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NEONATAL EXPOSURE TO TECHNICAL METHOXYCHLOR ALTERS PREGNANCY OUTCOME IN FEMALE MICE

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Abstract — This study was designed to determine the ability of female mice who were exposed neonatally to the pesticide methoxychlor (MXC) to mate, ovulate, and become pregnant upon reaching sexual maturity. One-day-old female mice (5 to 8/group) were exposed daily by intraperitoneal (ip) injection for 14 d to either sesame oil or 10 μg estradiol-17β or 0.1, 0.5 or 1.0 mg MXC suspended in sesame oil. The MXC exposures corresponded to 14 to 71, 68 to 357, or 135 to 714 mg/kg body weight, respectively. Three months later, female mice were placed with proven breeder males and checked daily for vaginal plugs. Mated female mice were sacrificed 18 d after the appearance of a vaginal plug to evaluate pregnancy. Uteri were examined for the presence of living fetuses and/or resorption sites. Ovaries were removed and prepared for histologic evaluation and tabulation of corpora lutea. All mice from all three MXC-treated groups did in fact mate, in comparison with only one of those exposed neonatally to estradiol. Increasing the dose of MXC produced a decreased number of pregnant animals at 18 d following mating. The mean number of live fetuses/litter was reduced in the 0.5 and 1.0 mg MXC-treated groups. Corpora lutea were significantly reduced in ovaries from only the 1.0 mg MXC group and the estradiol group. No effects of treatment were seen at 0.1 mg MXC. It is concluded that neonatal exposure to MXC does not interfere with mating. Instead, significant alterations are seen in initiating and/or maintaining pregnancy. The deleterious effects on pregnancy may be due to the influence of neonatal MXC treatments on the hypothalamic–pituitary–ovarian axis as well as on possible alteration of the uterine environment. © 1998 Elsevier Science Inc.

Key Words: methoxychlor; pregnancy; toxicology; ovary; uterus.

INTRODUCTION

Methoxychlor (MXC) is a chlorinated hydrocarbon pesticide widely used in this country on vegetables, fruit, and shade trees (1). This pesticide is considered to be a proestrogen that is metabolized in the liver into estrogenic compounds (2). Because of the estrogenicity of these metabolites, exposure of either the neonatal or the adult organism to high doses of MXC may place the female reproductive system at risk. MXC effects on the female reproductive system included the induction of precocious vaginal opening and persistent vaginal estrus in mice following 10 daily neonatal exposures totaling 80 and 160 mg/kg (3). Treating adult rodents with MXC increased lipid accumulation in ovarian cells (4) and hypertrophy of uterine epithelial cells in adult mice (5), in addition to implantation failure (6) and inhibition of the decidual cell response in rats (7). These alterations, although detrimental to normal reproductive activity during periods of actual exposure, have, for the most part, proved to be transitory. At least as far as MXC-induced effects on the adult female reproductive system are concerned, once exposure ceases, reproductive activities appear to return to normal.

How much more sensitive to MXC is the reproductive system in its early developmental stages? Does exposure of the newborn to this pesticide carry similar or increased risks to the female reproductive system as those seen following exposure of the adult? Exposure of the neonatal female mouse to MXC has resulted in some immediate effects directed at the reproductive system. Eroschenko (8) and Eroschenko and Cooke (3) have reported morphologic and biochemical alterations of the reproductive tract of immature females shortly after MXC exposure. Are such effects reversible or do such exposures of newborn females to MXC exert long-term effects on the reproductive system and compromise normal fertility and fecundity at sexual maturity?

We have recently shown that MXC administered to neonatal female mice produced variable effects in adults. A low MXC dose (0.1 mg) was augmentary in that the ovaries exhibited increased weight and were filled with corpora lutea and developing follicles. High MXC doses

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(0.5 mg and 1.0 mg), on the other hand, had an inhibitory effect on the ovaries in adult females, as seen by ovarian atrophy, relative ovarian weight loss, and depletion of corpora lutea (9). However, mice from this group could still be induced to ovulate by the administration of exogenous gonadotropins, although at a significantly reduced rate (10). Thus, the purpose of the present investigation was to compare the effects of neonatal exposure to estradiol or different doses of MXC on the ability of females to mate, ovulate, become impregnated, and produce living fetuses upon reaching sexual maturity. The results will indicate whether exposure of a female rodent immediately after birth to different doses of MXC could elicit significant adverse effects that might render the reproductive activities of these animals inoperative.

MATERIALS AND METHODS

Pregnant CD-1 mice were obtained from Charles River Breeding Laboratories (Wilmington, MA) at approximately 13 d of pregnancy. These mice were maintained on a 12:12-h light:dark cycle. Food and water were provided ad libitum. All females were allowed to deliver normally. Within 24 h of birth, dams with their litters were randomly assigned to different experimental groups and chemical treatments. One-day-old pups were exposed intraperitoneally (ip) for 14 consecutive d to either sesame oil only, 10 μg estradiol or 0.1, 0.5, or 1.0 mg MXC. Because the newborn mice, which weigh about 1.4 grams, are too small to be gavaged, the chemicals were administered ip. Furthermore, administering ip injections to these animals lets us control the dose more precisely. Since the amount of MXC given to each group was constant throughout the experiment, the dose in mg/kg changed as the animals rapidly increased in weight. The average weight of the newborn mice was 1.4 g and the average body weight of pups on Day 15 was 7.4 g, based on the weight of over 75 immature mice. Thus, the doses of 0.1, 0.5, or 1.0 mg of technical MXC corresponded to a range during the experiment of 14 to 71, 68 to 357, or 135 to 714 mg/kg body weight, respectively, and the 10.0 μg dose of estradiol ranged from 1.4 to 7.1 mg/kg body weight.

All chemicals were first suspended in filtered sesame oil and injected in a 0.05 mL volume of the vehicle, using a 27-gauge needle. All litters were treated without regard to the sex of the offspring. Furthermore, the litters were not culled for body weight. At the end of the treatments, males were removed from the litter and sacrificed. The sample size of females for the different experimental groups ranged from five to eight mice each. Chemical doses employed were based on previous data which found that these dose levels produced a range of dose-dependent alterations in the reproductive systems of immature and mature female mice without altering mortality or body weights (3,8,9).

The MXC, which consisted of 50% active ingredient, was provided by Kincaid Laboratories (Nitro, WV). This active ingredient is composed of 44% 2,2 bis-(p-methoxyphenyl)-1,1,1,-trichlorethane and 6% other isomers. Extenders make up the remaining 50% of the technical grade product. This formulation is manufactured under the trade name Marlate. It was selected because it is the form of pesticide that is currently used in the environment. The group treated with the pharmacologic dose of estradiol (10.0 μg) served as a positive control.

Female offspring were weaned at 21 d. At three months of age, the neonatally exposed females were placed with proven breeder males and checked for a vaginal plug the next morning to verify mating. If a vaginal plug was observed, the female was placed in a separate cage for 18 d. If no vaginal plug was present after the first attempted mating, the process of placing the experimental female with a breeder male was continued for a maximum of two weeks. If no vaginal plug was observed after this time, the female was considered unable to mate. The day on which a vaginal plug was seen was considered to be Day 0 of gestation.

After 18 d of gestation, females were sacrificed. Both uterine horns were examined for fetuses and resorption sites. The number of fetuses were tabulated and their status (living or dead) evaluated. In addition, the left ovary of each female was removed, fixed in 10% neutral buffered formalin, and embedded in paraffin. Animals that failed to mate within the two-week period also had their ovaries removed for evaluation. Tissues were serially sectioned at 8 μm and stained with hematoxylin and eosin. Sections of the ovary were examined under a light microscope and evaluated for the presence of healthy or abnormal follicles and number of corpora lutea.

Statistical analyses of the mean number of living fetuses/litter, the mean number of resorptions/litter, and the mean number of corpora lutea within the different groups were carried out. The data were summarized by computing group means and standard errors of the means. The group standard deviations were tested for homogeneity, and, because the heterogeneity was not statistically significant, a pooled estimate of the group standard deviations was used. Standard errors of the means were computed from the pooled estimate of the group standard deviation. The group means were compared using the Kruskal-Wallis analysis of variance to ascertain whether differences in mean number of living fetuses and mean number of corpora lutea were statistically significant. Once the global test was determined to

Table 1. Pregnancy and ovarian response in three-month-old mice following neonatal exposure to 10 μg estradiol and different doses of methoxychlor (MXC)

Treatment	No. of animals	Percent pregnant	Mean no. corpora lutea ± SEM
Sesame oil	5	100	10.4 ± 2.0
Estradiol-17β	8	0.0[a]	0.75 ± 1.6[a]
0.1 mg MXC	7	85.7	9.3 ± 1.7
0.5 mg MXC	8	75.0	9.4 ± 1.6
1.0 mg MXC	8	25.0[a]	4.5 ± 1.6[a]

[a]Statistically significant ($P < 0.05$) compared to sesame oil control.

be statistically significant at the minimal 0.05 level of significance, pairwise comparisons were made to isolate the groups that were significantly different using the Duncan's multiple range test to control the overall level of significance at the 0.05 level.

Fisher's Exact Test is the appropriate test statistic for analysis of pregnancy rate because pregnancy is a binary outcome and the sample sizes are small. The overall significance level was controlled using the Bonferroni method for the multiple comparisons made using Fisher's Exact Test.

RESULTS

Neonates survived the 14 daily injections well. At three months of age, mating ability was not inhibited in any of the MXC-treated groups. Of the 23 female mice treated with the different doses of MXC, only one exposed to 1.0 mg MXC failed to mate within the two-week time period. On the other hand, only one of the eight females exposed to estradiol exhibited a vaginal plug during the time assigned to mating; this single female provided no evidence of pregnancy when examined 18 d later. All five sesame oil vehicle control mice mated as evidenced by the appearance of a vaginal plug.

Pregnancy was considered to have occurred if either fetuses (living or dead) or resorption sites were detected at sacrifice on Day 18 of gestation. All five of the sesame oil control females became pregnant. The sole estradiol-treated mouse found with a vaginal plug was not pregnant. In the group exposed neonatally to 0.1 mg MXC, 85.7% of the females became pregnant, whereas in the 0.5 and 1.0 mg groups, 75% and 25%, respectively, became pregnant (Table 1). Statistically, the reduction in the percent of females achieving pregnancy was significant in the group exposed to 1.0 mg MXC.

However, even though a female achieved a state of pregnancy, this did not ensure that living offspring would result. To assess the ability of the exposed mice to maintain pregnancy, the mean number of living fetuses/litter and of resorptions/litter were evaluated. These data were obtained from only those animals in each group that had become pregnant. Since the estradiol group contained no pregnant females, it was not used in these calculations. Additionally, although there was a significant decrease in the percent of females exposed neonatally to 1.0 mg MXC becoming pregnant, this fact in itself provided an insufficient number of animals with litters on which to carry out statistical measurements on the mean number of living fetuses/litter and the mean number of resorptions/litter. Therefore, this group was omitted from these calculations (Table 2).

Table 2. Maintenance of pregnancy in three-month-old mice following neonatal exposure to different doses of methoxychlor (MXC)

Treatment	No. of animals with litters	Mean no. living fetuses/ litter ± SEM	Mean no. resorptions/ litter ± SEM
Sesame oil	5	9.2 ± 2.2	0.0
0.1 mg MXC	6	13.0 ± 1.3	0.2 + 0.2
0.5 mg MXC	6	4.0 ± 1.8	2.2 + 0.2[a]

[a]Statistically significant ($P < 0.05$) compared to sesame oil control.

A pregnancy was considered successful if at least one living fetus was present at the time of sacrifice. All sesame oil control females had living fetuses at the time of sacrifice. The mean number of living fetuses/litter found in the sesame oil controls was 9.2 ± 2.2 (SEM). The six females treated neonatally with 0.1 mg MXC that became pregnant had a mean of 13.0±1.3 living fetuses/litter (Table 2). Although there was a large apparent reduction in the mean number of living fetuses/litter in the group exposed neonatally to 0.5 mg MXC (4.0 ± 1.8), this difference was not significant. Although not being able to compute the data statistically because of the small number of females exposed to 1.0 mg MXC that actually became pregnant, it should be noted that of the two animals that became pregnant in this group, only one had living fetuses, which numbered 11.

The appearance of resorbed fetuses in the uterus is an indication of pregnancy but is also an indication of a failure to maintain pregnancy. No resorptions were observed in the pregnant uteri of control mice (Table 2). With increasing doses of MXC exposure neonatally, there was an increase in the number of resorbed fetuses/litter. A dead fetus close to term was found in one of eight females treated with 0.1 mg MXC. However, there were 14 living fetuses in the uterus of this female. Taking all the number of animals with litters into account in this group, there was a mean of 0.2 ± 0.2 resorptions/litter which was not significantly different from the control group. A significant increase in the mean number of resorptions/litter (2.2 ± 0.2) was observed in the 0.5 mg MXC group, where a total of 13 resorptions were observed in the uteri of four out of six pregnant mice exposed to 0.5 mg MXC (Table 2). It is interesting to

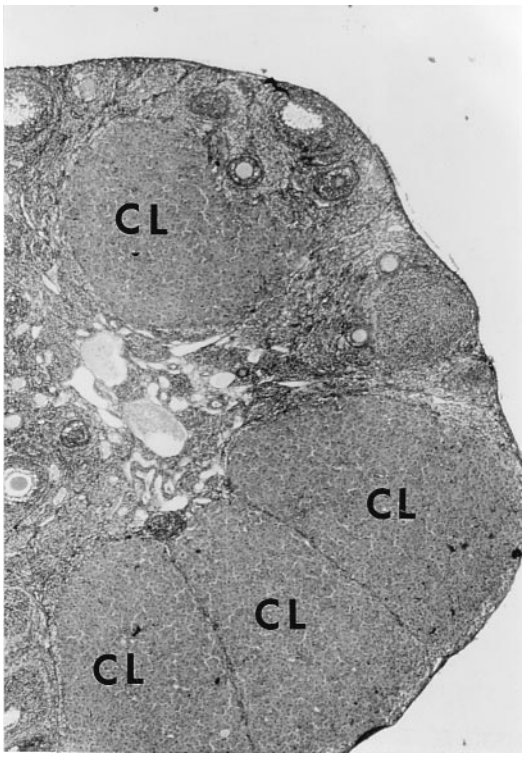

Fig. 1. Ovary from a control mouse treated with sesame oil during the neonatal period. Observe the numerous corpora lutea (CL) and the limited but basophilic interstitial tissue. X40

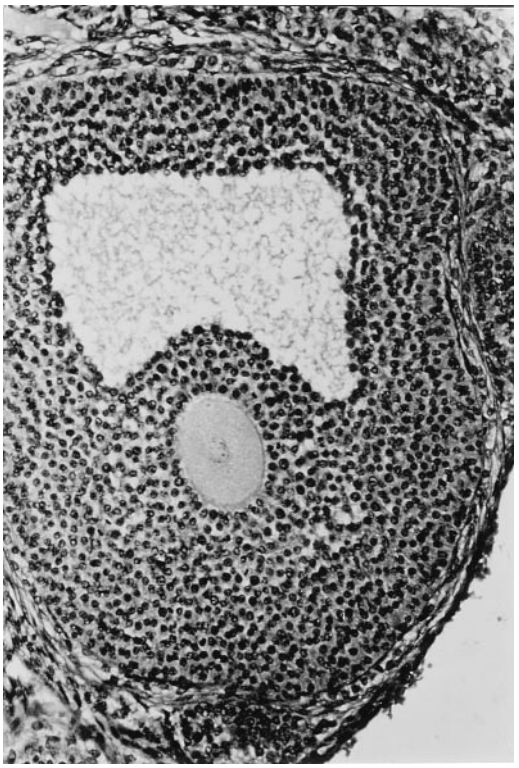

Fig. 2. Normal-appearing preovulatory follicle from a sesame oil control mouse. The follicle cells are healthy and form a thick wall surrounding the antrum. X180

note that of the two pregnant females exposed neonatally to 1.0 mg MXC, one had five resorbed fetuses in the uterus and the other had one resorbed fetus.

Histology of the control ovaries revealed numerous healthy preovulatory follicles and corpora lutea (Figure 1). Granulosa cells formed a thick wall surrounding the antrum of these preovulatory follicles, and there was little sign of any atresia in these follicles (Figure 2). The mean number of corpora lutea found was 10.4 ± 2.0 (Table 1).

In the estradiol-treated group, none of the females became pregnant. Ovaries of only three of the mice contained corpora lutea, with only one corpus luteum found in two of these mice (Table 1). The ovaries of the remaining five mice were devoid of corpora lutea and were filled with pale interstitial tissue (Figure 3). Numerous preovulatory follicles were observed, but most of them appeared atretic. However, there were also healthy medium-sized follicles in these ovaries.

The mean number of corpora lutea found in the groups exposed to 0.1 mg and 0.5 mg MXC was 9.3 ± 1.7 and 9.4 ± 1.6, respectively. These numbers were not significantly different from that of the control group. There was, however, a significant reduction in the mean number of corpora lutea in the group exposed to 1.0 mg MXC (4.5 ± 1.6). This represents a greater than 50% reduction in the number of corpora lutea when compared with the controls and the two lower doses of MXC.

The overall histologic appearance of the ovaries of the mice exposed postnatally to 0.1 mg MXC was similar to the sesame oil controls. Along with the numerous corpora lutea present, preovulatory follicles appeared normal, characterized by uniform follicular wall thickness all around the follicle (Figure 4).

Abnormal follicular wall configurations in large preovulatory follicles were common in mice exposed to 0.5 or 1.0 mg MXC. These follicles presented with specific characteristics common to both treatment groups. The follicle wall was thinned out in all areas (Figure 5) except at the cumulus oophorus, where it appeared normal. Even though these preovulatory follicles contained numerous pyknotic follicle cells, the ova in many of them appeared healthy.

Alterations within the cells of the follicle were not confined simply to the granulosa cells or those cells within the basement membrane of the follicle. The cells of the theca interna were characteristically seen as hypertrophied swollen cells all along the periphery of the follicle (Figures 6–8). This configuration was seen in almost all of the preovulatory follicles in those mice exposed to either 0.5 or 1.0 mg MXC.

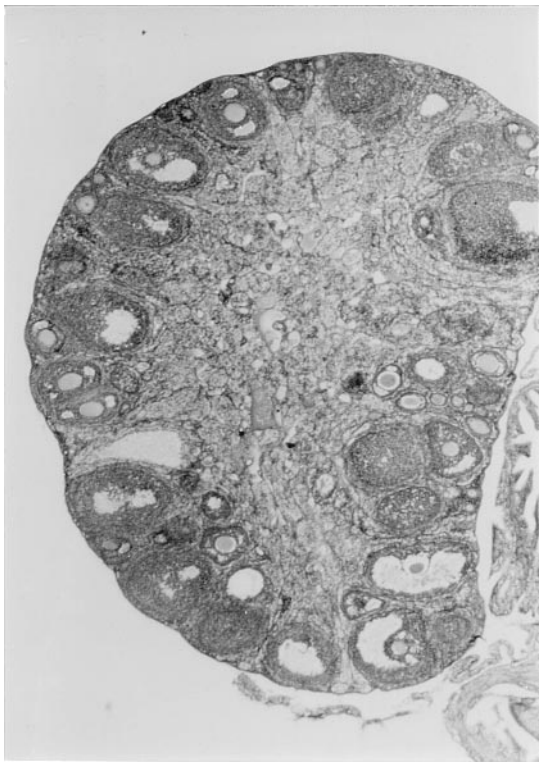

Fig. 3. Ovary from a mouse neonatally treated with estradiol-17β. The ovary is comprised of a vast area of pale interstitial tissue. Note the absence of corpora lutea. X44

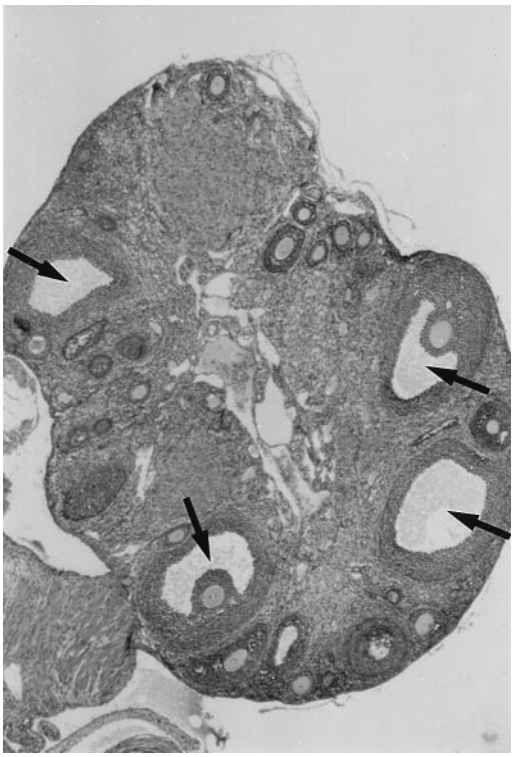

Fig. 4. Normal-appearing preovulatory follicles (arrows) in an ovary from a mouse treated with 0.1 mg MXC. Observe the uniform follicle wall thickness in these follicles. X44

Ovaries of the mice treated with 1.0 mg MXC were similar to those exposed to estradiol. The ovaries of mice from both these groups had few corpora lutea, and much of the ovary was comprised of interstitial tissue.

DISCUSSION

Exposure of female mice to MXC early in life produced significant alterations in ovarian morphology and fertility at sexual maturity. It has been reported earlier that 1.0 mg MXC exhibits about the same potency in affecting the immature uterus as 10.0 μg estradiol (3,8). This comparison in potency was established by evaluating and comparing effective doses of MXC and estradiol in inducing precocious vaginal opening in rodents (11–13), alterations in the estrous cycle, changes in reproductive tract morphology (5,8), and increases in uterine weight (2,14), glycogen content (15), and peroxidase activity (16).

There are reports, however, that indicate there are differences between MXC and estrogen activities in mammalian systems (17). In the present investigation, we also recorded several dissimilarities in the response of the female mouse to postnatal exposure to MXC and estradiol. At three months of age, female mice postnatally exposed to estradiol did not mate when placed with breeder males, whereas females exposed to different doses of MXC mated normally. That there was an interference with mating behavior in those female mice exposed postnatally to estradiol is not surprising because such treated mice exhibit changes in receptivity, lordosis, and sterility (18–20). In addition, large doses of estrogen given to neonatal rats invoke premature aging (21,22).

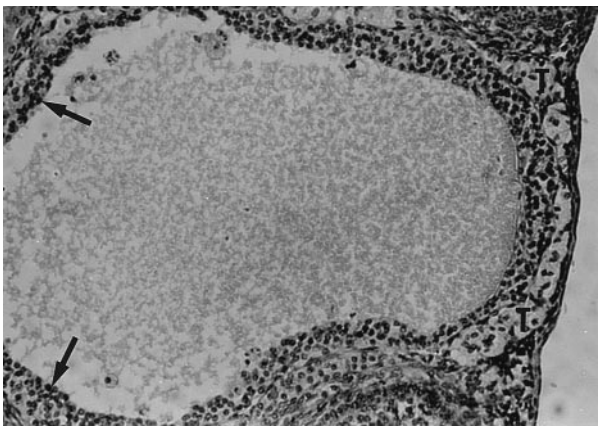

Fig. 5. Preovulatory-like follicle in an ovary of a mouse exposed to 0.5 mg MXC. The thinned-out follicle cell layers (arrows) and the hypertrophied theca (T) are readily apparent. X180

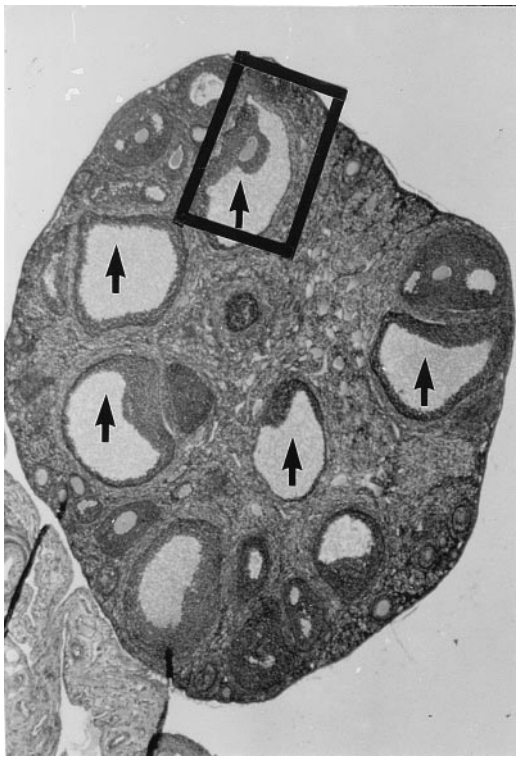

Fig. 6. Large preovulatory-like follicles (arrows) in an ovary of a mouse treated neonatally with 1.0 mg MXC. Follicular walls are thinned out and filled with pyknotic cells. Smaller follicles appear healthy. Note pale-staining interstitial tissue. X44

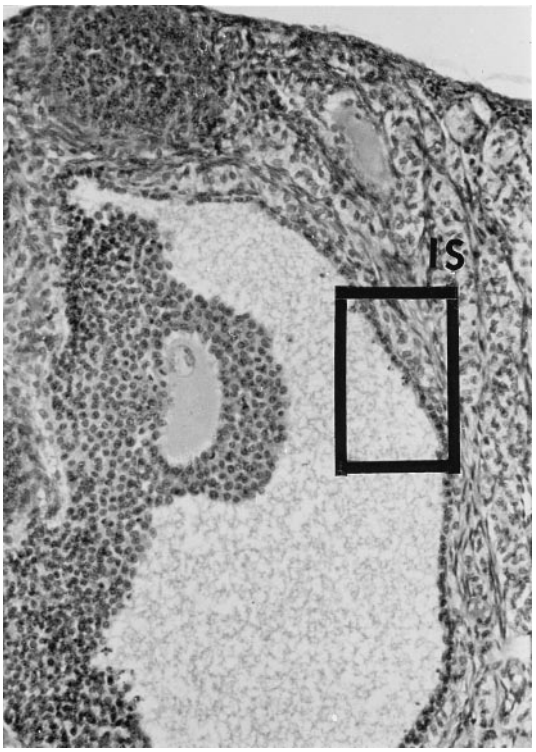

Fig. 7. High-power view of the area indicated in Figure 6 showing decimation of part of the follicle wall in a large preovulatory follicle containing healthy oocyte within the cumulus. Note pale interstitial tissue (IS). X180

Conversely, exposures to different MXC doses did not induce estrogen-like inhibition to mating. According to published data, it appears that increasing MXC doses would not have prevented mating. The highest concentration employed in the present study was 135.1 mg/kg body weight. Gray et al. (13) reported that sperm were detected in MXC-treated female rats that were acyclic. They used doses equivalent to 100 and 200 mg/kg body weight/d. In those females receiving the 200 mg/kg body weight/d dose, five of the 14 were found with sperm. Four of the five, however, failed to become pregnant. In our study, females exposed postnatally to MXC, although they mated successfully, did, however, exhibit compromised fertility. With increasing dose of MXC, there was a decrease in the pregnancy rate compared with the sesame oil control group in the 1.0 mg MXC group. No effects were observed in the 0.1 mg MXC group with regard to the mean number of living fetuses/litter. However, as the dose of MXC increased there was a reduction in this number. The MXC-induced effects on decreased pregnancy rate and mean number of living fetuses/litter apparently involved not only the reproductive organs, but also the hypothalamic–pituitary axis.

In our earlier work, we showed that neonatal exposures of female mice to different doses of MXC, in addition to altering ovarian morphology, initially interfered with normal estrous cycles and later caused permanent vaginal estrus (9). In a more recent study with rats and MXC, it was shown that the levels of estradiol were the same between control and MXC groups during estrus (23). This suggests that ovarian follicles differentiated and functioned similarly in control and MXC-treated mice. However, when the levels of the gonadotropic hormone FSH and the ovarian hormone progesterone were checked, it was seen that there were significant changes between control and treated rats. The anovulation and decreased number of corpora lutea were believed to be due to the effects of the high MXC doses on the hypothalamic–pituitary axis because both the FSH and progesterone plasma levels were significantly depressed at estrus (23). Thus, the comparison in our experiment of altered ovarian morphology, decreased number of corpora lutea, and decreased number of fetuses/litter between control and experimental groups would indicate that neonatal MXC exposures eventually interfered with normal hypothalamic–pituitary function in sexually mature animals.

In an earlier study, we failed to observe any corpora lutea in ovaries of three-month-old mice exposed to either 0.5 or 1.0 mg MXC during the first two weeks of

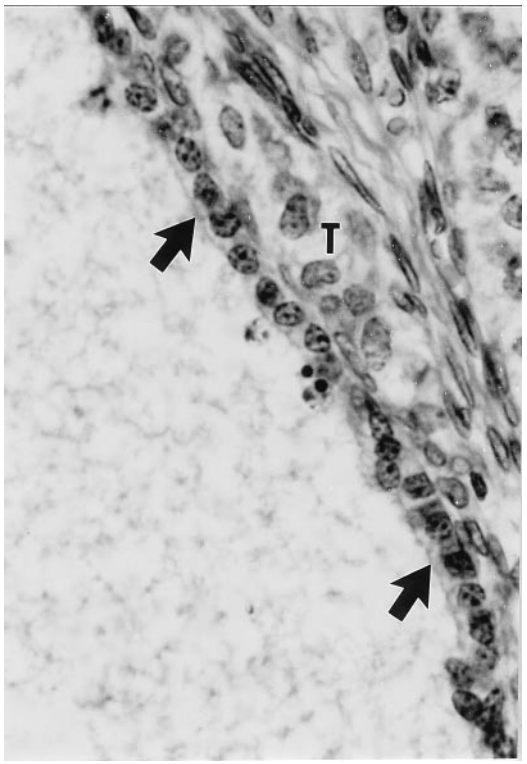

Fig. 8. High-power view of the area depicted in Figure 7 showing thinned-out follicle wall composed of follicle cells (arrows). The theca cells (T) are hypertrophied. X708

life (9). However, when such MXC-treated mice were exposed to a superovulatory regimen of exogenous gonadotropins, the ovaries ovulated and displayed fresh corpora lutea (10). This indicates that even with MXC treatment, there were preovulatory follicles that were able to respond to increased gonadotropin stimulation. The present experiment shows that, while MXC had an inhibitory effect on the ovaries, sufficient gonadotropin levels were apparently present in the MXC-treated mice to induce ovulations. Furthermore, some of the ovulated oocytes were viable and were fertilized. The fact that corpora lutea were found in the ovaries in the present experiment and not in the earlier study (9) could be due to the fact that the entire ovary was examined in this study, whereas only half of each ovary was observed in the earlier study (9).

The present investigation is the first to suggest that there is a dose-dependent response between neonatal exposures to MXC and the ability of mice to maintain pregnancy. In addition to MXC effects on the hypothalamus, pituitary, and ovary, the toxicant also affected uterine functions. This is evidenced by the fact that although some females treated with the two higher MXC doses did become pregnant and have successful implantations, they did experience an increased number of resorptions. The increased resorptions with increased dose of MXC could be due to reduction in plasma progesterone levels, as reported in female rats following exposure to MXC (23,24). Proper progesterone levels are essential for the maintenance of pregnancy and the well-being of the developing embryo (25). Other possible causes could be the change in uterine environment following MXC exposures, especially early in life.

Treatments of neonates with MXC produced a different pattern of uterine protein secretions when compared with estradiol (26). This altered uterine environment, combined with decreased progesterone levels (23, 24), could have produced a uterus that was hostile to embryo development. We have shown that when protein contents were analyzed after estradiol or MXC stimulation of the immature mouse uterus, MXC altered the secretory protein patterns (26). Thus, the toxic influence of MXC could interfere with reproduction by affecting ovarian functions and the uterine environment. At this time, we do not know how much the internal uterine environment of a mouse must be altered for it to become hostile to developing fetuses. However, the fact that MXC can alter the production of certain proteins could be a contributing factor in the present experiment to the increasing number of resorbed fetuses observed in the treated mice.

In other experiments, MXC has been shown to target both the pregnant and nonpregnant uteri of mice exposed as adults; MXC inhibited the decidual cell response in the uterus of pseudopregnant rats exposed to MXC (7). The decrease in the mean number of living fetuses/litter with the higher doses of MXC could also be due to the increased rate of embryo transport through the reproductive tract (27). More recently, MXC was found to induce an inflammatory reaction in the decidua of mice exposed early in pregnancy, ultimately resulting in death and detachment of the embryos (28). MXC also targets the uterine endometrium of nonpregnant mice as reflected by hypertrophy of the uterine endometrium (5).

Perhaps the most distinguishing feature of ovaries in mice treated with the two higher doses of MXC was the appearance and condition of the large preovulatory follicles. In these two MXC-treated groups, a large number of these follicles exhibited atresia. Although the entire follicle population is normally undergoing atresia in untreated animals, the atretic process in the preovulatory follicles of MXC-treated mice appeared different. The follicles were characterized by a healthy-appearing cumulus oophorus and oocyte, while that portion of the follicle opposite the cumulus exhibited a thinned out follicle wall containing numerous pyknotic nuclei. The healthy-appearing follicle cells and the healthy oocyte within these follicles, even in light of the numerous pyknotic follicular cells at the opposite pole of the

follicle, indicate that the important part of the follicle appeared viable. Thus, in the present study, at least in the group exposed to 1.0 mg MXC, there may be an effect on the ovulatory process. No entrapped oocytes were found within the corpora lutea, as seen in luteinized unruptured follicle syndrome, indicating that the contained oocyte was ovulated; it would appear that these ovaries were still able to ovulate. Eroschenko et al. (10) reported that such ovaries could ovulate, albeit a smaller number of oocytes following exposure to a superovulatory exogenous gonadotropic regimen.

The appearance of the theca interna cells surrounding these preovulatory follicles was also markedly altered in MXC-exposed mice. An apparent hypertrophy of thecal cells surrounding the follicle was seen, indicating that theca cells may also be the target of MXC action. Since theca cells contain the receptors for LH (29), it is possible that the MXC-induced hypertrophy of these cells alters their receptor function and compromises the LH effect on the ovary, resulting in altered ovulatory processes. Decreased presence of corpora lutea and decreased progesterone levels (23,24) would appear to support this conclusion.

The presence of an abnormally large amount of interstitial tissue in the ovaries of mice treated with estradiol or the two higher doses of MXC reflects increased atresia. Ovarian interstitial tissue is derived from follicular and theca cells of degenerating follicles. This abundance of interstitial tissue reflects an increased amount of atresia in these types of follicles, indicating that MXC might also interfere with reproductive processes in exposed animals by accelerating follicular atresia, leading to follicular depletion and decreased ovulation.

Thus, with increasing doses of MXC administered during the neonatal period, a significant effect of the pesticide is directed toward the function of the reproductive system of the adult animal. The induced toxic responses are manifested within the ovary and the uterus. These effects may be considered direct. However, some of the changes in ovarian morphology and early reports of decreased hormonal levels after MXC exposures also indicate that the toxicant has an indirect effect on the reproductive organs via the hypothalamic-pituitary axis. What is apparent, however, is the fact that even though the external appearance and mating behavior of these animals appear normal, neonatal exposure to MXC for as little as two weeks can severely affect the fertility of the exposed individual upon reaching sexual maturity.

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