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Effect of Mirex On Induced Ovulation In Immature Rats (38550)

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(Introduced by William Hansel)

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The chlorinated insecticide, Mirex, is currently being utilized to control the imported fire ant in the Southeastern United States. Like other pesticides that accumulate in nontarget organisms, its use has increased concern regarding its potential adverse effects on mammalian reproductive processes. Several authors have documented the ability of chlorinated hydrocarbons, such as Mirex, to alter estrous cycles, and to reduce litter size in laboratory animals when the pesticides are included in the diet (1–5). However, the mechanism by which these compounds influence reproductive function is not clear. Possibilities include an effect on neural mechanisms controlling gonadotrophin release, a direct action on the gonads, and increased metabolic activity of the liver resulting in altered steroid feedback systems. Consistent with these proposals, organochlorines have been found to be both neurotoxic (6–8), and capable of inducing hepatic microsomal enzymes (9–13). One or a combination of these mechanisms could account for the detrimental effects of pesticides on fertility.

In spite of numerous studies concerning reproductive failures caused by chronic feeding of pesticides, little consideration has been given to the effect of these compounds on ovulation. The present study was designed to investigate the effect of Mirex on PMS-induced ovulation in immature rats and to provide more definitive evidence regarding a possible site of action. Portions of the data were presented at the 1973 Southern Section Meeting of the American Society of Animal Science (14).

Materials and Methods. Twenty-eight day old, Long Evan strain rats were used in this study. The animals were maintained in an ambient temperature of 68–72 F and a light–dark cycle of 13 hr light and 11 hr dark. The light period extended from 7 AM to 8 PM. Purina Laboratory Chow and water were available *ad libitum.*

Pregnant mare serum (PMS) was administered in saline to immature females on day 28 (0 hr) at a dose level of 45 IU. Mirex[3] was suspended in a carboxy methylcellulose vehicle and injected sc at various dose levels and times. The experimental treatments are illustrated in Tables I–IV. When Human Chorionic Gonadotrophin (HCG) was given it was suspended in saline at a concentration of 25 IU/0.1 ml and injected sc 60 hr after PMS. Except where otherwise designated, animals were killed 72 hr following the injection of PMS. Following sacrifice the oviducts were removed, examined for the presence of a swollen segment and the ova counted according to the technique of Zarrow *et al.* (15). Body weights were obtained when the animals were killed.

Data were analyzed by analysis of variance, Dunnett's and Student's *t* tests (16).

Results and Discussion. The events believed to follow PMS administration to immature rats are follicular development and estrogen production, facilitation by estrogen of the release of ovulatory levels of LH, and subsequently, ovulation. This sequence of events can be blocked by administration of compounds affecting the central nervous system, such as barbiturates, tranquilizers, and steroids (17–21). Similarly, the data presented in this report indicate that the inhibition of PMS-induced ovulation by an acute injection of Mirex resulted from an action of the insecticide on neural mechanisms controlling LH release.

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[3] Mirex = dodecachlooctahdro-1,3,4,metheno-2H-cyclobuta(cd)pentalene. Obtained from Mr. Jerry O'Neal, USDA Southern Methods Laboratory, APHIS, Gulfport, MI 39501.

TABLE I. EFFECT OF INCREASED LEVELS OF MIREX ON PMS-INDUCED OVULATION IN IMMATURE RATS.

Dose Mirex[a] (mg)	No. of rats	Body weight[b] (g)	Ova/rat[b]	% Rats ovulating
0.0	33	72.8 ± 1.7	32.9 ± 2.6	100
0.2	14	72.4 ± 3.1	31.2 ± 1.9	100
0.4	11	78.2 ± 3.1	20.0 ± 3.8[c]	82
0.8	15	72.5 ± 2.8	15.4 ± 2.8[d]	100
1.5	20	78.5 ± 2.3	17.4 ± 2.4[d]	100
3.0	22	83.4 ± 3.1	17.3 ± 2.5[d]	100
6.0	22	80.5 ± 2.6	18.2 ± 2.8[d]	96
12.0	12	68.9 ± 2.9	15.9 ± 3.8[d]	92
25.0	11	72.2 ± 2.3	9.5 ± 3.9[d]	100
50.0	11	75.9 ± 3.6	6.1 ± 1.9[d]	100

[a] Animals received Mirex in a carboxy methylcellulose suspension at doses indicated at 48 hr and were sacrificed at 72 hr after PMS. Control animals received carboxy methylcellulose vehicle.

[b] Values represent mean ± SEM.

[c] Significantly different from controls ($P < 0.05$).

[d] Significantly different from controls ($P < 0.01$).

TABLE II. EFFECT OF ADMINISTRATION OF HCG ON OVULATION IN PMS-MIREX TREATED IMMATURE RATS.

Dose Mirex[a] (mg)	No. of rats	Body weight[b] (g)	Ova/Rat[b]	% Rats ovulating
0.0	12	88.3 ± 1.2	35.2 ± 6.7	100
6.0	10	72.7 ± 1.6	34.7 ± 4.2	100
25.0	11	74.2 ± 2.1	29.7 ± 1.8	100
50.0	8	62.4 ± 4.0	44.6 ± 3.5	100

[a] Animals received Mirex in a carboxy methylcellulose suspension at doses indicated at 48 hr after PMS. Animals received 25 IU HCG 60 hr after PMS. All animals were sacrificed at 72 hr after PMS.

[b] Values represent mean ± SEM.

In the first experiment immature rats were injected with dosages of Mirex ranging from 0.2 to 50 mg at 48 hr to determine the minimum dose required to affect ovulation (Table I). Except for the 0.2 mg dose all treatment levels significantly reduced the numbers of ova recovered 72 hr after administration of PMS. Mirex had a progressively greater effect on ovulation as the level of the insecticide was increased. However,

TABLE III. EFFECT OF TIME OF ADMINISTRATION OF MIREX ON PMS-INDUCED OVULATION IN IMMATURE RATS.

Time of injection[a] (hr) after PMS	No. of rats	Body weight[b] (g)	Ova/rat[b]	% Rats ovulating
0 hr Control	12	77.1±2.9	30.3±1.2	100
0 hr Mirex	12	88.6±2.4	16.8±3.1[c]	92
24 hr Control	17	77.2±1.2	31.9±1.6	100
24 hr Mirex	17	73.1±1.2	14.7±2.2[c]	88
48 hr Control	33	72.8±1.7	32.9±2.6	100
48 hr Mirex	22	83.4±3.1	17.3±2.5[c]	100
58 hr Control	14	75.3±2.2	29.7±1.5	100
58 hr Mirex	15	75.6±2.0	9.3±2.0[c]	80
63 hr Control	13	67.0±3.2	31.0±1.6	100
63 hr Mirex	12	77.0±2.9	32.5±1.3	100

[a] Mirex treated animals received 3 mg Mirex in a carboxy methylcellulose suspension at times indicated. Control animals received the carboxy methylcellulose vehicle.

[b] Values represent mean ± SEM.

[c] Significantly different from controls ($P < 0.05$).

TABLE IV. EFFECT OF TIME OF SACRIFICE ON NUMBER OF OVA RECOVERED IN PMS-MIREX TREATED IMMATURE RATS.

Time of sacrifice[a] (hr) after PMS	No. of rats	Body weight[b] (g)	Ova/rat[b]
60 hr Control	8	72.4 ± 3.9	0.0
60 hr Mirex	8	67.2 ± 3.2	0.0
72 hr Control	33	72.8 ± 1.7	32.9 ± 2.6
72 hr Mirex	22	83.4 ± 3.1	17.3 ± 2.5[c]
96 hr Control	11	89.2 ± 1.4	11.2 ± 5.2
96 hr Mirex	10	82.9 ± 1.6	13.3 ± 3.6
120 hr Control	16	77.5 ± 2.7	9.0 ± 1.5
120 hr Mirex	12	72.2 ± 2.8	6.8 ± 2.2
132 hr Control	10	71.3 ± 3.8	1.9 ± 1.5
132 hr Mirex	12	74.4 ± 2.6	2.6 ± 1.3
144 hr Control	9	63.7 ± 7.6	3.7 ± 1.4
144 hr Mirex	10	75.6 ± 2.7	3.8 ± 1.3

[a] Mirex treated animals received 3 mg Mirex in a carboxy methylcellulose suspension at 48 hr following PMS and were sacrificed at times indicated. Control animals received the carboxy methylcellulose vehicle.

[b] Values represent mean ± SEM.

[c] Significantly different from controls ($P < 0.05$).

the inhibition was not linear as the numbers of ova shed were approximately 50% of the controls from the 0.8 mg to the 12 mg treatment level. The inhibition of ovulation was 70% and 80% when the amount of Mirex injected was increased to 25 and 50 mg, respectively. As 3 mg of Mirex was the intermediate dosage which reduced ovulation by 50%, it was selected as the treatment level for subsequent experiments.

A second experiment was conducted to establish whether Mirex was affecting the release of LH or inhibiting follicular rupture directly. HCG was administered after injections of 6, 25, and 50 mg of Mirex (Table II). Chorionic gonadotrophin overcame the inhibitory effects of Mirex, suggesting that the ovary was not a primary site of action of the insecticide, and giving support to the concept of inhibition of central nervous system mechanisms for LH release. However, as only a single dose of HCG was used, the possibility that Mirex elevated the threshold of response of the ovary to LH cannot be discounted. Nevertheless, the procedure of injecting HCG (or LH) has been utilized by several investigators to demonstrate a central inhibition, rather than an ovarian block, of ovulation by phenobarbital (21) and progesterone (20).

Substantive evidence for a central site of action of Mirex was obtained in a third experiment when the insecticide was administered at varying time periods before and after PMS-induced LH release (Table III). Ovulating hormone release has been shown to occur approximately 56 hr after PMS, or under controlled lighting conditions between 2:00 PM and 5:00 PM on the day preceding ovulation (19, 21, 22). These investigators either administered a nervous depressant or hypophysectomized the animals at different times following PMS. Using ova numbers as an endpoint, they found that once the ovulatory surge of LH was released neither drugs nor surgical removal of the pituitary could block ovulation. Likewise, in our study when Mirex was injected from 0 to 58 hr after PMS, a significant inhibition of ovulation resulted. If Mirex was administered after the expected release of LH (63 hr), ovulation was not affected. In contrast, Wedig and Gay (13) reported that chlordane injections failed to prevent ovulation or the preovulatory LH rise in PMS-primed immature rats. However, as ova were not quantitated in their study it is possible that ovulation was not completely blocked.

Although our data are indicative of a CNS site of action, the possibility that an alteration in hepatic function could be responsible for the reduction in ovulation cannot be ruled out. It has been suggested by several investigators that an increase in microsomal enzymes induced by various pharmacologic agents could increase estrogen metabolism and thus eliminate its positive feedback on LH release (21, 23, 24). However, it seems unlikely to us that sufficient modification of hepatic function could have occurred in the interval between 58 and 63 hr to account for the 70% inhibition and no inhibition, respectively. Wedig and Gay (13) also discounted a hepatic mechanism as a cause for phenobarbital blocked PMS-induced ovulation.

To assess whether an injection of Mirex at 48 hr resulted in a premature or delayed release of LH, animals were killed at different times following PMS (Table IV). Early release of LH was contraindicated by a failure to find ova at 60 hr. Likewise, no delay in LH release was evident at intervals to 144 hr post PMS administration. The numbers of ova shed were the same for control and Mirex-injected rats except for those killed at 72 hr. This is counter to the advancement or postponement of ovulation caused by injections of progesterone (21, 25, 26), or the 24 hr delay in LH release following injections of sodium pentobarbital in PMS-primed rats (17). These results suggest that an acute injection of Mirex results in a persistent inhibition of ovulation by some unknown action on nervous mechanisms which influence the release of LH.

There were no significant differences in any experiments in ovarian and uterine weights between controls and Mirex-treated animals.

Summary. PMS-induced ovulation was significantly inhibited in 28-day-old immature rats by acute administration of the chlorinated pesticide Mirex. Numbers of ova recovered were reduced by 40–80% from controls with dose levels of Mirex ranging from

0.4 to 50 mg per animal. When injections of Mirex 48 hr after PMS were followed by HCG the inhibitory effect of the insecticide was overcome, suggesting that the ovary is not the primary site of action. Injections of Mirex at intervals preceding the PMS-induced LH surge resulted in an inhibition of ovulation. Administration of the insecticide after the expected release of LH was ineffective. There was no indication of a premature or delayed release of LH due to injection of Mirex. The data suggest that Mirex inhibits PMS-induced ovulation by an unknown effect upon neural mechanisms controlling the release of LH.

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