# The Cumulation and Disappearance of Mirex Residues I. In Tissues of Roosters Fed Four Concentrations of Mirex In Their Feed

by

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Mirex (dodecachlorooctahydro-1,2,4-metheno-2H-cyclobuta (cd) pentalene) has been reported as highly cumulative and slowly metabolized by GAINES and KIMBROUGH (1970).

BAETCHE et al. (1972) consider mirex a persistent pesticide. The few samples they obtained of poultry meat and beef tissues from areas recently treated with mirex showed a maximum of 0.55 ppm mirex in one sample of poultry liver. They point out that because of lack of information regarding tissue levels of mirex encountered with the feeding of LD 50 dosages, it is not possible to interpret the meaning of levels observed in samples discussed in their report. Also, they cite toxicity data of 200 to 500 ppm dietary mirex for at least 30 days, indicating toxicity of mirex is low in avian species.

In a recent report (1972), the U. S. Department of Agriculture stated that the imported fire ant is a serious pest that adversely affects human health and the agricultural economy of nine southern states. This report indicates that the Environmental Defense Fund (EDF) questions the behavior of mirex in the environment and the hazard it presents to human health and cites the lack of data regarding these areas.

Unless something is known of the rate of input required to obtain residues found in the environment, monitoring for mirex in non-target organisms is of questionable value. The present studies are an attempt to measure the cumulation and disappearance of mirex levels in products destined for human consumption, using a known rate of input.

## METHODS AND MATERIALS

Seventy-five young leghorn roosters were used. Mirex was placed in refined soybean oil to obtain a 1% stock solution. The stock solution was then diluted with additional oil to obtain four levels of mirex in the experimental ration. Proper mixing of ration was obtained by adding the correct amount of mirex in oil to corn meal, mixing one hour in a small converted cement mixer, adding



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premix to granular poultry ration and mixing an additional hour in a one-ton vertical mixer. Sufficient ration was mixed to last each test group ninety days. One group was fed the untreated ration throughout the study. Roosters were housed in individual hanging cages. Decontamination of houses and cages was with a steam cleaner followed by an alcohol:acetone (10:1) rinse.

Control roosters and those given four levels of mirex in the experimental ration were sacrificed, one per group, every four weeks for 20 weeks. After 20 weeks, some roosters were taken off mirex and slaughtered every two weeks through 12 weeks of feed-off. Others remained on the experimental ration and were slaughtered after 26 and 32 weeks of mirex input. Tissues sampled were breast, liver, kidney and abdominal fat (includes preening gland). Kidney samples were disected out as intact as possible without peripheral fat. No attempt was made to measure fat content of organ and muscle tissue.

Samples were frozen immediately, and delivered by air conditioned Government vehicle to the Environmental Quality Laboratory, Brownsville, Texas.

## ANALYTICAL PROCEDURES

### Extraction

Representative samples of liver, kidney or fat were macerated in blender jars for two minutes with 200 ml of Nanograde hexane, then transferred to half-gallon Mason jars with 300 ml of the solvent. Anhydrous sodium sulfate was added and the samples were extracted on a concentric rotator for two hours. Solvent was filtered through glass wool into amber sample bottles for refrigerated storage.

Breast tissue - Representative 50 g samples of finely ground breast tissue were transferred to half-gallon Mason jars, 500 ml of Nanograde hexane and 100 g of anhydrous sodium sulfate were added, with extraction on a concentric rotor for two hours. The extracts were filtered through glass wool into amber sample bottles and refrigerated.

### Cleanup

Aliquots representing 7.5 g of sample material were treated with 10 ml of concentrated sulfuric acid to destroy fats and oils. After draining the acid, the hexane extracts were washed with distilled water, the water was discarded, and the solution was dried over anhydrous sodium sulfate. Aliquots representing 5 g of sample material were transferred to chromatographic columns containing a mixture of 15 g of activated (8 hours @ 120°C) 60/100 mesh PR grade

Florisil and 2 g of anhydrous sodium sulfate. The mirex was diluted from the adsorbent with 100 ml of Nanograde hexane.

Gas Chromatographic Analysis

The purified extracts were evaporated through Snyder columns, transferred quantitatively to 15 ml centrifuge tubes and diluted to the appropriate volume and aliquot injected on the gas-liquid chromatograph. Resulting peaks from the samples were compared with chromatograms obtained from known concentrations of mirex and residues calculated based on height of peaks obtained.

The gas chromatograph was a Tracor Model Mt-220 equipped with a Ni-63 electron capture detector. In order to select out interferring peaks, five columns were used (1) a 6' x ¼" glass column containing 3% DC-200 on 100/120 mesh Gas Chrom-Q (Applied Science Laboratories, State College, Pa.); (2) a 6' x ¼"glass column containing 5% QF-1 on 100/120 mesh Gas Chrom-Q (Applied Science); (3) a 6' x ¼" glass column containing 3% OV-1 on 100/120 Gas Chrom-Q (Applied Science); (4) a 6' x ¼" glass column containing 5% OV-210 on 100/120 mesh Gas Chrom-Q (Applied Science); and (5) a 6' x ¼" glass column containing an 11% mixture of OV-17 and QF-1 on 80/100 mesh Gas Chrom-Q (Applied Science). Never more than two columns were used at a given time. Isothermal temperatures were 200, 300 and 250°C for the columns, detectors and injectors, respectively. Nitrogen carrier gas flow rate was 80cc/min. Sensitivity was adjusted to obtain approximately 40-50% of full-scale recorder pen deflection with an injection of 0.30 ng of mirex. Recorder chart speed was 30 inches per hour. A series of controls consisting of a solvent check, untreated sample, and mirex-fortified sample were processed as the regular samples to maintain a close monitoring of any contamination and to determine mirex recovery through the analytical procedure. Average recoveries of 88.51% were obtained for liver and kidney, and 88.45% for fat and breast. All residues reported were corrected for percentage recovery. No interfering peaks were observed in either the solvent checks or non-fortified samples.

## RESULTS AND DISCUSSION

When feed consumption was checked to provide an estimate for mirex feeding levels, birds were evidently not acclimated to hanging cages. After mirex was introduced into diet, feed consumption was about one-third less than anticipated for all groups, but very consistent within and between groups. Birds remained healthy, and only two died, apparently of natural causes, during the experiment.

Roosters were weighed at the beginning of the study and when they were slaughtered. Average weight gain per bird for all groups was 85.2 g. Averages including liver weights were obtained for the group fed the highest level of mirex and compared with control birds as follows:

| Averages of 15 Roosters | Body Weight at Slaughter (kg) | Weight Gain (g) | Liver Weight (g) | Liver as % Body Weight |
|---|---|---|---|---|
| Fed 7.21 ppm | 2.05 | 85 | 33.4 | 1.64 |
| Control Ration | 2.09 | 81 | 32.4 | 1.52 |

Obvious tissue differences such as light colored liver or dark colored abdominal fat were noted and recorded. No relationship could be determined between mirex input levels and these tissue differences.

Residues of mirex in breast tissue were low compared with other tissue samples as shown in TABLE 1. At least 26 weeks was required to exceed 2 ppm in breast tissue at the 7.21 ppm level of input. It is unlikely that environmental input levels would be as high as the 0.71 ppm feeding level. At least 20 weeks was required to exceed 0.2 ppm in breast tissue at this feeding level.

Typical of organochlorine pesticides, mirex shows a propensity for cumulation in fatty tissue. At the 7.21 ppm feeding level there was evidence that the saturation level had been reached in the fat after 26 weeks (TABLE 1). However, only one rooster was sacrificed in each group and differences could be physiological. The rooster sacrificed at 26 weeks had gained about twice as much body weight as the rooster killed at 32 weeks.

TABLE 2 shows the disappearance of mirex residues after removal of mirex from the diet. MEHENDALE et al. (1972) studied the metabolization of mirex as a single dose administered to rats. After 7 days, tissues and organs contained 34% of the total dose. They concluded that mirex is poorly absorbed by the gut of rats but once in the tissue 50% of the original level remained at 100 days.

TABLE 2 shows that after a prolonged period of mirex input residues disappear rather rapidly from fatty tissues within the first two weeks after removal of mirex from the diet.

At the 7.21 ppm level of input, as shown in Figure 1, 70% of the residues disappeared after two weeks. Good weight gainers apparently retained higher levels of mirex longer. Two roosters fed 7.21 ppm in their ration were slaughtered 12 weeks after removal of mirex from their feed. One bird lost 113 g of body weight and contained 73.37 ppm mirex in fatty tissue. The other rooster gained 283 g and contained 228.41 ppm in the fat.

TABLE I

THE CUMULATION OF MIREX IN TISSUES OF ROOSTERS FED FOUR LEVELS IN THEIR DIET

| Weeks on Feed | Tissue | Mirex in Tissues of Birds Fed [a] | | | |
|---|---|---|---|---|---|
| | | 0.007 ppm | 0.06 ppm | 0.71 ppm | 7.21 ppm |
| 4 | Breast | 0.00 | 0.01 | 0.02 | 0.08 |
| | Liver | 0.00 | 0.05 | 0.33 | 3.17 |
| | Kidney | 0.01 | 0.03 | 0.69 | 1.91 |
| | Fat[b] | 0.13 | 0.57 | 3.04 | 12.67 |
| 8 | Breast | 0.00 | 0.01 | 0.03 | 0.46 |
| | Liver | 0.01 | 0.04 | 0.94 | 6.96 |
| | Kidney | 0.02 | 0.48 | 1.73 | 18.70 |
| | Fat[b] | 0.54 | 1.15 | 11.93 | 158.11 |
| 12 | Breast | 0.00 | 0.01 | 0.07 | 1.00 |
| | Liver | 0.02 | 0.24 | 1.10 | 13.10 |
| | Kidney | 0.03 | 0.05 | 1.87 | 21.40 |
| | Fat[b] | 0.28 | - - | 19.14 | 262.82 |
| 16 | Breast | 0.01 | 0.02 | 0.12 | 0.60 |
| | Liver | 0.01 | 0.10 | 3.07 | 12.03 |
| | Kidney | 0.01 | 0.31 | 1.23 | 15.17 |
| | Fat[b] | 0.05 | 1.86 | 23.88 | 455.08 |
| 20 | Breast | 0.00 | 0.02 | 0.22 | 1.97 |
| | Liver | 0.04 | 0.09 | 2.54 | 19.32 |
| | Kidney | 0.01 | 0.23 | 3.21 | 20.67 |
| | Fat[b] | 0.72 | 4.43 | 48.77 | 531.02 |
| 26 | Breast | 0.00 | 0.02 | 0.07 | 1.99 |
| | Combined[c] | 0.06 | 0.18 | 2.64 | 23.28 |
| | Fat[b] | 0.26 | 6.76 | 10.92 | 993.89 |
| 32 | Breast | 0.00 | 0.03 | 0.16 | 5.40 |
| | Combined | 0.01 | 0.33 | 1.61 | 26.53 |
| | Fat[b] | 0.35 | 7.80 | 46.34 | 751.03 |

a Based on 0.01 ppm lower limits of detection, no mirex was found in tissues of pretreatment and control birds.

b Includes abdominal fat and preening gland.

c Average of residues in liver and kidney.

TABLE 2

THE DISAPPEARANCE OF MIREX RESIDUES IN TISSUES OF ROOSTERS FED FOUR LEVELS IN THEIR FEED FOR TWENTY WEEKS

| Weeks on Feed | Tissue | Mirex in Tissues of Birds Fed[a] 0.007 ppm | 0.06 ppm | 0.71 ppm | 7.21 ppm |
|---|---|---|---|---|---|
| 20-week Residues | Breast | 0.00 | 0.02 | 0.22 | 1.97 |
| | Liver | 0.04 | 0.09 | 2.54 | 19.32 |
| | Kidney | 0.01 | 0.23 | 3.21 | 20.67 |
| | Fat[b] | 0.72 | 4.43 | 48.77 | 531.02 |
| MIREX REMOVED FROM DIET OF ROOSTERS | | | | | |
| 2 | Breast | 0.00 | 0.02 | 0.14 | 0.64 |
| | Liver | 0.01 | 0.38 | 0.59 | 2.05 |
| | Kidney | 0.02 | 0.18 | 1.59 | 6.55 |
| | Fat[b] | 0.46 | - - | 3.77 | 130.59 |
| 4 | Breast | 0.00 | 0.01 | - - | 0.20 |
| | Combined[c] | 0.00 | 0.69 | - - | 4.87 |
| | Fat[b] | 0.07 | 1.15 | - - | 80.85 |
| 6 | Breast | 0.00 | 0.01 | 0.01 | 0.40 |
| | Combined | 0.02 | 0.06 | 0.18 | 5.82 |
| | Fat[b] | 0.79 | 1.38 | 4.64 | 14.52 |
| 8 | Breast | 0.00 | 0.01 | 0.09 | 0.77 |
| | Liver | 0.06 | 0.06 | 0.76 | 9.30 |
| | Kidney | 0.00 | 0.29 | 1.62 | 14.71 |
| | Fat[b] | 0.33 | 4.51 | 34.83 | 165.30 |
| 10 | Breast | 0.00 | 0.00 | 0.02 | 0.61 |
| | Liver | 0.00 | 0.04 | 1.28 | 6.49 |
| | Kidney | 0.03 | 0.10 | 0.99 | 7.69 |
| | Fat[b] | 0.41 | 2.10 | 12.34 | 120.63 |
| 12 | Breast | 0.00 | 0.01 | 0.19 | 0.88[d] |
| | Liver | 0.01 | 0.11 | 0.66 | 8.38[d] |
| | Kidney | 0.03 | 0.12 | 1.31 | 17.71[d] |
| | Fat[b] | 0.40 | 3.86 | 27.16 | 150.89[d] |

a, b, and c - See Table 1.

d Average of residues in tissue samples of two roosters.

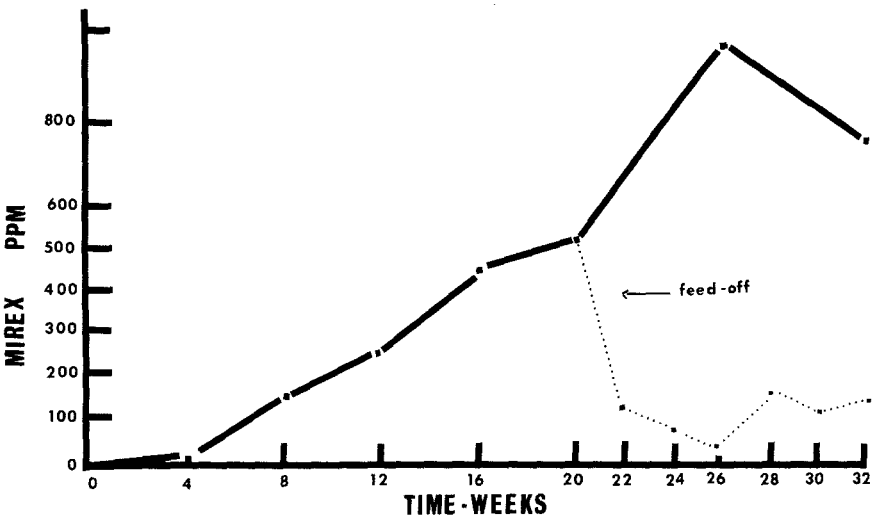


Figure 1. The rate of cumulation and disappearance of mirex in fat of roosters fed 7.21 ppm in their ration.

The 20-week residue levels in fat when feed-off began (TABLE 2) were subjected to Linear Regression analysis. Considering that X = ppm in the feed and Y = ppm in the fat, then Y = a ∱ bX, the Y intercept a = -1.0508, the slope b = ∱ 73.7628, and the correlation coefficient r = ∱ 0.9999767. The latter indicates an extraordinarily good straight line fit between amount fed and amount of residue accumulated.

After 12 weeks of feed-off, TABLE 2 shows that about half the residues in tissues of roosters fed mirex have disappeared.

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