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• *Original Contribution*

RESPONSE OF ADULT MURINE UTERINE EPITHELIUM TO 50% METHOXYCHLOR

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Abstract — This study was designed to assess the response of uterine epithelia of adult mice to a 4-week exposure of 50% methoxychlor (MXC) to ascertain whether significant changes were induced by 50% MXC that might compromise future implantation. Sexually mature virgin female mice were exposed to 0.1, 0.5, 1.0, 2.5, or 5.0 mg MXC via oral gavage for 5 consecutive days for 4 weeks. Controls received either sesame oil or 25 μg estradiol-17β (E-17β) also by gavage. At sacrifice, segments from each uterine horn were prepared for morphometric studies or for transmission electron microscopy. Results revealed a dose-dependent increase in the heights of uterine epithelial cells. Epithelial cell heights of the two groups treated with the highest doses of the pesticide were similar to that of the E-17β-treated group. Electron microscopy revealed increased vacuolization and swelling of mitochondria in cells of the 2.5 and 5.0 mg treated groups when compared to either of the control groups. In addition, there were effects on the number and size of microvilli in the uterine epithelial cells. The present study clearly demonstrates that a 4-week exposure of adult female mice to 50% MXC elicits significant estrogenic and toxic effects on the uterine epithelium.

Key Words: methoxychlor; uterus; toxicology; ultrastructure.

INTRODUCTION

Methoxychlor (1,1,1-trichloro 2,2 bis[*p*-methoxyphenyl] ethane is a chlorinated hydrocarbon pesticide widely used on vegetables, fruit and shade trees, and for insect control in dairy barns (1). Although its toxicity has been considered to be less than that of DDT and chlordecone, it is a proestrogen that is metabolized to compounds possessing estrogenic activity (2).

Exposure of the prenatal and newborn female mouse to methoxychlor (MXC) has elicited effects on the reproductive system. Eroschenko and Cooke (3) and Eroschenko and Rourke (4) have reported increased reproductive tract weights, accelerated vaginal opening, complete vaginal cornification, and uterine cell and oviductal epithelium hypertrophy as a result of MXC exposure early in development.

The reproductive tract of the newborn female mouse is highly sensitive to estrogens and estrogenic compounds, such as diethylstilbestrol (5). However, it is not known if the adult female reproductive tract is as sensitive to MXC as the developing perinatal structure. In other words, can MXC induce alterations in the adult female reproductive tract, specifically, the uterus, so as to affect immediate or future reproductive activity?

Evidence does exist that MXC exerts toxic effects on the adult female reproductive system. Recently, Martinez and Swartz (6,7) reported that following 4 weeks of exposure to this pesticide, adult mouse ovaries decreased in weight and contained fewer healthy large follicles. Ultrastructural alterations in these ovaries included increased lipid accumulations in interstitial and theca cells of exposed adult mice.

This study was designed to determine whether the adult uterus, already functioning in a normal physiologic mode, is susceptible to 50% MXC or if the toxic response of the uterus is limited to perinatal exposure. More specifically, the uterine epithelium of adult mice exposed to this organochlorine pesticide for 4 weeks was evaluated morphometrically and ultrastructurally in order to ascertain whether induced alterations in this uterine lining might be of

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such a nature as to interfere with implantation and compromise a future pregnancy.

MATERIALS AND METHODS

Adult virgin female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA), 7 weeks old, were used in this investigation. They were housed in animal quarters with a 12 : 12 h light : dark cycle. Food and water were provided ad libitum. After a 7-day period of acclimatization, mice were randomly distributed into seven groups. Groups were exposed via oral gavage to either sesame oil, estradiol-17β (E-17β; Sigma Chemical Company, St. Louis, MO) or 50% methoxychlor (MXC) for 4 weeks. The doses of 50% MXC used were 0.1, 0.5, 1.0, 2.5, or 5.0 mg. All chemicals were dissolved in sesame oil and administered in a 0.2 mL volume each day of exposure. The doses of this pesticide are approximately equivalent to 4, 20, 40, 100, and 200 mg/kg, respectively. The MXC, which consisted of 50% active ingredient was provided by Kincaid Laboratories (Nitro, WV). This formulation is manufactured under the trade name Marlate. It was selected because it is this form of the pesticide that is available commercially for the farmer and the pesticide applicator and, as a result, the one commonly used in the field. The group treated with the pharmacologic dose of E-17β (25 μg) served as a positive control in verifying whether the effects observed in the mice treated with 50% MXC 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 an exposure regimen for an individual in the workplace.

Animals were killed by cervical dislocation 24 h after the final exposure. Because the uterine epithelium varies in its morphologic configuration during the different stages of the estrous cycle, only animals in estrus were utilized for consistency. Uteri were removed promptly and fixed using two different techniques. The middle third of one horn of each uterus was fixed in 10% neutral buffered formalin for light microscopy and subsequent morphometric evaluation. The other horn was fixed in cold (4 °C) glutaraldehyde for 24 h in preparation for transmission electron microscopy.

Uterine tissue destined for morphometric studies was embedded in paraffin and serially sectioned at 6 μm, followed by staining of the sections with hematoxylin and eosin. Every 15th section was employed for acquiring morphometric data. In each 15th section, the heights of uterine epithelial cells were measured in 10 random locations around the uterine lumen. Each height was measured from the basement membrane to the apex of the cell. The total number of sections examined from each animal was 15, and because 10 random epithelial cells were measured in each section, there was a total of 150 measurements per uterus. The morphometric studies were performed using a Bioquant Image Analysis System IV Program.

The data were summarized by computing group means and standard errors of 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 cell height were statistically significant. Once the global test was determined to 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 Bonferroni approach to control the overall level of significance at the 0.05 level.

Uteri destined for ultrastructural studies were postfixed in 2% osmium tetroxide in 0.2 M cacodylate buffer (pH 7.2). They were dehydrated and then embedded in Polybed 812/Araldite capsules. The sections obtained were stained with uranyl acetate and lead citrate and observed on a Phillips CM10 electron microscope.

The uterine epithelial cells of three or four animals from each group were evaluated for ultrastructural morphologic alterations by observing the integrity of structures such as mitochondria, Golgi, microvilli, and endoplasmic reticulum in the differently treated groups.

RESULTS

There was a dose-related response of the uterine epithelium to 50% MXC. The mean height of the uterine epithelial cells exposed to sesame oil ± SEM was 26.0 ± 1.0 μm (Figure 1). Note that the estimate of the standard error of the mean used here is taken to be the square root of the pooled value of the mean squared error where pooling is over all seven groups. Those animals exposed for 4 weeks to either 0.1, 0.5, or 1.0 mg displayed no significant differences

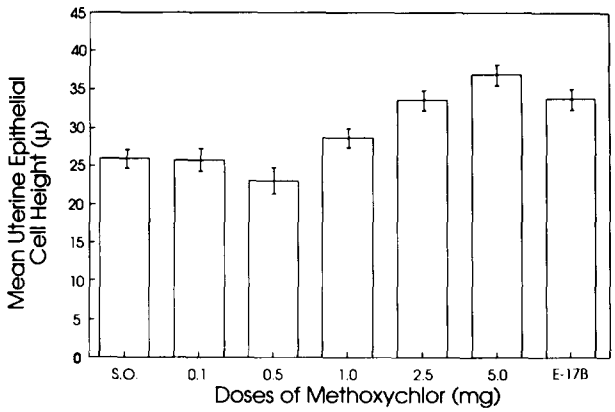

Fig. 1. Graph depicting the mean uterine epithelial cell height along with the standard error of the mean in response to 4-week exposure to different doses of 50% methoxychlor. Differences in the groups treated with estradiol, 2.5, and 5.0 of the pesticide are statistically significant when compared with the sesame oil group.

in uterine epithelial cell height when compared to sesame oil controls (Figure 1). However, the uteri of mice exposed to the two highest doses of this pesticide (2.5 and 5.0 mg) were characterized by hypertrophied uterine lining cells having a mean height of 33.6 ± 1.2 μm and 36.8 ± 1.2 μm, respectively. These values were significantly higher than that of the sesame oil controls ($P < 0.05$). In fact, the increased response seen in the uterine epithelial cells of mice exposed to these higher doses of 50% MXC was similar to that of the group exposed to estradiol-17β, the mean uterine epithelial cell height of which was also significantly higher than those of the sesame oil controls. The mean height of the uterine epithelial cells in the 5.0-mg group was not significantly different than that observed in the E-17β group (36.8 ± 1.2 vs. 33.7 ± 1.2 μm).

Electron microscopic studies revealed differences in the morphology of the uterine epithelial cells from animals treated with 50% MXC when compared with cells from sesame oil or E-17β-treated animals (control groups). Cells from the sesame oil group possessed short, apical microvilli, intact mitochondria, and well-developed Golgi complexes (Figure 2). The mitochondria were randomly distributed throughout the cytoplasm. Few vacuoles were contained in these cells. The endoplasmic reticulum was of the rough type and appeared normal. In cells of the E-17β-treated animals (Figure 3), Golgi complexes were somewhat dilated, and microvilli appeared taller when compared with those of the sesame oil group. There was an abundance of healthy mitochondria concentrated in the apical portion of the uterine epithelial cells. The endoplasmic reticulum appeared normal, and there were few vacuoles present.

The uterine epithelial cells of mice treated with 0.1 mg 50% MXC shared ultrastructural characteristics with those of both the sesame oil and estradiol-treated groups. They had microvilli similar to those seen in the estradiol group, but the mitochondria were distributed evenly throughout the cell like those of the sesame oil group (Figure 4). Cells from animals treated with 0.5 mg MXC demonstrated fewer microvilli and more vacuoles than either of the control groups. Mitochondria were randomly distributed throughout the cytoplasm in this group (Figure 5). Endoplasmic reticulum and Golgi complexes were dilated, and microvilli were sparse or nonexistent in some areas of the uterine epithelium in animals treated with 1.0 mg of the pesticide (Figure 6). Mitochondria still appeared normal and were found throughout the cytoplasm.

Cells from mice treated with 2.5 mg 50% MXC had few microvilli, swollen Golgi complexes and

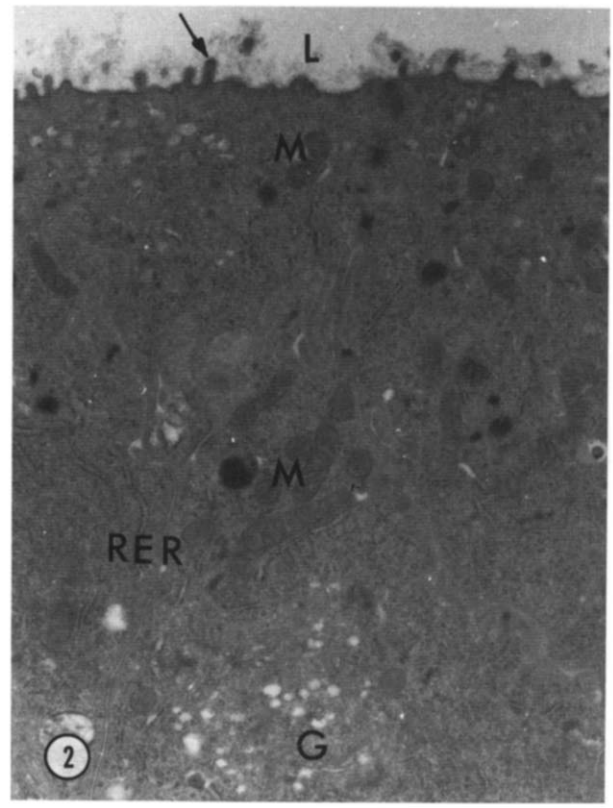

Fig. 2. Uterine epithelium from a mouse treated with sesame oil. Note microvilli (arrow) protruding into lumen (L), healthy mitochondria (M) scattered throughout the cytoplasm, rough endoplasmic reticulum (RER), and a well-developed Golgi complex (G). ×13,000

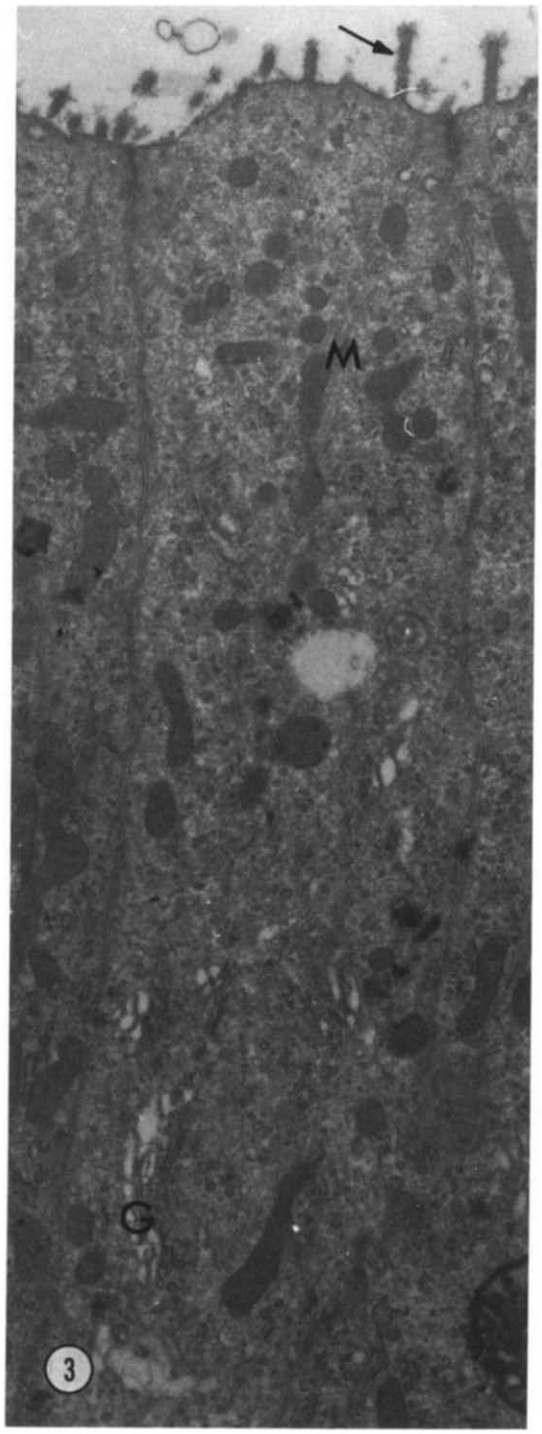

Fig. 3. Uterine epithelium from a mouse treated with E-17β. Mitochondria (M) are concentrated near the apex of the cell. Microvilli (arrow) appear taller than those of the sesame oil-treated group. The Golgi complex (G) appears somewhat dilated. ×13,000

endoplasmic reticulum, and an increase in vacuoles. Additionally, some of the mitochondria were enlarged and disrupted while others appeared normal (Figure 7). The group treated with the highest dose of the pesticide (5.0 mg) was characterized by significant ultrastructural aberrations. The uterine epithelial cells were filled with swollen, damaged mitochondria and a large number of vacuoles (Figure 8). The endoplasmic reticulum and the Golgi were dilated, and very few normal mitochondria were seen. The apical surfaces of these cells were very irregular with some thick blocklike microvilli.

DISCUSSION

The murine uterine epithelium is a dynamic structure, the morphology and biochemical activity of which are regulated by estrogen and progesterone. During the estrous cycle, the hormone-regulated events result in cycles of proliferation, differentiation, and death of uterine cells (8). The uterine epithelium reaches its maximum height from estrogen stimulation during estrus. In order to ensure proper comparisons of such a changing entity, all animals in the study were sacrificed during the estrus stage.

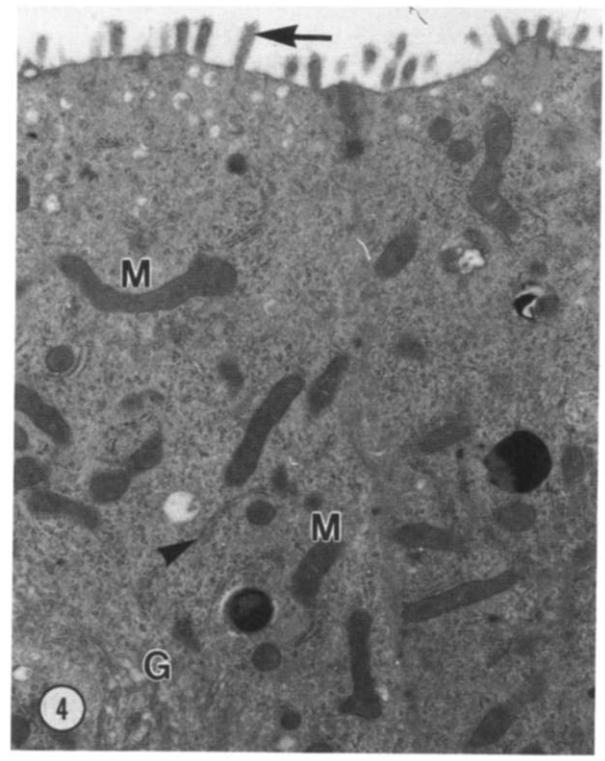

Fig. 4. Epithelial cells from the uterus of a mouse treated with 0.1 mg 50% MXC. Mitochondria (M) are numerous and appear healthy. Microvilli (arrow) are tall as in the E-17β-treated group (Figure 3). The Golgi complex (G) appears similar to those of the sesame oil and the E-17β-treated groups. ×13,000

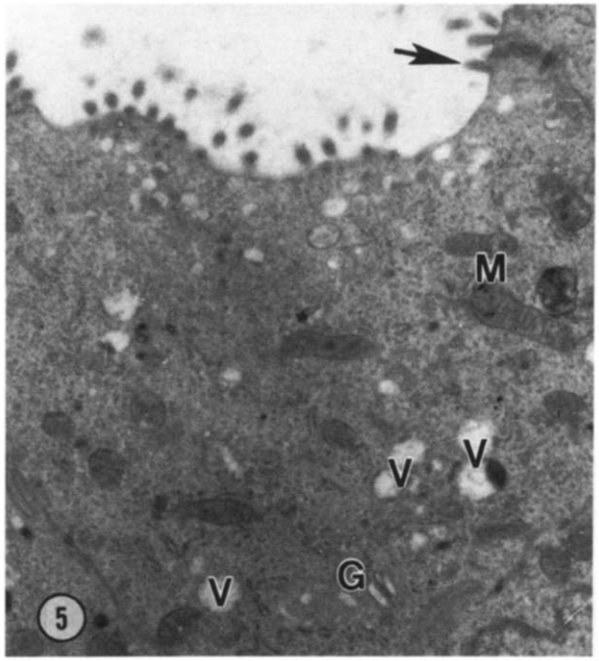

Fig. 5. Uterine epithelium from a mouse treated with 0.5 mg 50% MXC. There are fewer microvilli (arrow) and more vacuoles (V) than in the control groups (Figures 2 and 3). Mitochondria (M) appear normal, and the Golgi complex is well-developed. ×13,000

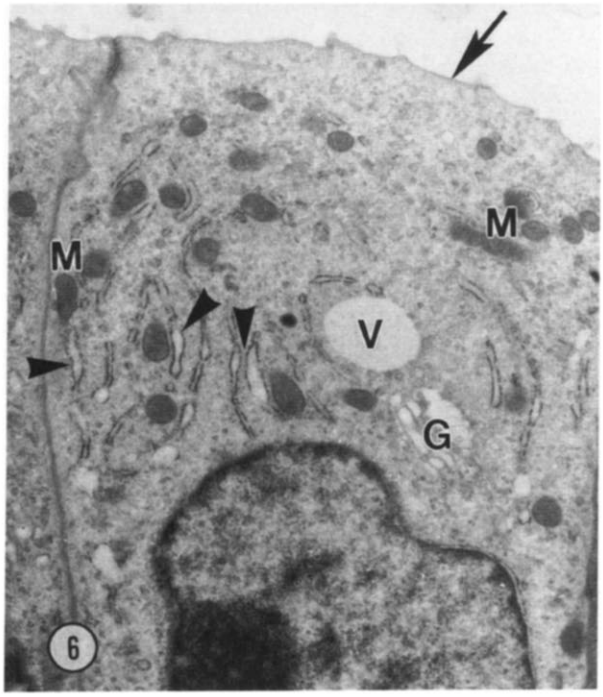

Fig. 6. No microvilli are evident on the surface (arrow) of the uterine epithelium from a mouse treated with 1.0 mg 50% MXC. The Golgi complex (G) and rough endoplasmic reticulum (arrowheads) appear swollen. A large vacuole (V) is evident; however, all mitochondria (M) appear normal. ×13,000

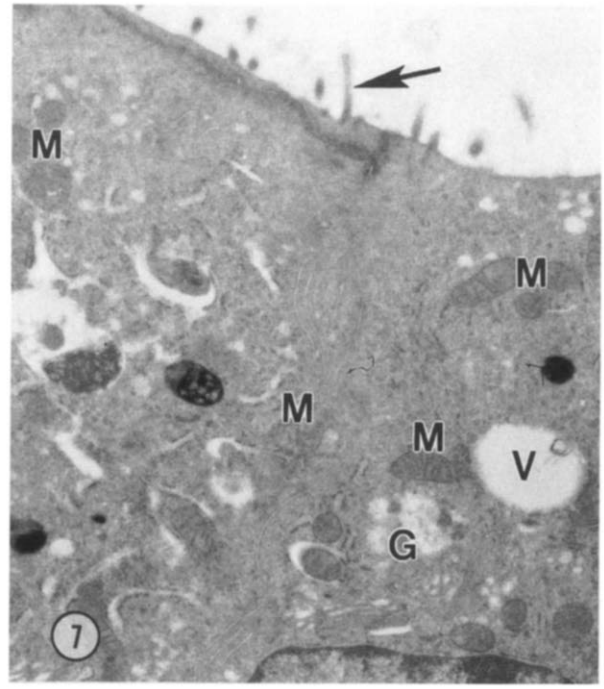

Fig. 7. Uterine epithelium from a mouse treated with 2.5 mg 50% MXC. There are few microvilli present (arrow). The mitochondria (M) on the right side of the micrograph appear normal, while those in the center and on the left side appear to be somewhat disrupted. The Golgi complex (G) is swollen, and a large vacuole (V) is present. ×13,000

Exogenous substances possessing estrogenic activity have been shown to exert estrogenlike effects on the female reproductive tract. Synthetic estrogens, like diethylstilbestrol (DES), some organochlorine pesticides like DDT (9,10) and chlordecone (11), and naturally occurring estrogenic substances from plants like coumestrol (12) possess estrogenic activity. MXC is another organochlorine pesticide that has been shown to possess estrogenic activity, presumably through its metabolites (13,14), but it has been considered to be less toxic than either DDT or chlordecone (15). However, MXC does induce significant alterations on the reproductive system (3,16–18).

Little information is available regarding exposure of the adult mammalian female reproductive system to MXC. Cummings and Gray (17) and Gray et al. (18), working with adult female rats, observed that purified MXC inhibited the decidual response in the uterus and induced uterine atrophy. However, this was determined simply by weighing the uteri. There was no attempt to assess the morphologic alterations underlying these inhibitory processes. Although the lack of a decidual response results from

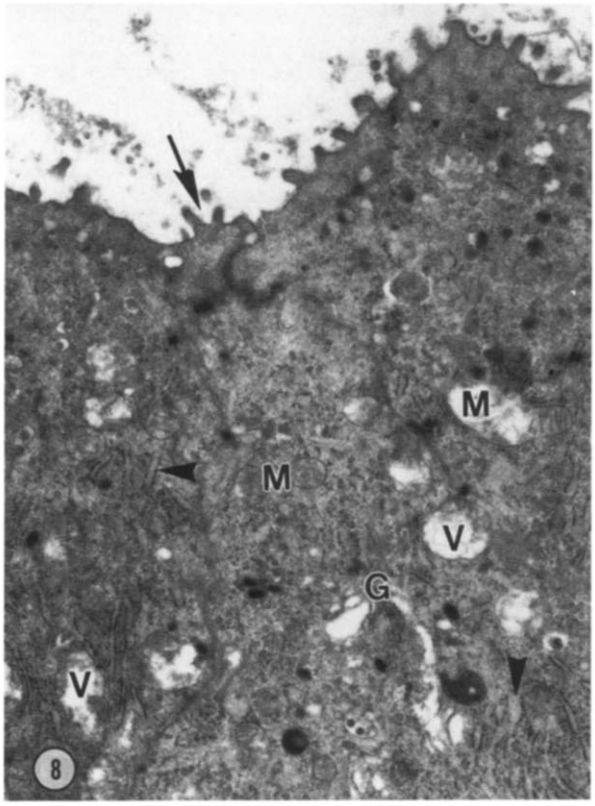

Fig. 8. Uterine epithelium from a mouse treated with 5.0 mg 50% MXC. Note irregular apical surface of cell with thick, blocklike microvilli (arrow) and vacuoles (V). Mitochondria (M) appear in various stages of degeneration. The Golgi complex (G) and rough endoplasmic reticulum (arrowheads) appears swollen. ×13,000

an excess of endogenous estrogen and exogenous MXC, the underlying subcellular effects of this within the uterine tissue are unknown. Of course, the absence of such a response would make implantation impossible.

Rourke et al. (19) observed reproductive tract alterations in ovariectomized mice when exposed to MXC for 14 consecutive days. In this model endogenous estrogen was eliminated from the system, and there was not competition for the estrogen receptor, allowing MXC to bind freely to the receptors. As a result, these authors reported uterine hypertrophy and alterations in protein secretory patterns within the uterus.

In the present study, MXC was forced to compete with endogenous estrogen for binding sites on estrogen receptors, because the ovaries were not removed. Significant cellular hypertrophy of the uterine epithelial cells was observed in those mice treated for 4 weeks with either 2.5 or 5.0 mg 50% MXC as well as those treated with E-17β when compared with sesame oil controls. This is similar to the response seen in animals esposed to MXC neonatally and in those ovariectomized mice exposed in adulthood. This cellular hypertrophy appears to be an estrogenic response quite similar to that seen in the E-17β-treated group.

Paralleling the cellular hypertrophy observed in the present study were specific ultrastructural characteristics that were dose-related. The uterine epithelium of the group receiving the lowest dose of the pesticide possessed tall microvilli similar to that of E-17β-treated animals. Estrogen has been reported to induce tall regular microvilli in uterine epithelial cells (20). This may be a reflection of the estrogenicity of MXC expressed at this low dose. At the higher doses, there was a loss and shortening of the microvilli that may have been a toxic response, overriding the estrogenic response.

Beginning with the 0.5 mg group, subtle specific ultrastructural changes became apparent. Fewer microvilli and an increasing number of vacuoles were observed. Eroschenko (21), using scanning electron microscopy, observed an absence of microvilli in some of the uterine epithelial cells exposed to high doses of MXC. In the 2.5-mg group in this study some of the mitochondria were enlarged and disrupted. Finally, the group exposed to 5.0 mg of this pesticide contained few healthy-looking mitochondria, the majority being swollen and vacuolated. Thus, we see a pattern of toxicity beginning with a decrease in microvilli and finally ending with a maximal effect of mitochondrial distortion along with swelling of the RER and Golgi.

Mitochondria have been implicated as targets for specific pesticides. Eroschenko and Becker (16) reported mitochondrial damage in oviducts of immature quail exposed to chlordecone. Byczkowski (22) found damaged mitochondrial membranes in rat liver mitochondria exposed to DDT. Bal (23) observed defective mitochondria in seminiferous tubules of rats exposed to MXC. MXC has been shown to inhibit oxidative phosphorylation and ATPases in mitochondrial fractions obtained from other tissues, such as liver and cardiac muscle (24), which probably signifies the demise of the cells.

The dilated RER and Golgi probably reflect an attempt to increase secretory activity. Such configurations of these organelles have appeared as a result of estrogen stimulation (25), which induces increased secretory activity. Gossypol, a phenolic pigment with reported antifertility properties in both males and females, induced swollen RER in chronically treated women (26).

RER and Golgi complexes are actively engaged

in protein synthesis and in packaging of such protein for secretion. Morphologic alterations in these organelles may result in changes in protein secretion within the uterus of MXC-treated mice (19).

In conclusion, this study has shown that 50% MXC, in the regimen administered, exhibits both estrogenic and toxic effects on the reproductive tract of the intact adult female. However, it still needs to be ascertained which components of technical MXC are responsible for the observed effects on the uterine epithelium. They may be due to the inherent toxicity of MXC, the products of MXC metabolism, estrogenic contaminants in technical MXC, or to any combination of these factors. With increasing dosage, more and more toxic effects become visible following 4 weeks of exposure. Even with the presently employed regimen of 5 days of exposure to this pesticide followed by 2 days of no exposure, 4 weeks of such exposure eventually takes its toll on the reproductive tract. Therefore, even though MXC is rapidly eliminated from the system, there appears to be a significant effect on the adult system. It appears unlikely that such uteri could accept a fertilized egg for implantation because of the observed alterations in the uterine epithelium. Whether these toxic effects would have a permanent effect on the future fertility of the individual, as seen in those animals exposed perinatally, cannot be determined from this study.

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REFERENCES

1. McEwen FL, Stephenson GR. The use and significance of pesticides in the environment. New York: John Wiley & Sons; 1979.
2. Tullner WW. Uterotrophic action of the insecticide methoxychlor. Science. 1961;133:647–8.
3. Eroschenko VP, Cooke PS. Morphological and biochemical alterations in reproductive tracts of neonatal female mice treated with the pesticide methoxychlor. Biol Reprod. 1990;42:573–83.
4. Rourke AW. Uterine ultrastructure and patterns of protein secretion following estradiol or methoxychlor treatments. Anat Rec. 1991;229:26A.
5. Ennis BW, Davies J. Reproductive tract abnormalities in rats treated neonatally with DES. Am J Anat. 1982;164:145–54.
6. Martinez EM, Swartz WJ. Effects of methoxychlor on the reproductive system of the adult mouse: 1. Gross and histologic observations. Reprod Toxicol. 1991;5:139–47.
7. Martinez EM, Swartz WJ. Effects of methoxychlor on the reproductive system of the mouse: 2. Ultrastructural observations. Reprod Toxicol. 1992;6:93–8.
8. Allen E. The oestrous cycle in the mouse. Am J Anat. 1922;30:297–371.
9. Bitman J, Cecil HC, Harris SJ, Fries GF. Estrogenic activity of *o,p'*-DDT in the mammalian uterus and avian oviduct. Science. 1968;18:371–2.
10. Robison AK, Schmidt WA, Stancel GM. Estrogenic activity of DDT: Estrogen–receptor profiles and the responses of individual uterine cell types following *o,p'*-DDT administration. J Toxicol Environ Health. 1985;16:493–508.
11. Gellert RJ. Kepone, mirex, dieldrin and aldrin: estrogenic activity and the induction of persistent vaginal estrus and anovulation in rats following neonatal treatments. Environ Res. 1978;16:131–8.
12. Burroughs CD, Bern HA, Stokstad ELR. Prolonged vaginal cornification and other changes in mice treated neonatally with coumestrol, a plant estrogen. J Toxicol Environ Health. 1985;15:51–61.
13. Welch RM, Levin W, Conney AH. Estrogenic action of DDT and its analogs. Toxicol Appl Pharmacol. 1969;14:358–67.
14. Bulger WH, Feil VJ, Kupfer D. Role of hepatic monooxygenases in generating estrogenic metabolites from methoxychlor and from its identified contaminants. Mol Pharmacol. 1985;27:115–24.
15. Ball HS, Mungkornkarn P. Chronic toxicity effects of methoxychlor on the reproductive system of the rat. Proc Int Cong Toxicol. 1978;1:446–7.
16. Eroschenko VP, Becker GM. Chlordecone (kepone) induced ultrastructural and morphometric alterations in the cells of the immature quail oviduct. Teratology. 1984;33:19–32.
17. Cummings AM, Gray LE Jr. Methoxychlor affects the decidual cell response of the uterus but not other progestational parameters in female rats. Toxicol Appl Pharmacol. 1987; 90:330–6.
18. Gray LE Jr, Ostby JS, Ferrell JM, Sigmon RE, Goldman JM. Methoxychlor induces estrogen-like alterations of behavior and the reproductive tract in the female rat and hamster: effects on sex behavior, running wheel activity, and uterine morphology. Toxicol Appl Pharmacol. 1988;96:525–40.
19. Rourke AW, Eroschenko VP, Washburn LJ. Protein secretions in mouse uterus after methoxychlor or estradiol exposure. Reprod Toxicol. 1991;5:437–42.
20. Luxford KA, Murphy CR. Changes in the apical microfilaments of rat uterine epithelial cells in response to estradiol and progesterone. Anat Rec. 1992;233:521–6.
21. Eroschenko VP. Ultrastructure of vagina and uterus in young mice after methoxychlor exposure. Reprod Toxicol. 1991;5: 427–34.
22. Byczkowski JZ. Biochemical and ultrastructural changes in biological membranes induced by *p,p'*-DDT (chlorophenoytanum) and its metabolites. Pol J Pharmacol. 1977;29:411–7.
23. Bal HS. Effect of methoxychlor on reproductive systems of the rat. Proc Soc Exptl Biol Med. 1984;176:187–96.
24. Desaiah D, Cutkomp LK, Yap HH, Koch RB. Inhibition of oligomycin-sensitive magnesium adenosine triphosphate activity in fish by polychlorinated biphenyls. Biochem Pharmacol. 1972;21:857–65.
25. Alkhalaf M, Chaminadas G, Propper AY, Adessi GL. Ultrastructural changes induced by oestradiol-17β, progesterone and oestrone-3-sulphate in guinea-pig endometrial glandular cells grown in primary culture. J Endocrinol. 1989;122: 439–44.
26. Bender HS, Caceci T, Misra HP. An ultrastructural study of the effects of gossypol on the endometrium of the female rat. J Ultrastruct Mol Struct Res. 1988;101:137–44.