Fate of Mirex-^{14}C in Japanese Quail[1]

by

G. WAYNE IVIE[2], H. WYMAN DOROUGH[3], and HARRY E. BRYANT

University of Kentucky
Lexington, Ky 40506

Mirex (1,3,4-metheno-dodecachloro, octohydro-2H-cyclobuta [c,d] pentalene) residues have been detected in birds and other wildlife 1 year after the material was applied for the control of the imported fire ant (BAETCKE et al., 1972). While the insecticide was found in one or more tissues of all but one of the 42 birds analyzed, the levels varied considerably depending upon species. For example, adipose tissue from the brown thrasher contained 20 to 60 ppm of mirex while the maximum level in the bobwhite quail was 3 ppm.

The fact that mirex does occur as a residue in wild populations of birds when used under practical conditions requires that more information be obtained on the fate of this insecticide in avian species. In the current study, total accountability of mirex given to Japanese quail was attempted by administering mirex-^{14}C as a single oral dose to adult male and female birds. The nature and levels of residues in the excreta, eggs and tissues were determined during a 84-day post-treatment period.

MATERIALS AND METHODS

Treatment and Sampling. Fifteen Japanese quail of each sex were treated orally with mirex-^{14}C (5.68 mCi/mMole; Mallinckrodt Chemical Works, St. Louis, Mo.). The treatments, 1.5 µCi/bird in 0.5 ml corn oil via a tube into the crop, were equivalent to approximately 1.2 mg/kg of body weight for the males and about 1.1 mg/kg for the slightly larger females. All of the birds were 10 weeks old and the females had been laying for about 4 weeks. Four treated birds of each sex were held in separate cages to allow collection of feces and eggs from individual birds. The remaining

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2 Present Address: Veterinary Toxicology and Entomology Research Laboratory, ARS, USDA, College Station, Texas.

3 To whom correspondence should be addressed.

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birds were held in a common cage. The quail were provided water and a commercial laying mash ad libitum. Feces samples from the isolated birds were collected daily for 7 days, and eggs were collected daily for the duration of the study. For tissue analysis, 2 males and 2 females from the common holding cage were sacrificed at designated intervals, and all samples frozen until analyzed.

Analysis. Total radiocarbon content in tissues and egg shell was determined by oxygen combustion in a Beckman Biological Materials Oxidizer. The ^{14}carbon dioxide was trapped by passing the combustion gases through a solution of 2:1 2-methoxyethanol:2-aminoethanol, and aliquots of the solution were radioassayed by liquid scintillation counting. The efficiency of the oxidizer was determined during each run by combusting samples from control birds to which mirex-^{14}C had been added. All values were corrected to 100%.

Radiocarbon in the feces, egg white and yolk was quantitated by direct liquid scintillation counting of 100 mg of fresh, thoroughly mixed material. Oxygen combustion confirmed that direct scintillation counting was an accurate means of determining levels of radioactivity in these samples.

For determination of the chemical nature of radiocarbon residues in feces, egg yolk, liver and whole birds after defeathering, the material to be extracted was homogenized in 50% ethanol for 5 minutes, then partitioned 3 times with 1:1 mixture of absolute ether: petroleum ether. The combined organic extracts were partitioned twice with 2% sodium chloride, then dried with anhydrous sodium sulfate. After stripping the solvent and dissolving the resulting oil in hexane, the extract was placed on a column containing 30 g of aluminum oxide (5% deactivated) above 30 g of PR grade Florisil (GIBSON, et al., 1972) and eluted with hexane. Fat samples from the neck and crop region of individual birds was extracted with hot hexane, and the extract was prepared for thin layer chromatography (TLC) and gas-liquid chromatography (GLC) as described above. Tissue solids after extraction were combusted to quantitate unextractable radiocarbon.

TLC was accomplished on precoated silica gel F-254 chromatoplates (0.25 mm gel thickness, E. Merck, Darmstadt, Germany) with heptane as the solvent. After development of the plates, radioactive areas on the gel were located by radioautography. GLC analyses were performed on a Varian Aerograph Model 1440 equipped with a 5 ft. x 1/8 in. stainless steel column. The column and operating parameters were 2% SP 2401 on chromosorb W-HP 100/120 mesh, injector 210°C, column 180°C, detector 205°C, with nitrogen flow maintained at 14 ml/min.

RESULTS

Following treatment with the mirex, the birds showed no perceptible toxicity symptoms, and egg production was not affected. The 4 females maintained in separate cages produced an average of 0.82 eggs/bird/day for the 84 days during which production was recorded.

Elimination. Mirex was rapidly absorbed from the digestive tract of quail following oral administration as indicated by only moderate amounts of the dose eliminated in the feces (Table 1). Only 12% of the dose was eliminated in the feces of females, while approximately twice that amount was detected in feces of males. Of the total radiocarbon excreted during the week following treatment, about 90% was in samples collected within the first 24 hours. Since elimination appeared to be essentially complete by 7 days, the samples were no longer radioassayed.

Egg yolks provided a ready avenue for excretion of residues following treatment of quail with mirex-^{14}C. Approximately 50% of the dose occurred in the yolks of eggs laid during the first week after treatment; after 84 days, 85% of the dose had been eliminated from the body by this route (Table 1). Only trace quantities of ^{14}C-residues were detected in the egg white and none in the shell.

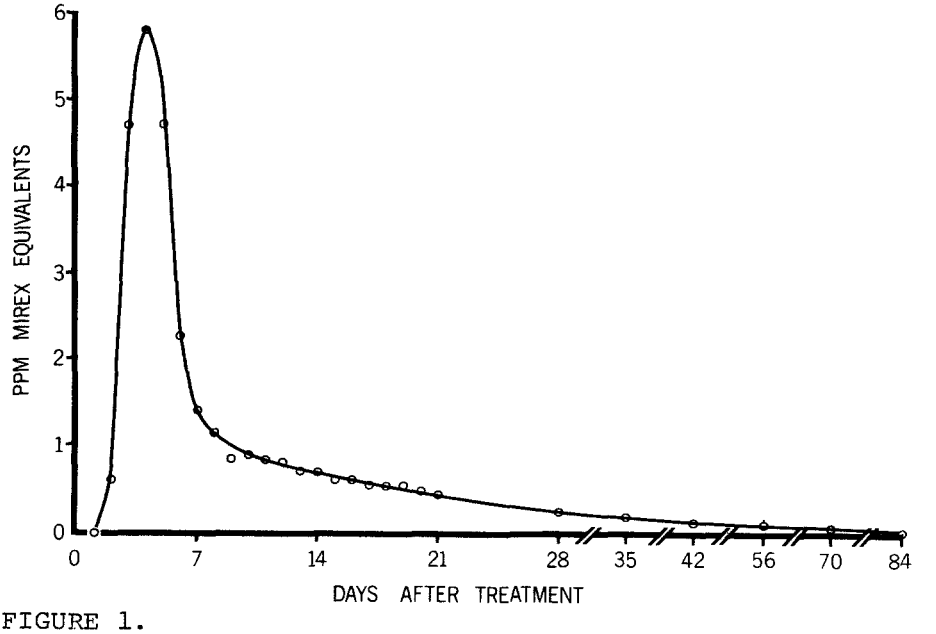

FIGURE 1.
Radiocarbon residues in egg yolk of Japanese Quail after treatment with mirex-^{14}C at 1.1 mg per kg.

TABLE 1.

Fate of a single oral dose of mirex-^{14}C administered to Japanese quail.

Days after treatment	Percent of administered radiocarbon					
	Eliminated from body, cum. %				Remaining in body[b]	Total recovered
	Feces[a]	Eggs				
		White[a]	Yolk	Shell[a]		
1						
Males	25	-	-	-	73	98
Females	10	0	0	0	86	96
3						
Males	26	-	-	-	71	97
Females	11	.09	13	0	76	100
7						
Males	26	-	-	-	68	94
Females	12	.14	48	0	38	98
28						
Males	26	-	-	-	52	78
Females	12	-	12	-	20	104
84						
Males	26	-	-	-	53	79
Females	12	-	85	-	2	99

[a] Samples not analyzed after 7 days.

[b] Based on whole-body analysis of birds.

Radioactive residues reached a maximum concentration in the yolk of 5.8 ppm mirex equivalents on the fourth day after treatment (Fig. 1). Thereafter, the level dropped rapidly and after 7 days had declined to 1.4 ppm. At termination of the study, 84 days after administering the radiolabeled mirex, small levels of radiocarbon (0.01 ppm) were still being excreted in egg yolk.

Tissues. Whole-body analysis revealed that both male and female quail retained radiocarbon in the body throughout the 84-day study and, as expected, levels in males were much higher than in females (Table 1). Radiocarbon administered to females was almost totally accounted for in the eggs, feces, and whole body samples, but about 20% of the administered dose could not be accounted for in males after the seventh day. Because low levels of radiocarbon were being excreted in the feces when collection of samples was terminated 7 days after treatment, it is likely that continued low-level elimination in the feces accounted, at least in part, for the loss of radiocarbon noted in the males. Feathers were excluded from whole-body analyses, but oxygen combustion showed that they contained no radioactive residues.

Residue levels in all tissues, with the exception of bodyfat, were highest the first day after treatment and then decreased rapidly (Table 2). Radioactive residues in the bodyfat of females decreased much faster than in males and at the end of the study the levels were about 30-fold higher in the males than in the females.

Differences in residue levels between males and females also were observed in the muscle and liver. During the first week after treatment, males showed

Table 2. ^{14}C-Residues in tissues of Japanese quail treated with a single dose of mirex-^{14}C.[a]

Days after treatment	PPM mirex equivalents in males (M) and females (F)[b]									
	Fat		Liver		Kidney		Muscle		Brain	
	M	F	M	F	M	F	M	F	M	F
1	8.4	10.0	1.01	3.50	0.53	0.52	1.24	0.47	0.06	0.04
3	16.5	7.9	.63	1.45	.27	.26	.22	.10	0	.01
7	12.4	9.5	.44	.62	.02	.10	.21	.04	.01	0
14	12.4	6.5	.19	.43	.12	.03	.18	0	0	0
28	11.2	2.4	.19	.35	.14	0	.14	.01	0	0
56	8.2	1.5	.10	.02	.27	.01	.09	.02	.01	0
84	6.5	.2	.11	.02	.11	0	.09	0	0	0

[a] Dosage - males 1.2 mg/kg, females 1.1 mg/kg.

[b] Zero indicates residues less than 0.01 ppm.

generally higher residues in muscle than did females, but the reverse relationship was observed in the liver. Radiocarbon levels in the brain and kidney did not appear to differ significantly between the two sexes.

Chemical Nature of the Residues. The extraction procedure employed provided essentially quantitative recovery of the radioactive residues from individual tissues, egg yolk, and feces. Recoveries of residues from the whole body analyses were 97% or greater. TLC and GLC analysis of all extracts revealed the presence of only unmetabolized mirex.

DISCUSSION

The current studies demonstrated that mature male and female Japanese quail differ in their patterns of absorption, storage, and excretion of orally-administered mirex. These differences were attributed largely to the fact that high levels of mirex were incorporated into the egg yolk, thus providing for more efficient elimination of the insecticide from the female. It is possible that this mirex would be transferred through the eggs to succeeding generations.

Female quail absorbed mirex from the digestive tract more completely than did males, probably because more active absorption processes are associated with egg production. Mirex was rapidly taken up by the bodyfat in both sexes, but particularly so in the male. Since no channels comparable to egg production exist in males for elimination of the very lipophilic mirex, the compound dissipated slowly from the bodyfat.

Because of the large quantities of mirex in the egg yolk, especially during the first week after treatment, most of the absorbed mirex was excreted by the females. The initial high level of mirex in the yolk might be explained by the fact that development of the ovum occurs over a period of 7 to 10 days before the egg is laid (ROMANOFF, 1949). Therefore, eggs laid the first week after treatment were in an active developmental stage when maximum mirex residues were present in the body. These residues were readily available for incorporation into the yolk material. Egg yolks formed later were exposed only to that mirex slowly released from storage in other tissues.

As time after treatment progressed, mirex levels in all tissues of the female declined rapidly as the mirex was transferred to the eggs. Residue dissipation from tissues of the male occurred much slower and more than half of the administered mirex remained in the body even after 84 days.

The lack of mirex metabolism observed in the current studies was consistent with earlier findings that rats do not degrade mirex following oral administration (GIBSON et al., 1972; MEHENDALE et al., 1972). The small amounts of unextractable radiocarbon detected in certain samples was probably unchanged mirex and reflects the efficiency of the extraction procedure employed. No more than 3% of the total radioactive content of any sample remained after extraction and usually it was less than 1%. These data clearly show that residue and/or monitoring studies using non-radioactive mirex should represent "total" mirex residues if procedures of extraction, cleanup and analysis are adequate for the quantitation of mirex, per se.

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