, hatchability, or chick weight between control and any of the treatment groups. Observations on the time of hatch and embryonic development did not reveal any treatment effect on the length of hatching time or on embryonic abnormalities.

The results of the progeny test are shown in Table X. The data indicate that progeny survival during the prelay and the laying period was not influenced by the feeding of mirex to the parent birds. Statistical analysis of the egg production and fertility data shows that the feeding of mirex to the parent birds did not affect these characteristics in the offspring. The hatchability data did indicate that the progeny of quail fed 0.3 ppm of mirex produced eggs that hatched significantly less than did the progeny of control birds. This difference is apparently due to factors other than the mirex treatment because the feeding of the two higher mirex levels (3.0 and 30.0 ppm) did not affect the hatchability of fertile eggs.

The high mirex levels in the eggs, and the extreme metabolic stability of the compound, suggested that residues

Table X. Effect of Mirex in Breeding Diet on Progeny Mortality and Reproductive Performance in Quail

Mirex level, ppm	Mortality to 34 days	Mortality laying phase	Hen day egg production, %	Fertility, %	Hatchability of fertile eggs
0	3/47[a]	3/18	59.50	88.12	68.35
0.3	2/67	1/18	58.10	88.83	61.88[b]
3.0	6/68	0/18	56.40	90.67	68.05
30.0	3/66	0/18	55.20	91.24	69.75

[a] Number of birds that died out of the number started. [b] This value significantly different ($P \leq 0.05$) than control.

Table XI. Mirex-^{14}C Equivalents in the Tissues and Egg Yolk of Japanese Quail Hatched from Eggs Laid by Birds Fed 30 ppm of Mirex-^{14}C in the Diet for 4 Months[a]

Age of birds, weeks	Ppm of mirex equivalents[b]						
	Liver		Kidney		Fat		
	Male	Female	Male	Female	Male	Female	Egg yolk
1	31.4[c]		*d*		1076.9[c]		*d*
2	4.2	4.6	9.5	5.6	301.9	368.4	
3	4.0	3.5	1.3	2.9	125.6	134.5	
4	0.1	4.0	1.2	2.5	58.2	73.6	
6	0.1	2.3	3.2	1.6	56.9	63.2	5.9
8	2.1	1.2	2.6	2.4	19.1	16.0	3.4
12	1.1	1.0	2.0	2.2	22.0	8.3	1.2

[a] No residues were detected in the brain and muscle. [b] Data from the 0.3- and 3.0-ppm feeding levels were 1/100th and 1/10th the levels shown here. [c] Sex of bird could not be determined. [d] Prior to laying age.

would be detectable in the F_1 generation chicks. Indeed, residues in certain tissues of these chicks were quite high (Table XI). One-week-old chicks hatched from eggs of parents fed 30 ppm of dietary mirex exhibited body fat residues of greater than 1000 ppm of mirex. The abdominal fat analyzed in the young chicks contained the absorbed yolk sac, which most likely contributed to the high mirex residues observed. Because the chicks were not further exposed to the insecticide, residues rapidly decreased on a parts per million basis due to chick growth and concurrent dilution and probably excretion of residues. However, when the female chicks reached laying age, significant amounts of mirex were observed in the egg yolk, and these residues continued to be detectable through at least the first 6 weeks of egg production (Table XI).

DISCUSSION

The current studies involving extended dietary exposure of mirex to rats, quail, and fish give further indication that this insecticide is highly stable to biochemical degradation, and also demonstrate that mirex shows very high potential for accumulation in certain vertebrate tissues. The use of uniformly labeled mirex-^{14}C in these studies permitted the detection of virtually any type of degradation product, but hundreds of analyses over the 16-month period of this investigation showed no indication of mirex metabolism. Only the identity of the low levels of radiocarbon not extractable from certain tissues (Table VI) remains uncertain. However, even if these residues consist in part of metabolic products of mirex, it seems apparent from these and earlier studies that mirex is probably the most biochemically stable organic pesticide known.

Mirex exhibited a very great tendency toward accumulation at high levels in adipose tissues of rats and quail. The mirex concentrations in fat of rats and male birds after 16 months of continuous treatment were about 120- and 200-fold greater than dietary intake levels, respectively. Perhaps more important, there was no indication of a plateau in tissue residue concentration when the study was terminated (Figure 1, Tables I–III). Thus, the data from even these extended studies do not permit an estimation of maximum tissue residues which might be obtained.

The levels of mirex residues accumulated by rats and birds in these studies were considerably higher than those reported for other common lipophilic pesticides. Dietary exposure of DDT to rats (Ortega *et al.*, 1956) resulted in DDT-DDE residues in the body fat about 5- to 25-fold higher than dietary intake levels. In most cases, a plateau of residues was observed within 6 months. Dieldrin levels in the fat of rats reached a plateau after about 1 month of dietary treatment, and maximum residues in the fat were only about fivefold higher than those in the diet (Diechmann *et al.*, 1968).

Following cessation of treatment, body burdens of mirex in rats dissipate at a much slower rate than does either DDT or dieldrin. Only about 40% of the mirex in most rat tissues was dissipated during a 10-month period after treatment stopped (Figure 1, Table II), while in the DDT studies cited above, the comparable period for dissipation of DDT residues in fat of rats was shown to be on the order of 3 months. Dieldrin residues in adipose tissue of rats had a half-life of only 1–2 weeks following cessation of treatment (Robinson *et al.*, 1969).

Studies with female quail indicate that dietary mirex will be readily excreted at high levels through the eggs of birds, which confirms our earlier studies involving administration of single mirex doses to quail (Ivie *et al.*, 1974). Because much of the consumed mirex is eliminated in the egg yolk of female birds in full egg production, it is possible that consumption of even very low mirex levels by birds may result in significant mirex residues in the eggs.

It was demonstrated that consumption of mirex by female quail results in residues appearing in the F_1 generation, and that without additional mirex treatment the F_1 birds pass significant mirex residues into the egg yolk. This in itself is an interesting analytical exercise, but it further indicates the potential interactions of mirex with the reproductive process in birds. Previous studies have shown that feeding mirex to laying hens for 16 weeks at 300 or 600 ppm in the diet resulted in reduced hatchability and chick survival (Naber and Ware, 1965) The current studies, in which more moderate mirex levels were fed to quail, indicated that the compound had no effect on reproduction at levels in the diet as high as 30 ppm, with corresponding egg yolk residues of approximately 200 ppm.

Polar photoproducts of mirex were excreted from rats much more rapidly and showed less tendency to be re-

tained in body tissues than either mirex or its more nonpolar photoproducts. Thus, any environmental degradation of mirex to these derivatives might be expected to result in a lessening of potential ecological hazards.

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Isolation of 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane and an Octachloro Toxicant from Technical Toxaphene

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Technical toxaphene was subjected to the following sequence of chromatographic steps to isolate those components which are of the highest acute toxicity to mice treated intraperitoneally: liquid-liquid partition column, adsorption column, liquid-liquid partition column, adsorption column, and preparative gas-liquid chromatography. Two crystalline toxicants were isolated, one a $C_{10}H_{10}Cl_8$ component 14-fold more toxic to mice than toxaphene and the other a $C_{10}H_{11}Cl_7$ component with a sixfold greater toxicity than toxaphene. These two compounds are four- and twofold more toxic, respectively, than toxaphene to houseflies treated topically. The $C_{10}H_{11}Cl_7$ component is identified as 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane. The $C_{10}H_{10}Cl_8$ and $C_{10}H_{11}Cl_7$ toxicants, which make up about 6 and 3%, respectively, of technical toxaphene, appear to contribute significantly to its mammalian toxicity. These chromatographic procedures should be appropriate to isolate any individual component of toxaphene provided it is stable under the chromatographic conditions employed and a suitable monitoring technique is available.

Toxaphene produced by chlorination of camphene to an overall average composition of $C_{10}H_{10}Cl_8$ (Buntin, 1951) is extensively used in insect control even though its chemical composition is poorly understood (Guyer *et al.*, 1971). Examination of technical toxaphene by silica gel column chromatography followed by coupled gas-liquid chromatography (glc)-mass spectroscopy reveals a complex mixture of at least 175 C_{10} polychloro compounds made up of $C_{10}H_8Cl_{10}$, $C_{10}H_9Cl_9$, $C_{10}H_7Cl_9$, $C_{10}H_{10}Cl_8$, $C_{10}H_8Cl_8$, $C_{10}H_{11}Cl_7$, $C_{10}H_9Cl_7$, $C_{10}H_{12}Cl_6$, and $C_{10}H_{10}Cl_6$ derivatives (Casida *et al.*, 1974). A procedure is needed for isolation of individual toxaphene components to permit determination of their structure, residual persistence, metabolic fate, and toxicity. The difficulties in achieving such isolations are evident on considering the number of components in technical toxaphene and the likelihood that many of them are closely related compounds.

This report describes the isolation and properties of two components of technical toxaphene. A mouse intraperitoneal (ip) acute toxicity assay is used to monitor the purification since the toxaphene components which are most toxic to mammals warrant special attention in evaluating the hazards associated with toxaphene residues.

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MATERIALS AND METHODS

Chemicals. Technical toxaphene (reference standard, sample X 16189-49) and [^{14}C]toxaphene (1.35 mCi/g; from chlorination of [8-^{14}C]camphene; sample X-19098-4-2R) were provided by Hercules Incorporated, Wilmington, Del. Seven toxaphene fractions (I–VII) were obtained by chromatographing the technical material on a silica gel column with hexane as the developer (Knox *et al.*, 1974); this chromatographic procedure is similar to one referred to later as adsorption chromatography system II. The eluted materials were combined in the order of elution such that each fraction contained one-seventh of the total chlorine content of technical toxaphene (Knox *et al.*, 1974).

Chromatography. *Thin-Layer Chromatography (Tlc).* Silica gel 60 F-254 tlc chromatoplates (20 × 20 cm, 0.25 mm layer thickness, EM Laboratories Inc., Elmsford, N. Y.) were used either without treatment or after coating the gel by dipping the chromatoplates for 5 sec into a 20% (v/v) solution of β-methoxypropionitrile (MPN) in dichloromethane or a 15% (v/v) solution of dimethylformamide (DMF) in acetone and then placing the plates in a horizontal position for solvent evaporation. The chromatoplates after spotting with technical toxaphene or fractions thereof (10–100 μg) were developed for 17 cm with one of the following solvent systems: pentane with a first development for 9 cm and drying before a second development in the same direction for 17 cm; hexane; hexane saturated with formic acid;