The Cumulation and Disappearance of Mirex Residues. II. In Milk and Tissues of Cows 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 (solenopsis invicta Buren) and other ants, LOFGREN et al. (1963, 1964), USDA (1972), ALLEY (1973). Due to its present use over this large section of the United States, much controversy has developed pertaining to the use of this insecticide. Studies by various researchers have produced residue data concerning the monitoring of mirex in milk and tissues of various animals. GAINES and KIMBROUGH (1970) determined that mirex in milk of suckling rats produced a high incidence of cataracts; LOFGREN et al. (1964) reported on studies conducted by M. M. Darley monitoring mirex residues in milk. Cows which grazed on fields treated with 4.2 gm mirex/acre showed residues in milk below the detectable limits of 3 ppb. DOROUGH and IVIE (1974), in a study to determine mirex residues following the feeding of mirex-14C in gelatin capsules for 28 days to a lactating cow, reported residues of radio-labeled mirex in milk, tissue, urine and feces of the cow. This study produced evidence indicating the major pathway of excretion of mirex residues was in the feces, with only minute quantities being detected in the milk (0.58 ppm), slightly higher concentrations in tissue (0.21 ppm in fat) and no detectable residues in the urine of the cow.

Additional studies by HAWTHORNE et al. (1973) showed absence of mirex residues in whole milk above the detectable limits of 0.3 ppb. The milk samples were collected from cows which had grazed in areas treated two to five times with 1.7 ppm per acre mirex as a bait. In a report by BAETCHE et al. (1972), residues of 16 ppb mirex were found in the lipid fraction of cows' milk. Since cows' milk is consumed to a great extent by humans, including infants, further studies were necessary to determine if significant mirex residues appear in the milk of dairy cows consuming known quantities of the insecticide under normal feeding conditions.

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

This study was designed to monitor accumulation of mirex residues in milk of the cows. Fat samples were also analyzed to determine the extent of storage of the insecticide in fatty tissues which could later be eliminated in the milk. Additional data was obtained from tissue of calves receiving milk from these cows.

METHODS AND MATERIALS

Three dairy cows were obtained from a pesticide-free herd for the milk study. Individual stanchions were used for the cows; stanchions and premises were decontaminated with a steam cleaner followed by rinsing with a 10% solution of acetone in ethyl alcohol. An ammonia base antibacterial agent was used daily to clean and sterilize the automatic milking machine.

A mixture of 16% protein grain ration was treated separately with two concentrations of mirex in soybean oil (0.01 and 1.00 ppm). Two of the cows were fed the rations individually during the study while one cow was fed the untreated feed to serve as a control. Mirex concentrations were based on average feed consumption by the dairy cows.

Daily samples of one pint of warm whole milk each were collected from untreated and treated cows. These samples were stored in a refrigerator and composited by treatment weekly (seven pints per cow), then frozen until analyzed. Cows were fed the experimental rations for 31 weeks at which time the study was terminated.

Fat samples were collected by omentectomy ten days after termination of the 31 week feeding study. The tissue was frozen and transported to the analytical laboratory where it was maintained in a frozen condition pending residue analysis.

Fat samples were also collected as described above from the calf receiving milk from the cow on the 1.00 ppm ration and the one receiving milk from the control cow 20 weeks after feeding began. The two calves were then slaughtered after 31 weeks feeding and samples of renal fat, omental fat, loin, liver and kidney tissue collected. All tissue samples were frozen pending subsequent residue analysis.

ANALYTICAL PROCEDURES

Extraction

Representative 400 gram samples of whole milk were weighed into two-liter separatory funnels and extracted utilizing the procedure of MILLS (1961) modified as described below: ammonium oxalate was used instead of potassium oxalate, Nanograde isopropanol was used instead of methanol, and the mirex extracted with three portions of a 1:3 (v/v) mixture of Nanograde diethyl ether in hexane. The hexane extracts were dried by filtering through anhydrous sodium sulfate and stored in amber sample bottles in a refrigerator.

Tissue samples were extracted according to the procedure of MEDLEY et al. (1974). Extracts were dried and stored as described for the milk samples.

Cleanup

Aliquots representing 7.5 grams of milk 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) and MARKIN et al. (1972).

Gas Chromatographic Analysis

Samples were analyzed by electron-capture gas chromatography utilizing essentially the same procedure previously described by MEDLEY et al. (1974). Questionable peaks with identical retention times as mirex were subjected to confirmation procedures utilizing dual-column and partitioning coefficient (p-value) of BOWMAN and BEROZA (1966). No peaks were reported which were not at least twice the background "noise" level. The same system of control samples was employed in these procedures as described in the previous paper. Average mirex recovery was 86.9% for the milk, 101.6% for the calf fat, 89.5% for the calf liver, 101.6% for the calf kidney, 83.3% for the calf loin and 78.0% for the fat from the dairy cows. All residues were corrected for these recoveries; no interferences were detected in the solvents; traces of mirex, apparently from cross-contamination, were detected in omental fat, back fat, renal fat and kidney samples from calves receiving milk from cows feeding on uncontaminated feed. These values are reported in the tables and residues were corrected for these values. Several unidentified peaks appeared on the GC chromatograms from samples of treated cattle tissue with identical peaks also appearing in chromatograms from samples of untreated tissue, therefore it is highly unlikely that they were a result of mirex metabolites but were from other pesticides or other material in the tissue.

TABLE 1

Mirex Residues in Milk from Cows Fed Two Levels of the Insecticide in Their Daily Rations

Feeding Interval, Weeks	Feeding Level, ppm	Residue ppm[a/], [b/], [c/]
Pretreatment	-	<0.01
	-	<0.01
	-	<0.01
1	Control	<0.01
	0.01	0.02
	1.00	0.02
4	Control	<0.01
	0.01	<0.01
	1.00	0.02
8	Control	<0.01
	0.01	0.01
	1.00	0.01
12	Control	<0.01
	0.01	<0.01
	1.00	0.01
16	Control	<0.01
	0.01	0.02
	1.00	0.03
20	Control	<0.01
	0.01	0.01
	1.00	0.02
24	Control	<0.01
	0.01	0.02
	1.00	0.05
28	Control	<0.01
	0.01	0.01
	1.00	0.06
31	Control	<0.01
	0.01	0.01
	1.00	0.08

a/ Corrected for mirex recovery from fortified samples.
b/ Lower limits of sensitivity = 0.01 ppm.
c/ Average of two replicates.

TABLE 2

Mirex Residues in Fat of Cows Fed Two Levels of the Insecticide in Daily Rations and in Tissues of Calves Receiving Milk from the Control Cow and the 1.00 ppm Cow

Feeding Interval, Weeks	Feeding Level, ppm	Residue, ppm, in: [a], [b] Back Fat	Renal Fat	Omental Fat	Loin	Liver	Kidney
				COWS			
31[c]	Control	-	-	<0.01	-	-	-
	0.01	-	-	0.06	-	-	-
	1.00	-	-	1.87	-	-	-
				CALVES			
	Control	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
20	1.00	-	-	1.82	-	-	-
31	1.00	1.41	2.52	1.67	0.92	0.08	0.76

[a] Corrected for recovery from mirex fortified tissue.
[b] Lower limits of sensitivity = 0.01 ppm.
[c] Samples collected 10 days after termination of feeding study.

RESULTS AND DISCUSSION

Table 1 presents residue data obtained from milk samples collected from cows fed two concentrations of the insecticide in their rations. Although mirex and other organochlorine pesticides show a tendency to concentrate in fatty tissues of animals, this was not the case for milk samples, even for feeding levels as high as one part per million when treated feed was consumed for 31 weeks. No residues exceeding 0.08 ppm were detected in any of the milk samples.

Table 2 shows residue data for omental fat samples collected from the control, 0.01 ppm and 1.00 ppm cows after 31 weeks consumption of contaminated feed, at which time the study was terminated. These results indicated a low accumulation of mirex for both feeding levels, 0.06 ppm for the 0.01 ppm animal and 1.87 ppm for the 1.00 ppm cow. Residues of mirex were determined in omental fat of a calf receiving milk from the 1.00 ppm cow after 20 weeks feeding and in tissues of back fat, renal fat, omental fat,loin, kidney and liver of this calf after 31 weeks. Residues of 1.82 ppm were detected in the back fat, renal fat, omental fat, loin, liver and kidney tissues, respectively, after slaughter at the 31 week feeding interval.

Contrary to numerous reports indicating the accumulation of mirex residues in fatty tissues, excessive residues did not accumulate in the milk and fatty tissues of the cows and calves. Apparently during the digestive process some type of reaction occurs which does not occur in nonruminant animals.

Based on results obtained from these studies, it was concluded that mirex does not present a major problem when consumed with daily food of ruminant-type mammals, since low residues were detected in both the milk and tissues from animals feeding on rations contaminated with the insecticide.

Additional studies should be conducted to determine the accumulation and decline of mirex residues in milk and fat of dairy cows when they are fed the mirex in daily rations, then removed from treated feed to determine the rate of disappearance. Tests should also be conducted to determine if non-ruminant animals accumulate residues as slowly as the dairy cows, and if so, also determine rates of decline. Additional information is also necessary to determine routes of disappearance of the mirex, whether it is in the urine or feces as water soluble metabolites or how it is eliminated from ruminant animals.

ACKNOWLEDGMENTS

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

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