Barbiturate-Induced Sleeping Times, Liver Weights, and Reproduction of Cottontail Rabbits after Mirex Ingestion

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Mirex (Dodeca-chlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalene) is a chlorinated hydrocarbon insecticide used in the southeastern United States almost exclusively for the control of one pest--the imported fire ant (Solenopsis invicta Buren) (MARKIN et al. 1974). Controlled experiments with Mirex have shown that exposure to low amounts of this insecticide may adversely affect the reproductive success of mice (WARE and GOOD 1967) and rats (GAINES and KIMBROUGH 1970).

Increased activity or synthesis of hepatic microsomal enzymes after exposure to drugs or environmental pollutants is known to increase the in vitro metabolism of many sex hormones (CONNEY and BURNS 1972). It has been hypothesized that an alteration of steroid metabolism due to hepatic microsomal enzyme induction by a wide array of pollutants may explain, at least in part, the effects of environmental pollutants on reproduction and fertility of many birds and mammals (PEAKALL 1967, CONNEY and BURNS 1972).

The most commonly used in vivo method for determining hepatic microsomal enzyme activity is the measurement of barbiturate-induced sleeping times. To our knowledge, no work has been done with barbiturate-induced sleeping times following Mirex exposure. Various investigators (BAKER et al. 1972, CHADWICK and FREAL 1974) have utilized in vitro methods to demonstrate hepatic microsomal enzyme induction in response to Mirex exposure. The objective of this study was to examine hepatic microsomal enzyme induction in the cottontail rabbit (Sylvilagus floridanus) using sodium pentobarbital-induced sleeping times during and after cessation of dietary exposure to Mirex.

MATERIALS AND METHODS

Wild cottontail rabbits were live-trapped and allowed at least 3 weeks in which to acclimate to laboratory cages, feed, sulfaquinoxaline-treated water, and controlled temperature and lighting conditions. The experiment was designed as a 2 X 2 factorial. Two levels of Mirex treatment (0 vs. 20 ppm) and two levels of nutritional treatment (ad libitum vs. 25% restricted diet) were employed. Technical Mirex (97%, supplied by Allied Chemical Corp., Houston, Texas) was dissolved in acetone and applied to commercial rabbit pellets while being mixed in a large feed mixer. The feed for the

Bulletin of Environmental Contamination & Toxicology,
0007-4861/78/0019-0223 $1.20

Mirex-treated-ad libitum rabbits received 20 mg of Mirex per kg of feed (i.e. 20 ppm). Since the Mirex-treated-restricted rabbits were to receive 25% less than the ad libitum rabbits consumed, 26.6 ppm was included in their diets. The restricted diets were adjusted weekly based on the amount of feed consumed by the respective Mirex-treated and control rabbits on ad libitum diets. Control rabbits received feed to which equal amounts of acetone without Mirex had been applied.

During the eighth week of treatment, 29 female rabbits were selected at random, weighed, and injected intraperitoneally with sodium pentobarbital (Nembutal^{R}) at a level of 45 mg per kg body weight. Sleeping time was measured as the time elapsed from loss to regaining of righting ability. At the end of 8 weeks treatment, the females were assigned randomly among four 0.1 ha outdoor penned enclosures with 16 untreated males for breeding purposes. They remained in the outdoor pens for 3 weeks during which untreated feed was provided. The rabbits were then retrapped and the females were brought to the laboratory, weighed, and sodium pentobarbital-induced sleeping times were again measured. The rabbits were sacrificed and livers were removed, preserved in 10% formalin, and later weighed. As reproductive characteristics, ovarian weights, number of corpora lutea (i.e. ovulation rates), and number and age of fetuses in utero were determined. The heads were removed and frozen for later analysis of brains to indicate relative concentrations of Mirex residues. A method employing gas-liquid chromatography with electron capture detection similar to the one described by MEDLEY et al. (1974) was used to determine Mirex residues. Petroleum ether was used as the extracting solvent. The samples were partitioned with petroleum ether saturated acetonitrile and cleaned up through activated 5% Florisil. Recovery for the procedure was 85.3% for pure Mirex and 25% for rabbit brains (n = 1). As only relative Mirex concentrations were of interest, the residues were not corrected for procedural losses.

RESULTS

After the third week of treatment, there was an apparent reduction in the weekly feed consumption values recorded for the Mirex-treated rabbits on ad libitum diets as compared to the control-ad libitum rabbits. This decrease in feed intake persisted throughout the remainder of the 8 week laboratory treatment period; however, the differences observed were not statistically significant ($P>0.05$).

None of the reproductive characteristics examined was significantly affected by Mirex treatment. The nutritional treatment did significantly affect many of the reproductive characteristics examined. These results have been published elsewhere (WARREN and KIRKPATRICK 1976).

Sodium pentobarbital-induced sleeping times and liver weights are presented in Table 1. The sleeping times recorded during the eighth week of treatment as well as those recorded after the

TABLE 1

Liver weights at sacrifice and sodium pentobarbital-induced sleeping times of female rabbits during and after cessation of Mirex treatment (mean ± S.E.)

			Sodium pentobarbital-induced sleeping times			
			After 8 weeks on treatment		3 weeks after cessation of treatment	
Group	n	Liver wt. (g)[a]	No. which slept	Minutes slept[b]	No. which slept	Minutes slept[b]
Control						
Ad libitum	8	35.3±3.6	7	178.6±13.7	5	140.6± 9.9
Restricted	7	29.3±2.8	7	213.1±11.5	5	182.0±18.3
Mirex-treated						
Ad libitum	6	39.5±2.9	2	95.5± 3.5	6	82.0±10.7
Restricted	8	39.6±3.8	6	97.5±10.9	5	70.6± 9.5

[a] $p<0.05$, ANOVA F-test for control vs. Mirex-treated.
[b] $p<0.01$, ANOVA F-test for control vs. Mirex-treated.

cessation of Mirex treatment for 3 weeks were significantly ($P<0.01$) less for Mirex-treated rabbits. All rabbits seemed to sleep for shorter periods after having been in the outdoor pens for 3 weeks than when sleeping times were measured in the laboratory. A paired "t" test of these differences failed to indicate significance, however. Liver weights for Mirex-treated rabbits were significantly ($P<0.05$) greater than for controls. Nutritional restriction for 11 weeks had no significant effect on either sleeping times or liver weights.

Table 2 presents the relative residues of Mirex recorded in the brains of rabbits in this experiment. Mirex-treated rabbits had significantly ($P<0.01$) higher concentrations of Mirex in their brains than did the control rabbits. No effect due to nutritional treatment was evident in the brain residues.

TABLE 2

Brain residues of control and Mirex-treated female rabbits on ad libitum and restricted diets. (mean ± S.E.)

Group	n	Mirex concentration (ppm)[a]
Control		
Ad libitum	8	0.008 ± 0.003
Restricted	7	0.003 ± 0.002
Mirex-treated[b]		
Ad libitum	6	4.206 ± 0.615
Restricted	8	4.528 ± 0.593

[a] $p<0.01$, ANOVA F-test for control vs. Mirex-treated.
[b] Brains were collected 3 weeks after cessation of Mirex-treatment.

DISCUSSION

Increased hepatic microsomal enzyme activity in the Mirex-treated rabbits apparently allowed them to metabolize the injected barbiturate faster and, as a result, sleep for shorter periods than the control rabbits. Previous work has shown that exposure to relatively low dosages of polychlorinated biphenyls also causes a decrease in barbiturate-induced sleeping times of rats (VILLENEUVE et al. 1972), mice (SANDERS et al. 1974), and cottontail rabbits (ZEPP et al. 1974).

In the present experiment, Mirex treatment had ceased for 3 weeks, yet the significant decrease in sleeping times for Mirex-treated rabbits still persisted. This indicates that Mirex was probably stored in adipose tissue and later released as fat reserves were mobilized, a conclusion which is in agreement with the persistent and accumulative properties of most chlorinated hydrocarbon insecticides.

Further evidence that Mirex treatment caused an increase in hepatic microsomal enzyme activity is provided by the greater liver weights recorded in Mirex-treated rabbits. Greater liver weights following exposure to low dosages of Mirex have been reported previously in mice (BYARD et al. 1974) and rats (GAINES and KIMBROUGH 1970, MEHENDALE et al. 1973). SANDERS et al. (1974) showed that sleeping times in mice treated with polychlorinated biphenyls were inversely related to liver weights. We observed a similar relationship in the current experiment. The greater liver weights recorded in this experiment were observed after the rabbits had been off Mirex treatment for 3 weeks.

Brain residues of chlorinated hydrocarbon pesticides are generally most precise for determining toxicity and indicate chronic exposure rates (STICKEL 1973). Previous work has been conducted on brain residues of Mirex. MEHENDALE et al. (1972) administered a single oral dose of 6mg/kg Mirex-^{14}C to rats. Seven days later, 0.07% of the original dose was located in brain tissue. IVIE et al. (1974) demonstrated that after dietary exposure to Mirex-^{14}C for 1 month, residues in brains of rats fed 30 ppm were approximately 28% of the dietary exposure level.

SUMMARY

Captive female cottontail rabbits on two nutritional regimes were fed 20 or 26.6 ppm Mirex for 8 weeks after which they were removed from treatment for 3 weeks and mated to untreated males. A nonsignificant decrease in feed consumption was observed in Mirex-treated rabbits. Mirex treatment did not significantly affect reproduction. Sodium pentobarbital-induced sleeping times measured after the rabbits had been on treatment for 8 weeks and again after treatment had ceased for 3 weeks were significantly shorter for Mirex-treated rabbits. Liver weights were significantly greater in the Mirex-treated rabbits. These results indicate that hepatic microsomal enzyme activity was increased due to Mirex exposure. Furthermore, since the effects were observed after Mirex treatment had ceased, it appears that Mirex was stored in adipose tissue and later released as fat reserves were mobilized. Brain residues of Mirex were significantly greater in Mirex-treated rabbits.

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