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EFFECTS OF METHOXYCHLOR ON THE REPRODUCTIVE SYSTEM OF THE ADULT FEMALE MOUSE
1. GROSS AND HISTOLOGIC OBSERVATIONS

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Abstract — The purpose of this study is to examine the effects of the pesticide methoxychlor (MXC) on the reproductive system of the adult female mouse. Sexually mature (7- to 8-week) virgin female CD-1 mice were exposed to 1.25, 2.5, or 5.0 mg MXC (50% technical grade) via oral gavage for 5 consecutive days each week for either 2 or 4 weeks. Control groups received either 0.025 mg estradiol-17β (E-17β) or the sesame oil vehicle for the same time period. Vaginal smears were taken daily, and weights were recorded weekly. Twenty-four hours following the final exposure, animals were sacrificed. Ovaries and reproductive tracts were removed and weighed. One ovary from each animal was prepared for light microscopic evaluation. Results revealed a dose dependency of MXC in inducing persistent vaginal estrus (PVE). Ovaries of MXC-exposed and E-17β-exposed animals weighed significantly less than the sesame oil controls. In addition, there was an increase in the number of atretic large follicles in the E-17β group and in those mice treated with the two highest doses of MXC, indicating a potential reduction in the immediate fertility of the animal. Thus, this commonly employed pesticide appears to mimic closely those effects on the female reproductive system induced by estrogens.

Key Words: methoxychlor; ovary; persistent estrus; toxicology.

INTRODUCTION

Methoxychlor [1,1,1-trichloro 2,2-bis (*p*-methoxyphenyl) ethane] is a chlorinated hydrocarbon pesticide developed to replace both DDT and chlordecone. Methoxychlor has been used on fruits and vegetables near harvest, on shade trees, in home gardens, and in dairy barns for housefly control. It is employed as either a larvicide or adulticide against black flies and mosquitoes (1).

Compared to DDT and chlordecone, methoxychlor is considered to be less toxic (2), however, it has been shown to elicit significant effects in the mammalian organism. Zaleska–Freljan et al. (3) reported hyperemia and fine-grained inflammatory infiltration in the liver, and vacuolar degeneration in the walls of proximal convoluted kidney tubules in 7- to 8-week old mice. In male and female rats ingesting 2000 ppm methoxychlor in foods for periods up to two years, a significant incidence of hepatocellular carcinomas of the liver was observed (4).

Reports demonstrating that the reproductive system was not immune to the effects of methoxychlor also began to surface. Prenatal exposure of rats resulted in an increase in the number of resorption sites in the uterus and anomalous fetuses (5). Methoxychlor impaired reproductive behavior in prenatally exposed male rats and caused atrophy and abnormal development of the testes when immature male rats were exposed (6). Inhibition of spermatogenesis and fatty degenerative changes in Sertoli cells were major findings in male rats exposed to methoxychlor as adults (7).

Most reports detailing the effects of methoxychlor on the female reproductive system resulted from exposure during prenatal or postnatal periods. Specific studies revealed that exposure at these times induces precocious vaginal opening in rats (6), and hypertrophied uterine and oviductal epithelium in female mice (8). Little information is available as to whether exposure of the adult female results in any deleterious effect on the female reproductive system.

Like DDT and chlordecone, methoxychlor has been shown to possess estrogenic activity (9,10,11). Both DDT and chlordecone caused toxic effects directed toward the female reproductive system, similar to those caused by a synthetic estrogen, diethylstilbestrol (DES). Prenatal exposure of female animals to DES-induced cysts (12), hemorrhagic polyovular follicles in mouse ovaries (13), and uterine metaplasia in rats (14). A deficiency of corpora lutea and hypertrophy of intersti-

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tial cell cytoplasm was also observed in mice as adults (15,16).

Methoxychlor is currently being manufactured in the United States and is used widely throughout the country. It is apparent that very little is known regarding its effects on the adult female reproductive system. Since it is a structural analog of both DDT and chlordecone and since it has been shown to possess estrogenic activity similar to DDT and chlordecone, it is important to ascertain whether methoxychlor might cause similar adverse effects on the ovaries and whether such effects might lead to its being banned from use. For this reason, this investigation will analyze the gross and histologic effects that exposure to methoxychlor produces in the reproductive system of adult female mice.

MATERIALS AND METHODS

Adult virgin female CD-1 mice (Charles River Breeding Laboratories Wilmington, MA), 7 weeks old, were used in this study. They were housed in animal quarters with a 12:12 light:dark cycle. Food and water were provided ad libitum. After a 7-day period of acclimatization, mice were randomly distributed into 10 groups containing at least 8 mice each. Five groups were treated for 2 weeks and the other five for 4 weeks. Groups were exposed via oral gavage to either sesame oil, estradiol-17β (E-17β; Sigma Chemical Company, St. Louis, MO), or different concentrations of methoxychlor (MXC) dissolved in 0.2 mL of sesame oil that was used as a vehicle for methoxychlor. The chemical doses used were the following: 0.025 mg E-17β and 1.25, 2.5, and 5.0 mg MXC. The doses of MXC were equivalent to approximately 200, 100, and 50 mg/kg. The methoxychlor, which consisted of 50% MXC, was provided by Dr. V. Eroschenko of the Department of Biology at the University of Idaho, Moscow, Idaho. The groups treated with the pharmacologic regimens of estradiol served as a positive control in verifying whether the effects observed in MXC-treated animals were due to the estrogenicity of the pesticide or to the inherent toxicity of the chemical itself.

Weekly procedures consisted of 5 consecutive days of exposure to the chemicals, followed by 2 days of no treatment. This timetable was established to mimic an ordinary 5-day work week, representing the maximum weekly exposure to which a female working with such a compound might be subjected.

Animals were weighed at the beginning of the experiment and then at weekly intervals. Vaginal smears were taken daily to assess the onset of persistent vaginal estrus (PVE). An animal was considered to exhibit PVE when its vaginal smear contained keratinized and/or nucleated epithelial cells without leukocytes for 4 consecutive days (17).

Animals were sacrificed by cervical dislocation 24 h after the final exposure. Ovaries were removed and weighed, and one ovary from each of the 8 to 10 animals included in each of the treatment groups was fixed in Bouin's fixative in preparation for light microscopy. All ovaries were obtained from animals in estrus on the day they were sacrificed. Tissues were embedded in paraffin, then serially sectioned at 8 μm and stained with hematoxylin and eosin. Sections of the ovary were examined under a light microscope and the general histologic appearance of the ovary was assessed.

In addition, each section was examined for the presence of large follicles (over 300 μm in diameter), which were tabulated and then classified as either healthy or atretic, according to the characteristics described by Mandl and Zuckerman (18,19) for antral follicles. Specifically, this characterization consisted of seeing one of the following: more than 5 pyknotic cells in the granulosa cell layer, a free-floating oocyte detached from the granulosa cells, or an obviously degenerated oocyte. The percentage of atretic large follicles was determined and then compared among the 5 groups for statistical significance using the square root transformation together with analysis of variance. Dunnett's test was used to control the overall significance level for comparing experimentals to controls.

The reproductive tracts, which included the uterine horns and vagina, were also removed and weighed in order to ascertain whether these secondary organs of reproduction were grossly affected by MXC as had been shown in mice exposed prenatally to estrogenic compounds (14).

In order to insure normalization of data, the weights of the organs were expressed in relationship to the total body weight of the animal and not just as the true weight of the organ. All statistical analyses employed the organ weight values expressed in relation to the total body weight. The ovaries and reproductive tracts of the 5 groups were compared for statistical significance using analysis of variance together with Dunnett's test for comparing experimentals to controls.

RESULTS

Following 2 weeks of exposure, the members of the sesame oil control group increased their weight a mean of 3.2% ± 0.9% (SEM). Those groups treated with 1.25 and 2.5 mg MXC increased their weight a mean of 2.2% ± 0.6% and 4.4% ± 0.6%, respectively, which was not significantly different from that of the sesame oil controls (Figure 1). However, both the group treated with E-17β and that treated with 5.0 mg MXC for 2 weeks showed a significant increase in the percent weight gain. Estradiol-treated animals increased their weight a mean of 6.7% ± 0.5%, and the 5.0 mg

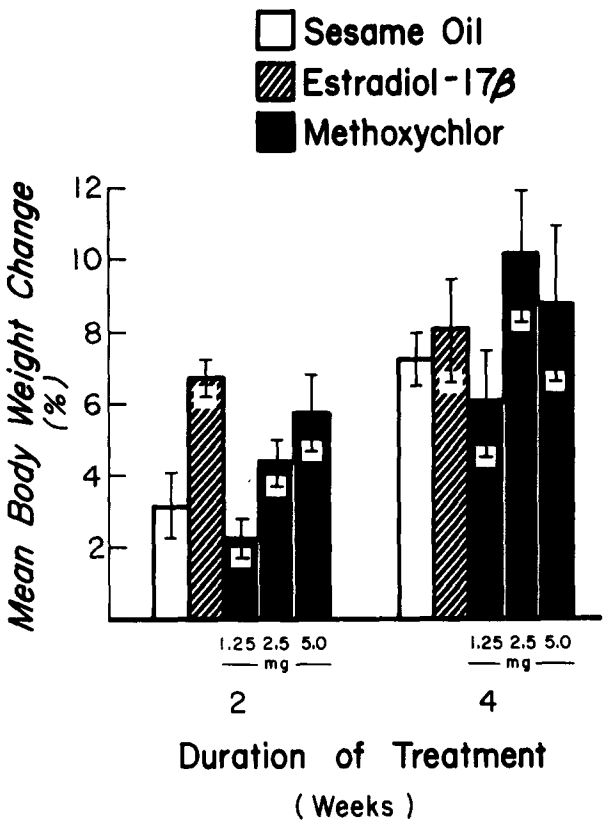

Fig. 1. Histogram showing the mean body weight change in the different treatment groups after 2 and 4 weeks of exposure to E-17β and MXC.

MXC-treated animals increased their weight 5.8% ± 1.1% during the first 2 weeks of exposure (Figure 1).

The estradiol-treated group and all 3 MXC-treated groups increased in weight by the end of the 4th week of exposure. The groups treated with 1.25, 2.5, and 5.0 mg MXC increased a mean of 6.0% ± 1.4%, 10.1% ± 1.8%, and 8.8% ± 2.1%. Mice treated with E-17β increased a mean of 8.0% ± 1.4% in weight over the 4-week period. None of these increases were significantly different from the sesame oil controls which, increased their weight a mean of 7.2% ± 0.8% (Figure 1).

The response to MXC with respect to the onset of persistent vaginal estrus (PVE) was related to the dose of MXC administered. Mice exposed to 5.0 mg MXC displayed PVE almost immediately, achieving this condition in a mean of 5.4 days following the beginning of MXC exposure. The rapidity with which these animals achieved PVE becomes readily apparent when one realizes that a minimum period of 4 days of constant estrus following the beginning of MXC exposure is necessary before an animal can be considered to be in PVE. This means that this group of mice entered estrus a mean of 1.4 days following the first MXC exposure, but it was

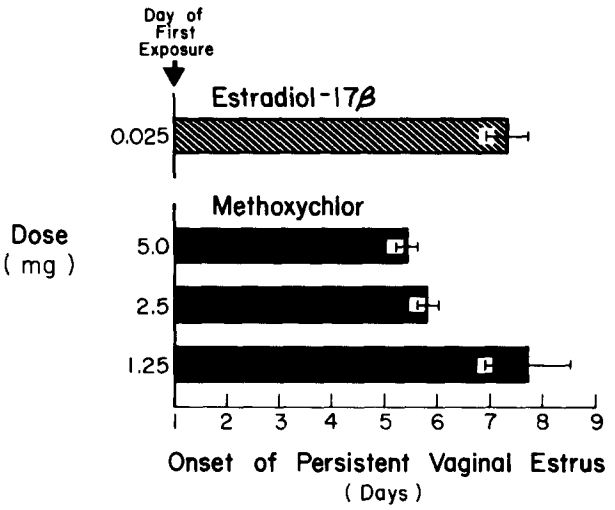

Fig. 2. Histogram depicting the mean number of days required by each treatment group to enter persistent vaginal estrus.

not until 4 subsequent days of estrus (5.4 days) that this group exhibited PVE. The 2 lower doses of MXC (1.25 and 2.5 mg) induced PVE in 7.7 and 5.8 days, respectively (Figure 2). The daily doses of 0.025 mg E-17β resulted in PVE in mice within a mean of 7.3 days. The sesame oil controls failed to demonstrate PVE throughout the duration of the experiment.

Analyzing the data obtained from ovarian weights following 2 weeks of exposure, no difference was observed in groups exposed to both MXC and estradiol when compared to sesame oil controls. The mean ovarian weights illustrated in Table 1 represent the weights of both ovaries combined. Four weeks of exposure was enough to significantly affect ovarian weight in the estradiol-treated animals and the groups receiving the 2 highest doses of MXC when compared to sesame oil controls (Table 1). At the end of the 4-week treatment period, the mean weight of the ovaries exposed to sesame oil had increased to 53.8 ± 3.1 mg/100 g body weight. These ovaries were significantly larger than those of both the estradiol-treated group and the 2.5 and 5.0 mg MXC-treated groups. Ovaries of animals exposed to E-17β had a mean weight of 40.9 ± 2.1 mg/100 g of body weight. The mean weights of the ovaries for the groups treated with 2.5 and 5.0 mg MXC were 35.7 ± 3.1 and 36.9 ± 5.3, respectively (Table 1). The group of mice treated with the lowest dosage of MXC (1.25 mg) had ovaries not significantly different in weight from the controls.

Macroscopically, the ovaries of the estradiol-treated mice and those treated with the higher doses of MXC presented an interesting picture. Most had a large amount of fat associated with them, especially after 4 weeks of exposure. In addition, the ovarian bursae were distended

Table 1. Tissue weights in mice treated with methoxychlor and estradiol-17β

Treatment	Dose (mg)	Number of mice	Tissue weight: Ovary mg	Ovary mg/100 g body wt	Reproductive tract mg	Reproductive tract mg/g body wt
2 weeks						
Sesame oil	--	10	10.8 ± 0.7[a]	43.1 ± 3.4	215.7 ± 28.3	8.5 ± 2.7
Estradiol-17β	0.025	16	9.5 ± 0.7	38.1 ± 2.2	209.0 ± 10.6	8.5 ± 0.5
Methoxychlor	1.25	9	9.8 ± 0.4	40.4 ± 1.2	222.4 ± 15.0	9.1 ± 0.4
Methoxychlor	2.5	12	9.7 ± 0.9	39.6 ± 3.3	209.5 ± 15.4	8.6 ± 0.6
Methoxychlor	5.0	13	8.9 ± 0.5	36.4 ± 2.2	234.6 ± 11.8	9.6 ± 0.5
4 weeks						
Sesame oil	--	8	13.2 ± 0.8	53.8 ± 3.1	242.1 ± 23.6	9.1 ± 1.4
Estradiol-17β	0.025	12	10.1 ± 0.6[b]	40.9 ± 2.1[b]	220.2 ± 9.6	8.9 ± 0.3
Methoxychlor	1.25	9	10.6 ± 1.0[b]	43.9 ± 4.3	234.0 ± 18.0	9.7 ± 0.7
Methoxychlor	2.5	9	8.7 ± 0.8[b]	35.7 ± 3.1[b]	214.9 ± 12.6	8.8 ± 0.4
Methoxychlor	5.0	9	9.5 ± 1.3[b]	36.9 ± 5.3[b]	233.5 ± 12.6	8.9 ± 0.4

[a]Standard error of mean.
[b]Significantly different from mean for sesame oil by Dunnett's test.

with fluid in a significant number of mice exposed to 2.5 and 5.0 mg MXC and E-17β.

A histologic view of the ovaries revealed a reduction in the number of large, fresh corpora lutea, at least in the ovaries treated with the two higher doses of MXC for 4 weeks when compared to numerous fresh corpora lutea found in sesame oil-treated mice (Figures 3 and 4). No quantitative tabulations, however, were conducted in this regard.

The mean number of large follicles found in the ovaries of all 5 groups did not differ following 2 weeks of exposure. These numbers ranged from a high of 44.4 ± 3.8 in the 5.0-mg MXC-treated group to a low of 36.6 ± 1.7 in those treated with 1.25 mg MXC (Table 2). The values for the other MXC-treated group, the E-17β-treated group, and the group exposed to sesame oil all fell in between these values. Interestingly enough, the former 2 groups also similarly presented the highest and lowest values for the percentage of atresia among these follicles. None were significantly different from the controls.

After 4 weeks of exposure, the mean number of follicles present again did not differ significantly among the 5 groups. In fact, there was little difference between

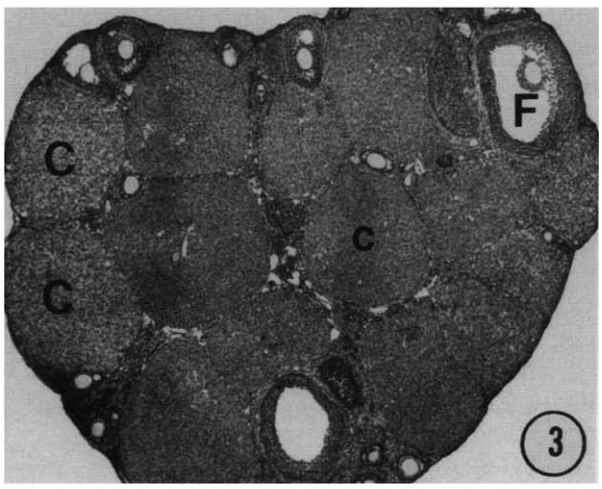

Fig. 3. Ovary of a mouse treated with sesame oil for 4 weeks containing many corpora lutea. Some fresh corpora lutea (C) can be observed as well as numerous older corpora lutea (c). Follicle (F). H and E stain. ×37.8

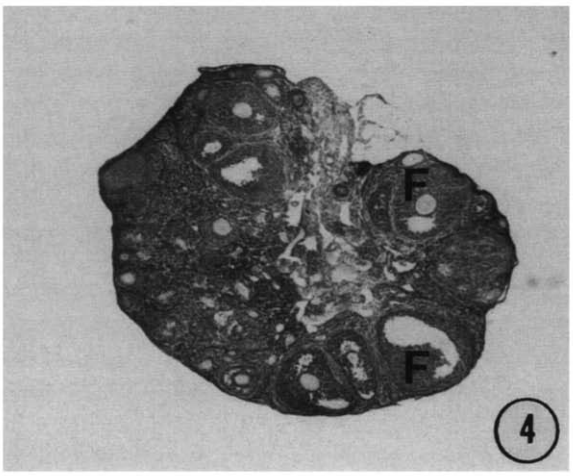

Fig. 4. Ovary of an animal treated with 5.0 mg MXC for 4 weeks. Note the absence of corpora lutea. Follicle (F). H and E stain. ×37.8

Table 2. Mean number and condition of large follicles in ovaries of mice exposed to methoxychlor for 2 and 4 weeks

Treatment	Dose (mg)	Large follicles			
		Total	Healthy	Atretic	% Atretic
2 weeks					
Sesame oil	--	37.5 ± 3.4[a]	17.4 ± 3.3	20.1 ± 2.7	55.6 ± 7.3
Estradiol-17β	0.025	39.0 ± 3.1	15.4 ± 1.4	23.6 ± 3.1	59.4 ± 4.1
Methoxychlor	1.25	36.6 ± 1.7	16.5 ± 1.3	20.1 ± 1.4	54.9 ± 2.9
Methoxychlor	2.5	41.6 ± 4.8	16.0 ± 2.8	24.4 ± 2.9	60.4 ± 4.9
Methoxychlor	5.0	44.4 ± 3.8	16.3 ± 2.0	28.1 ± 2.6[b]	63.2 ± 3.1
4 weeks					
Sesame oil	--	41.1 ± 3.0	22.5 ± 3.7	18.6 ± 2.7	46.3 ± 6.4
Estradiol-17β	0.025	40.0 ± 5.6	15.6 ± 2.4	24.4 ± 3.6	60.7 ± 2.3[b]
Methoxychlor	1.25	29.9 ± 5.6	13.1 ± 2.4[b]	16.8 ± 3.7	55.3 ± 4.1[c]
Methoxychlor	2.5	34.0 ± 4.1	14.1 ± 2.4[b]	19.9 ± 2.1	62.0 ± 4.0[b]
Methoxychlor	5.0	39.0 ± 3.9	15.9 ± 1.6	23.1 ± 2.8	58.6 ± 2.4[b]

[a]Standard error of the mean.
[b]Significantly different from mean for sesame oil by Dunnett's test.
[c]Difference from mean for sesame oil is borderline significant by Dunnett's test.

the values of each group following 2 and 4 weeks of exposure with the exception of the group treated with 1.25 mg MXC. In this group at 4 weeks, there was a mean of only 29.9 ± 5.6 large follicles present compared to 36.6 ± 1.7 at 2 weeks. In the group exposed to 2.5 mg MXC, 62.0% ± 4.0% of its large follicles were found to be atretic while the 5.0-mg MXC-treated group had 58.6% ± 2.4% of its large follicles in some stage of atresia. Similarly, the E-17β-treated group had slightly over 60% (60.7% ± 2.3%) of its large follicles undergoing atresia. These values were all significantly higher than the 46.3% ± 6.4% atresia seen in the sesame oil controls at this time. Those mice exposed to the lower dose of MXC exhibited only a borderline significance in the increase in the percentage of atresia in the large follicles present (Table 2).

The increase in atresia in large follicles in mice exposed to the higher doses of MXC was very apparent when viewing the tissue sections microscopically (Figure 5). The majority of these larger follicles were atretic, and this atresia was manifested in varying degrees. Some follicles displayed a very advanced stage of atresia in which many granulosa cells contained pyknotic nuclei with much cellular debris in the antrum and/or pseudocleavage of the oocyte (Figure 6). In other large follicles classified as atretic there was a minimal indication of the atretic process. In these follicles, the oocyte appeared healthy, and there was a minimal presence of pyknotic nuclei mainly in those granulosa cells near the follicular antrum. Cells of the cumulus oophorus appeared normal (Figure 7).

Although there was a significant increase in the amount of atresia in large follicles of the above-mentioned treated groups, there did not appear to be any effect on the small and medium-sized follicles in these groups (Figure 8).

There were no significant differences between the mean weights of the reproductive tracts among the different groups, whether they were exposed for 2 or 4 weeks (Table 1). At 2 weeks, the mean weights of the uterine horns and vaginae ranged from a high of 9.6 ± 0.5 mg/g body weight for the group receiving 5.0 mg MXC to a low of 8.5 ± 0.5 mg/g body weight for the group exposed to E-17β (Table 1).

DISCUSSION

The data presented here reveal that MXC, at least in the higher doses employed in this study, affects the adult female reproductive system in some ways similar

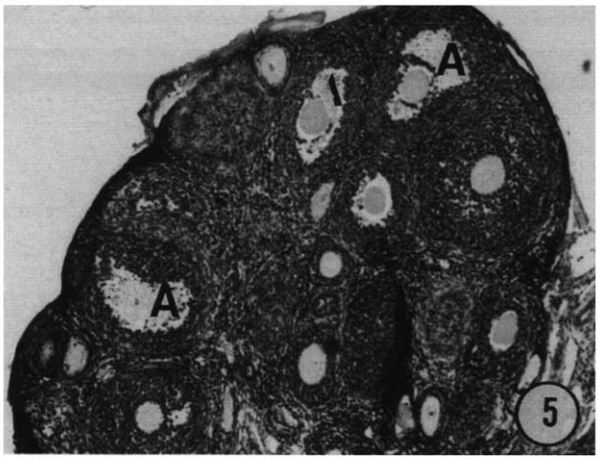

Fig. 5. Ovary of an animal treated with 5.0 mg MXC for 4 weeks. Observe the large number of atretic follicles (A) present containing pyknotic nuclei and some cellular debris in the antrum. H and E stain. ×75.6

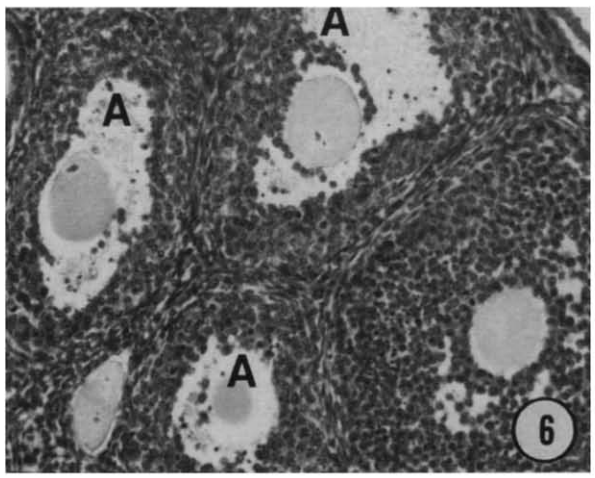

Fig. 6. Ovary of a 5.0-mg MXC-treated mouse after 4 weeks of exposure, showing advanced degeneration. Two of the atretic follicles (A) contain oocytes lying free in the antrum devoid of surrounding cumulus cells while the other atretic follicle has an oocyte with an incomplete layer of cumulus cells. H and E stain. ×75.6

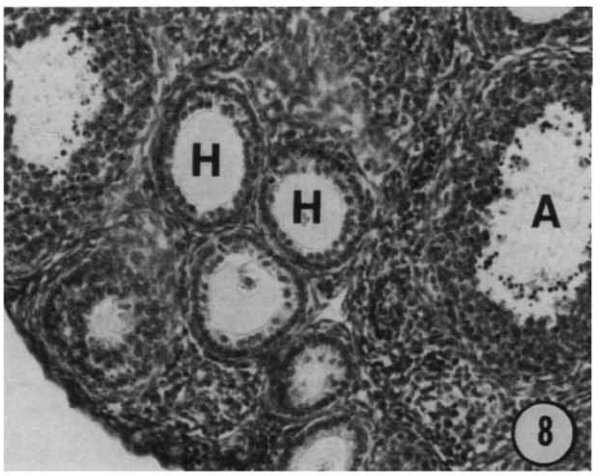

Fig. 8. Ovary of a 5.0-mg MXC-treated mouse after 4 weeks of exposure, containing small- and medium-sized healthy follicles (H). A large atretic follicle (A) can also be observed. H and E stain. ×189

to those induced by estrogenic substances such as estradiol and DES and other estrogenic pesticides such as DDT and chlordecone when administered to prenatal or postnatal females.

The present data revealed that exposure to MXC for as long as 4 weeks did not alter the normal weight gain compared to controls. This is significant since nutritional deficiencies have been shown to alter reproductive activities (20). Knuth and Friesen (21) reported that underfeeding resulted in loss of regular cyclicity in adult female animals. Quantitative alterations in food intake by adult female rats resulted in weight reduction, interrupted estrous cycles, and atrophy of the pituitary, uterus, and ovary (22,23). In the present study there was a significant increase in the percentage of body weight gain in both the E-17β and 5.0 mg MXC-treated mice following 2 weeks of exposure. The fact that there is an increase in percent body weight gain in these two groups is difficult to explain, especially in light of the fact that after 4 weeks of exposure, the percent body weight gain of these 2 groups was not significantly different from the controls. This differs from the results obtained by Hodge and colleagues (24) and Tullner and Edgcomb (25) which showed an adverse effect on body growth in male rats fed a 1% to 3% diet of MXC. This reduction in body weight appeared to be due to a decrease in food intake and not directly to the MXC. In the present study, there does not appear to be a nutritional deficiency that would be responsible for the observed reproductive effects.

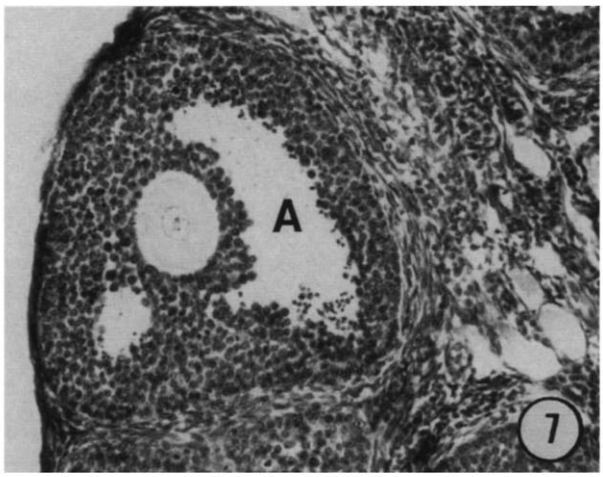

Fig. 7. Follicle of a mouse treated with 5.0 mg MXC for 4 weeks, displaying minimal atresia. The atretic follicle (A) present here contains a few pyknotic nuclei in granulosa cells near the antrum. H and E stain. ×189

The mice in this study were normal in appearance and behavior, unlike animals exposed to other estrogenic pesticides like chlordecone, which has been shown to induce severe tremors in addition to toxic effects on the reproductive system (28,29). It may be that the dose of MXC required to elicit such neurotoxic effects is much higher than that employed in this study. Swartz and colleagues (28) were able to observe reproductive effects of chlordecone at doses that did not induce any visible neuropathies.

In the present study, there was a rapid entrance of the MXC-exposed adult animal into PVE. The higher

the dose, the more rapidly the groups exhibited PVE. In fact, no matter what the dose of MXC, all animals were in PVE within 8 days of the beginning of the experiment. This would indicate that there was some estrogenicity of MXC. This agrees with studies by Bitman and Cecil (11), who reported the estrogenicity of MXC using the 18-h glycogen response in immature rat uteri.

Other pesticides have resulted in a similar alteration of the estrous cycle. Exposure of newborn rats to chlordecone-induced precocious vaginal opening and PVE (26,27). Uphouse and colleagues (17) and Swartz and colleagues (28) reported similar responses following exposure in adult rats and mice. The most potent estrogenic isomer of DDT is *o,p'*-DDT (10,29). This compound induced PVE and subsequent infertility when administered to newborn rats.

The present investigation revealed that following 4 weeks of exposure to MXC there was a significant decrease in the weights of the ovaries in those animals exposed to E-17β and the two higher doses of MXC. Histologic observations revealed that this decrease in weight was due primarily to the small number of corpora lutea in ovaries of these mice, since there appears to be little, if any, ovulation occurring in these animals. It has been shown that prenatal exposure to MXC results in an absence of corpora lutea in the adult (7).

Gellert (26) observed a similar decrease in ovarian weight in animals that were exposed to another estrogenic pesticide (chlordecone) prior to reaching puberty. He also attributed this reduction in weight to an absence of corpora lutea. Prenatal exposure to zearalenone, an estrogenic substance isolated from maize infected with fusarial mold, also caused a decrease in the weight of the gonads of rats (30). Haney and colleagues (16) observed a decrease in ovarian weight following prenatal exposure of mice to the synthetic estrogen, DES.

Estrogens have been shown to induce atresia in preovulatory follicles (31,32). In the present study, those animals exposed to E-17β for 4 weeks displayed an increase in the percentage of large follicles exhibiting atresia. Similarly, those groups of mice exposed to the two higher doses of MXC for 4 weeks contained ovaries with a significantly higher percentage of large atretic follicles than those of controls. Thus, both MXC and E-17β caused an increase in atresia in the large follicles, which was probably due to the estrogenicity of these agents. An increase in the percentage of large follicles displaying atresia has also resulted from exposure to the pesticide, chlordecone, which also possesses estrogenic activity (33). Dierschke and colleagues (34) reported that local application of E-17β unilaterally to adult rat ovaries resulted in a 51% reduction in the number of ovulations on the treated side. Since these treatments did not alter circulating levels of estrogens, they suggested that the action of estradiol had a direct effect on the ovary and, therefore, the reduction in ovulation was not a result of alterations in gonadotropic hormone secretion.

Interestingly enough, there were no significant differences between the total number of large follicles (healthy and atretic) among any of the groups. The concern for the increased atresia in the large follicles in the MXC-exposed animals centers around the fact that these constitute the pool from which oocytes are selected for ovulation. Therefore, in the event of an ovulatory stimulus (gonadotropins or possibly the cessation of pesticide exposure) there is a reduced number of oocytes available for ovulation, which might affect the immediate fertility of the female. Even if some of the oocytes from healthy follicles were induced to ovulate, it is unknown whether these eggs would be viable.

Although no attempt was made to tabulate small and medium follicles, it appeared from evaluation of the sections, that there was no apparent increase in atresia in these immature follicles in any of the groups. This being so, it would suggest that the possible immediate infertility observed due to the atresia in large follicles might be reversible once exposure to the pesticide ceases, since some of the medium-sized follicles would form the pool from which the new preovulatory follicles would arise.

It is not unusual for the toxicity of an agent to be limited to only one population of follicles. Such is the case with busulphan and endoxan, which selectively affected older secondary and tertiary follicles, causing degeneration, but which failed to exhibit any toxicity toward either early secondary follicles or primary follicles with large oocytes (35). Similarly, it was demonstrated that treatment with 3-methylcholanthrene in 4-week-old mice significantly decreased or depleted only primordial follicles (36).

Exposure of prenatal or immature animals to estrogenic substances such as DES has resulted in marked morphologic changes in the reproductive tracts of these animals as they reach adulthood. Such abnormalities include squamous metaplasia in the uterus (14), vaginal adenosis (37), and cystic endometrial hyperplasia (12). Exposure of neonatal female mice to MXC resulted in stratification of the vaginal epithelium, uterine swelling, and increased DNA content in the reproductive tract (8). Interestingly enough, an evaluation of an increase in weight of the reproductive tract following exposure of the immature female laboratory rodent to a specific chemical is employed as an assay for evaluating the estrogenicity of a compound (38).

No such changes were observed in the weights of the reproductive tracts in either the MXC-treated or the E-17β-treated mice when compared to that of controls, even after 4 weeks of exposure. Thus, adult exposure does not elicit the same weight increases in the repro-

ductive tract that neonatal exposure does. Estrogenic substances like zearalenone have been shown to interact with estrogen receptors in the uterine cytosol of both immature rats and mice (39,40). It may be that these receptors and their binding capacity change with age or that receptor sites are occupied by endogenous estrogens.

Methoxychlor, a widely used pesticide, is being employed as a substitute for the banned chlordecone. A similar chlorinated pesticide, DDT, had also been previously banned. All 3 of these compounds are similar in that they possess estrogenic activity. The property of estrogenicity itself in a compound provides a potential framework for inducing toxicity in the mammalian organism. Any agent that might compete with an endogenous steroid, estrogen in this case, might upset the delicate balance necessary for normal reproductive activity. Prenatal and neonatal exposure to MXC have resulted in adverse reproductive effects when such animals reach adulthood. These changes are similar to those induced by estrogens and other xenobiotics possessing estrogenic activity. Although these agents exhibit estrogenic effects, it is important to be aware that the general toxicity of each of them may be quite different.

The hypothalamic–pituitary–ovarian axis in an immature animal is in a state of development and differentiation and, thus, is extremely susceptible to exogenous agents. Exposure of such animals to sex hormones or substances possessing the activity of such hormones certainly affects the differentiation of the central nervous system and reproductive organs, with resultant abnormal reproductive patterns developing as adulthood is reached (41). The importance of the present study is that effects observed previously in animals exposed either prenatally or postnatally to estrogens or estrogenic substances are also seen in female mice exposed to MXC during adulthood following the completion of the growth and differentiation of the hypothalamic–pituitary–ovarian axis.

Whether such induced effects observed during the 2- or 4-week exposures of adult mice to MXC are reversible once exposure ceases is not known at the present time. Whether the observed effects occur as a result of a direct effect upon the ovary or whether only indirectly on this organ, possibly through action upon the hypothalamus and/or pituitary, cannot be ascertained from this study. What is apparent, however, is that the adult female can be adversely affected by this estrogenic substance, at least in the doses and by the route of administration employed in this experiment. Thus, necessary precautions should be employed by all adult females in using such substances, not just those who are pregnant, but those who are contemplating pregnancy.

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