The Cumulation and Disappearance of Mirex Residues. III. In Eggs and Tissues of Hens Fed Two Concentrations of the Insecticide in Their Diet

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INTRODUCTION

Mirex [dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta (c,d) pentalene] is an insecticide used extensively in the southeastern United States to control the imported fire ant and other ants, LOFGREN et al. (1963, 1964), USDA (1972), ALLEY (1973). This insecticide is metabolized rather rapidly by domestic fowls as reported by MEDLEY et al. (1974). This should partially explain why birds are not extremely sensitive to mirex. NABER and WARE (1965) determined that a daily consumption of 300-600 ppm of mirex was necessary to produce significant decrease in egg hatch and in survival of the chicks. However, a closely related organochlorine, Kepone (General Chemicals) [decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (c,d) pentalene 2-one] produces significant effects on avian reproduction at 150 ppm daily intake.

BAETCHE et al. (1972) conducted studies in which they produced evidence indicating 8-81% mortality of quail, mallards, pheasants and cowbirds fed diets containing 200-500 ppm mirex for 30-111 days.

Recent studies by HEATH and SPANN (1973) produced evidence showing that concentrations of 10 or 40 ppm mirex fed bobwhite quail or 1-10 ppm fed mallards did not significantly affect reproduction. Some suppression of egg production was found for the mallards but none for the bobwhites. These studies also produced residue information for the birds and eggs in the tests. Results indicated rapid transmittal to eggs from treated birds and also a significant accumulation of mirex residues in brains and carcasses of these birds.

In another study on the toxicity and persistence of mirex reported by STICKEL et al. (1973), significant residues were detected in brains and carcasses of birds fed levels of 750 ppm mirex. A sharp decrease in residues was seen following removal from treated feed.

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Bulletin of Environmental Contamination & Toxicology,
Vol. 14, No. 1 © 1975 by Springer-Verlag New York Inc.

The Environmental Defense Fund (EDF) objected to the use of mirex due to the human health hazard in as much as eggs are consumed by humans. Their objection was NABER and WARE's work which seemed insufficient evidence to adequately judge the human health hazard. Therefore, an experiment was subsequently conducted to determine the intake of mirex by birds to produce residues in their eggs, USDA (1972).

The studies reported in this paper were designed to monitor the accumulation of mirex residues in eggs and tissue of laying hens consuming known quantities of the insecticide in their daily rations. Data was obtained for residues in eggs and tissue of birds after removal from contaminated rations. This data should be of significant value in realistically evaluating the fate of feeding low-level quantities of mirex to domestic fowl and perhaps even wild birds.

METHODS AND MATERIALS

Forty-five laboratory reared, pesticide-free white leghorn hens were obtained for use in the egg study. Mixing of rations and care of birds has been previously described in a test utilizing roosters, MEDLEY et al. (1974). Two feeding levels of mirex, 0.01 and 1.00 ppm were used in this study. Hens were divided into 3 groups of 15 hens per group; in addition to the two treated groups, another group fed only the untreated feed was utilized as a control.

Eggs were collected daily from each of the three groups and refrigerated at 7°C. until transported to the laboratory where they were placed in a freezer and maintained in a frozen condition until analyzed. After 27 weeks feeding, 3 hens from each group were removed from the experimental diet to observe decline of mirex residues over a 13 week period. The remaining hens were maintained on the treated diet for the full 40-week period of the study. All of the hens were then slaughtered and samples of fat, breast, kidney and liver tissue collected for residue analysis. Tissue samples were delivered to the laboratory in a frozen condition and maintained in this state until analyzed.

Five hens died at various intervals during the study. Tissue samples were collected from each of these birds for residue analysis. Although mirex residues were detected in all of the hens, levels were not great enough to produce mortality. Highest residues were found in fatty tissues, with smaller quantities in the breast, kidney and liver samples. Two of the hens which died were from the control group, which showed the highest residues, 0.14 and 0.23 ppm in fat samples. This was probably due to cross contamination in the sample collection or processing procedures.

ANALYTICAL PROCEDURES

Extraction

Representative 100 gram egg samples were extracted in half-gallon Mason jars with 500 milliliters of a 4:1 mixture of Nanograde hexane and isopropyl alcohol by rotating concentrically for two hours. The alcohol and water were removed by washing 3 consecutive times with fresh 300 milliliter portions of distilled water; the extracts were dried by filtering through anhydrous granular sodium sulfate and stored in a refrigerator in amber sample bottles.

Procedures for extraction of tissue samples were described previously in a report by MEDLEY et al. (1974). Extracts were dried and stored as described for the egg samples.

Cleanup

Aliquots representing 7.5 grams of eggs or tissue were subjected to the sulfuric acid treatment to destroy fats and oils, then 5.0 gram aliquots cleaned up through activated Florisil (Floridin Company) columns as described previously by MEDLEY et al. (1974).

Gas Chromatographic Analysis

Samples were analyzed by electron-capture gas chromatography utilizing essentially the same procedure previously described by MEDLEY et al. (1974). Doubtful peaks with identical retention times as mirex were subjected to identification procedures utilizing dual-column and the partitioning coefficient (p-values) of BOWMAN and BEROZA (1966). No peaks were reported which were not at least twice the background level. The identical system of control samples was employed in these procedures as described in the previous paper. Average recoveries of 92.5% were obtained for the liver and kidneys, 89.2% for the fat, 92.3% for the breast of the hens and 87.4% for the eggs; all residues were corrected for

TABLE 1

Mirex Residues in Eggs from Hens Fed Two Levels of Mirex in Their Daily Rations

Sampling Interval, Weeks	Feeding Level, ppm	Residue ppm[a], [b], [c]
	RESIDUE EGGS[d]	
Pretreatment	-	<0.01
	-	<0.01
	-	<0.01
1	Control	<0.01
	0.01	<0.01
	1.06	0.07
4	Control	<0.01
	0.01	0.03
	1.06	0.83
8	Control	<0.01
	0.01	0.01
	1.06	0.80
12	Control	<0.01
	0.01	<0.01
	1.06	1.06
16	Control	<0.01
	0.01	0.01
	1.06	1.01
20	Control	<0.01
	0.01	0.01
	1.06	0.84
24	Control	<0.01
	0.01	0.02
	1.06	0.76
28	Control	<0.01
	0.01	0.03
	1.06	2.03

Sampling Interval, Weeks	Feeding Level, ppm	Residue ppm[a/], [b/], [c/]
32	Control	<0.01
	0.01	0.02
	1.06	1.50
33	Control	<0.01
	0.01	0.02
	1.06	1.20
37	Control	<0.01
	0.01	0.01
	- [e/]	-
	NON-RESIDUE EGGS[f/]	
35	Control	<0.01
	0.01	0.01
	1.06	0.78
40	Control	<0.01
	0.01	- [e/]
	1.06	0.66

a/ Corrected for mirex recovery from fortified eggs.
b/ Lower limits of sensitivity = 0.01 ppm.
c/ Average of two replicates.
d/ "Residue" eggs collected from hens fed treated feed for the entire 40 week test.
e/ Only one feeding level analyzed.
f/ "Non-residue" eggs collected from hens removed from treated feed after 27 weeks feeding and fed untreated rations for 13 weeks.

these recoveries. No interferences were detected in the solvent checks, however detectable quantities of apparent mirex were found in samples of fat, liver and kidney tissue from control hens in the non-residue group and the residue group for the 40 week sampling. Residues in this group have been corrected for these values. Apparently cross-contamination was introduced in collection and handling or processing the tissue samples.

RESULTS AND DISCUSSION

Table 1 presents data for mirex residues in eggs from hens receiving 2 concentrations of mirex in their daily rations for 37 weeks and also for eggs from hens which were removed from this contaminated feed after 27 weeks feeding and placed on untreated feed for 13 weeks. Within one week of feeding, significant residues appeared in the 1.06 ppm eggs (0.07 ppm), thereafter significant residues appeared in both the 0.01 and 1.06 ppm feeding levels. This trend of accumulation continued until the 28th week of feeding, at which time a maximum residue of 2.03 ppm was attained, then a gradual decrease in residues was seen which continued through 37 weeks when the study was terminated. A significant residue reduction was noted in eggs from hens removed from the treated feed, especially for the 1.06 ppm eggs. Residues declined from 1.20 ppm after 33 weeks to 0.66 ppm in eggs from the 1.06 ppm hens when the study was terminated after consuming untreated feed for 13 weeks following removal from the treated feed. Eggs from the 0.01 ppm hens did not show a significant decrease (0.02 vs 0.01 ppm) 8 weeks following removal from the treated feed.

Tissue samples were analyzed from birds slaughtered at the conclusion of the 39 weeks feeding study and from those slaughtered after feeding on treated rations for 27 weeks and placed on untreated feed for 13 weeks. Mirex residues in tissues of hens maintained on the treated feed for the 39 week study averaged 0.30, 0.01, 0.01 and 0.07 ppm in the fat, breast, liver and kidney, respectively for the 0.01 ppm hens and 24.79, 0.31, 1.93 and 3.43 ppm in the same tissues for the 1.06 ppm hens. These results represent an average of two replicates. Mirex residues in tissues from the eight hens removed from treated feed after 27 weeks and placed on untreated feed for 12 weeks averaged 0.28, 0.01, 0.01 and 0.03 ppm in fat, breast, liver and kidney, respectively for the 0.01 ppm birds and 15.15, 0.11, 0.49 and 2.16 ppm in the same tissue of the 1.06 ppm hens. These results represent an average of four replicates.

Typical of organochlorine pesticides, mirex exhibited a tendency to accumulate in fatty tissues and eggs. This was demonstrated by the high residues of mirex apppearing in the fatty tissues of the laying hens feeding on rations contaminated with the insecticide. However, once the birds were removed from treated feed an almost immediate decline in residues was noted.

Additional studies should be conducted to determine the pathways of disappearance of mirex from poultry and other birds. Although there were no indications of mirex metabolites, such as the partially dechlorinated insecticide in fatty tissues of the birds, studies designed differently might produce evidence of such compounds.

ACKNOWLEDGMENTS

The authors wish to express grateful appreciation to Harry Richardson for his expert technical assistance in the processing and extraction of these samples.

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