

Dept. of Anatomy and Histology,
Fac. Vet. Med., University of Zagazig
Head of Dept. M.A. Ali

NEONATAL EXPOSURE WITH METHOXYCHLOR OR ESTRADIOL IN MICE, AFFECTS THE UTERUS AFTER PUBERTY

(With 2 Tables & 16 Fig.)

By

A.A. ABUEL-ATTA

(Received at 18/5/1993)

تعرض الجرذان حديثى الولادة للميثوكسى كلور أو للاسترايول
يؤثر على تركيب ووظيفة الرحم بعد البلوغ

أحمد أبو العطا

ترجع أهمية الميثوكسى كلور التجارى إلى أنه يستخدم حالياً فى مجال واسع كمبيد للآفات وللحشرات بديلاً لل DDT وذلك لما يتميز به الميثوكسى كلور من سهولة تحلله فى البيئه وقلة سميته للانسان وقصر فترة نصف العمر له فى الثدييات. يحتوى الميثوكسى كلور التجارى المستخدم فى هذا البحث على ٥٠% مادة فعالة والباقى مواد أخرى لهم تأثير استروجينى على القناة التناسلية لاناث الجرذان فى الفترة الحساسه بعد الولاده. الأمر الذى دفعنا عند تصميم هذه تجربته أن نأخذ فى الاعتبار مقارنة تأثير حقن جرعة يومية من الميثوكسى كلور (١٠ ، ٥٠ ، ١٠٠ مجم) أو استرايول (١٠ ميكروجرام) كمعلق على زيت السمسم المعقم أو زيت السمسم فقط للحيوانات الضابطه وذلك حقناً يومياً (٥ ر ٢ سم) فى التجويف البريتونى باستخدام محقن التيوبركلين ولمدة ١٥ يوم ابتداء من اليوم الثانى بعد الولاده، ثم التحفظ وتربية اناث الجرذان المعامله حتى بلغت من العمر الثلاثة شهور. وتم أخذ أوزان الحيوانات قبل الانتهاء من تجربته. ولذلك كان الهدف من البحث هو معرفة التغيرات الهستولوجية والوظيفية فى رحم الجرذان عند عمر

ثلاث شهور والتي سبق حقنها بالميثوكسى كلور أو الاستراديول فى الفترة المبكره بعد الولاده. تم أخذ القناة التناسلية من اناث الجرذان وأخذ أوزانها وعمل تحليل احصائى لها. ثم تم تمثيل الرحم فى قطاعات بلاستيك مستخدماً ميثاكريلات الجليكول ثم صبغت وفحصت. وقد أسفرت نتائج البحث عن أن حقن الميثوكسى كلور تجارى فى الجرذان حديثى الولاده يحدث نقص معنوى فى أوزان القناة التناسلية الانثوية من تلك الجرذان بعد البلوغ خصوصاً بعد جرعة ٥ر. مجم، أما المعاملات الأخرى فلا تحدث تغيير معنوى لا فى أوزان الحيوانات ولا فى أوزان القناة التناسلية. وحقن الاستراديول يحدث تغيرات انعكاسية فى الغشاء الطلائى المبطن للرحم أو لغدد الرحم بالإضافة إلى ظهور غدد ذات تكوينات تشويهيه وعشوائيه مع ظهور تكوينات حويصليه فى بعض الغدد الرحمية. وبالنسبه للميثوكسى كلور التجارى يحدث زيادة فى تفرغات الغدد الرحمية، ظهور غدد متحوصله وذلك حيال الجرعات الصغيرة ار. مجم أما عند الجرعات العالية فتزداد عدد وحجم الغدد المتحوصله مع ظهور تغيرات أنعكاسيه فى الغشاء الطلائى وعند جرعة ار مجم فإن الغدد الرحمية تتفرع بطريقة عشوائية ومكثفه ومتشوهه مع زيادة المواد المفترزة داخلها بالإضافة إلى وجود عدم إنتظام فى تركيب وشكل طبقات الرحم. ومما سبق من نتائج، يتضح لنا أن الميثوكسى كلور التجارى يحدث تغييرات فى تركيب رحم الجرذان بعد بلوغها والتي سبق أن تعرضت له بعد ولادتها وهذه التغييرات تشبه إلى حد كبير تأثير الاستراديول وخاصة التغييرات الناتجة من الميثوكسى كلور ١ر مجم. ولما كانت تلك التغييرات ناتجة من تعرض نفس اناث الجرذان خلال الفترة الحرجه من نمو وتمايز القناة التناسلية بعد ولادتها إلى مثل هذه الكيماويات فإن هذه التغييرات الهستولوجية فى تركيب الرحم قد تتداخل وتؤثر على التناسل بل وقد تحدث عقم فى اناث القوارض، وبالتالي يمكن الاعتماد على الميثوكسى كلور فى مقاومة القوارض.



SUMMARY

The histomorphological features of the uterus of three months old mice (after puberty), previously to 17-B Estradiol (E) or different doses of technical grade Methoxychlor (T.g. Mx), was investigated. Early as one day-old, the neonates received a daily intraperitoneal injection for 14 successive days with a sterile sesame oil containing 10 ug (E), or 0.1, 0.5 & 1.0 mg (T.g. Mx) as treated groups, or sesame oil vehicle only as a control group. The 0.5 mg (Mx)-dose significantly decreased the weight of the reproductive tract of mice after puberty. The (E)-induced histological alterations were disorganization and focal metaplasia of endometrial and glandular epithelia. Cystic formations of some endometrial glands, and appearance of pale-stained cells throughout the disorganized glands, stroma and myometrium were also observed. The (Mx)-resulted changes were epithelial hyperplasia, disorganized endometrial glands, cystic glandular units with appearance of epithelial metaplasia in both endometrial and glandular epithelium. These alterations were increased at the highest (Mx)-doses.

INTRODUCTION

Since 1960, there was a need to replace the pesticide DDT, because it was highly persistent in the environment and toxic to birds and mammals. In addition, the insects became resistant to its application (BROWN, 1971). Recently, a pesticide Methoxychlor (Mx) substituted (DDT), being of low toxicity to non-target organism, rapidly metabolised in vivo, and degraded in the environment (KUPFER and BULGER, 1982). Short-term treatment with (Mx) stimulated the development of the neonatal female reproductive tract at lower doses, and induced abnormalities at higher doses (EROSCHENKO and COOKE, 1990). The aim of the current investigation, was to reveal how neonatal (E) or (MX) exposures, influence the histological structure of the uterus of mice after puberty.

MATERIAL AND METHODS

Technical grade Methoxychlor (T.g.Mx) was obtained from Dupont Nemours Inc. (Wilmington, DE, USA), and 17-B Estradiol (E)

was purchased from Sigma Chemical Inc. (St. Louis, Mo, USA). Neonates (one day old, obtained from pregnant mice (NIH Swiss strain from Harlan, IN, USA), were given food and water ad libitum and kept on 12h:12h light/dark cycle, during the period of treatment. One day-old neonates (Table 1), received daily intraperitoneal injection (for 14 successive days), of a sterilized 0.05 ml sesame oil, in which, 10 ug (E) or 0.1, 0.5, or 1.0 mg (T.g.Mx) were suspended. The control animals received sesame oil vehicle only. After sex differentiation, the male pups were discarded, and the female pups were survived till the end of the experiments. The determination of the chemical doses was similar to those used by *EROSCHENKO and COOKE (1990)*.

At termination, the three months-old adult female mice, were weighed, decapitated and their reproductive tracts (without ovaries) were excised, trimmed of fat and weighed. Uteruses were fixed in 10% neutral buffer formalin and embedded in plastic glycol methacrylate medium. Sections were made at 2-3 um thick and stained with hematoxylin and eosin, or periodic Schiff's (PAS) technique counterstained with hematoxylin, for the light microscopic examination. Statistical Analysis System "SAS" (1987), was adopted to interpretate the data.

Table 1: The total number of female mice (neonates) used per treatment.

Treatment	Number of animals
Sesame oil (control)	10
10 ug (E)	6
0.1 mg (t.g. Mx)	12
0.5 mg (t.g. Mx)	8
1.0 mg (t.g. Mx)	6

RESULTS

The wall of the uterus of the adult (3 months-old) female mice, presented three layers; an endometrium, a myometrium, and surrounded from outside by tunica serosa (Fig. 1) The endometrial features varied according to the follicular or the luteal phases of the estrous cycle. The lining epithelium of the uterus being regular, during the luteal phase (Fig. 1), or irregular (showing several pits), during the follicular phase (Fig. 2), was mainly of the psuedostratified variety possessing numerous supranuclear vacuoles (Fig. 3), or some infranuclear vacuoles and some mitotic figures during the follicular phase

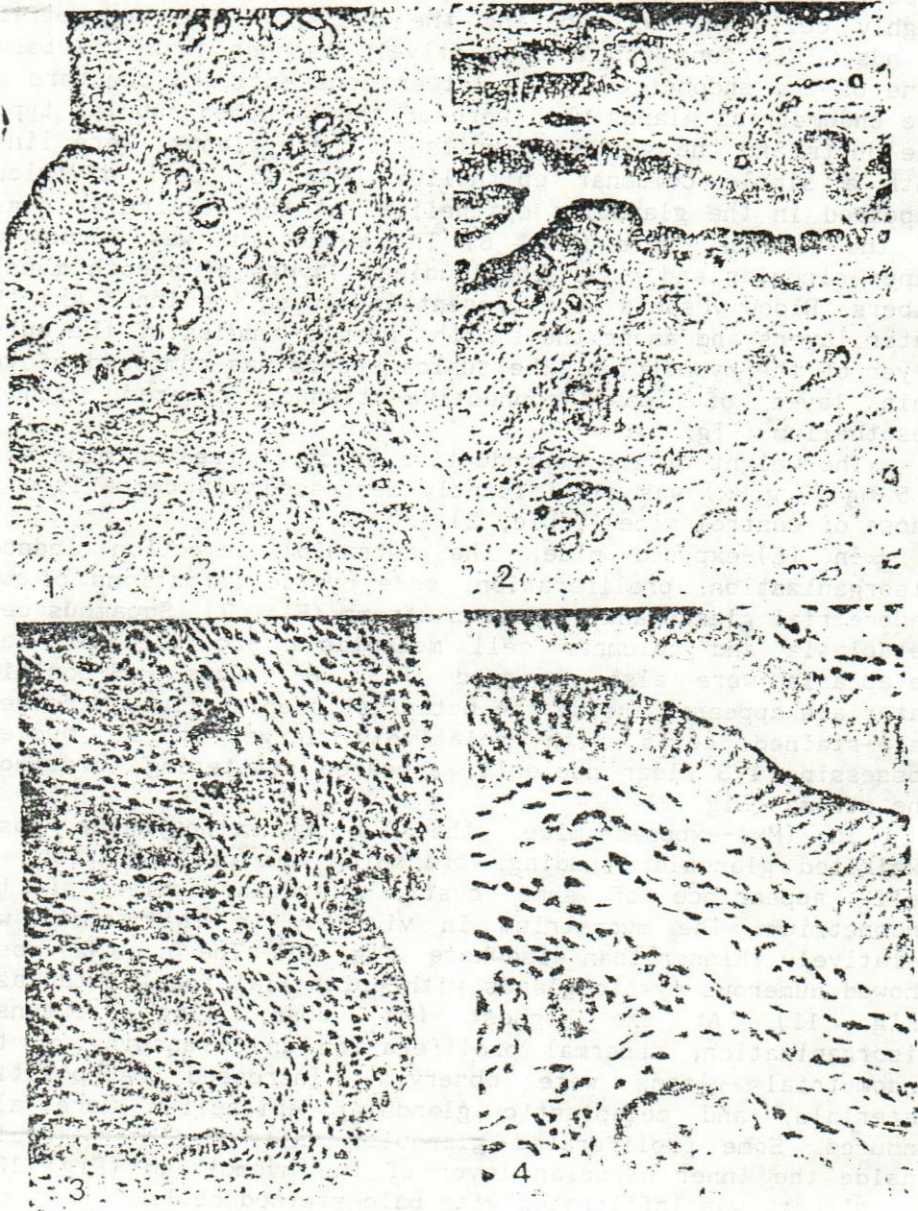
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(Fig. 4). However, some areas were lined with simple columnar or simple cuboidal epithelium (Fig. 5). Lymphocytes were present among the lining cells of the endometrium. The superficial layer of the lamina propria "functional zone", was highly cellular, accomodating the ducts of the endometrial glands. This zone appeared relatively thinner than the basal zone of the endometrium. The latter zone enclosed clusters of the endometrial glands which were of the branched tubular type. The ducts and the secretory units of these glands, were lined with a simple columnar epithelium (Fig. 5) Cell divisions appeared in the glandular epithelium, at the follicular phase of the estrous cycle (Fig. 6) The myometrium was formed of inner circular and outer longitudinal layers of smooth muscle fibers. Blood vessels were present inbetween the inner and the outer layers and among the smooth muscle bundles of the outer layer of the myometrium. The tunica serosa was composed of very thin layer of loose connective tissue, covered with a mesothelium (Fig. 1).

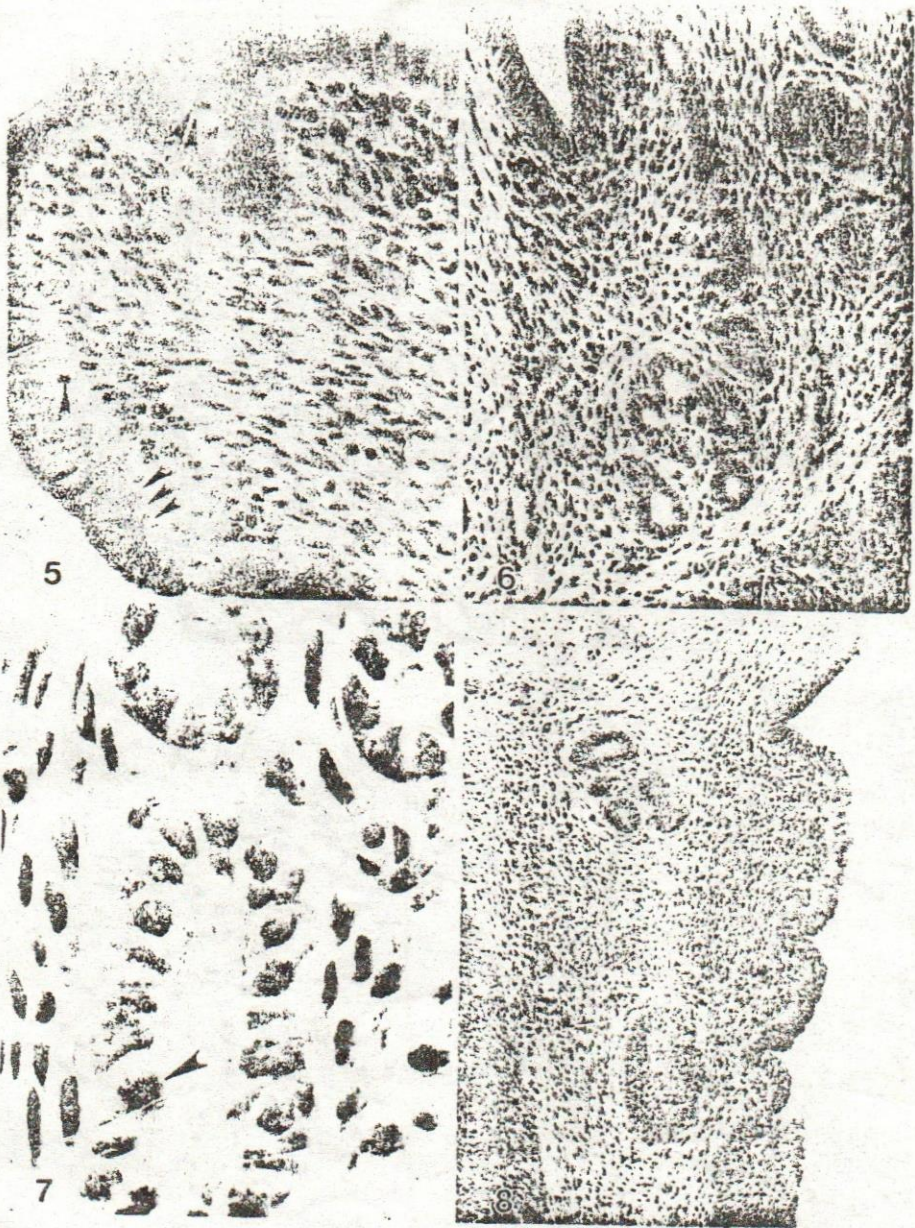
The weight of the reproductive tract of treated mice with 0.5 mg (T.g.Mx) was significantly decreased, in comparison to those of control mice (Table, 2).

In (E)-exposed mice, the estradiol injection induced disorganization, proliferation, some cystic formations of the endometrial glands and edematous stroma (Fig. 7). Squamous cell metaplasia and columnar cell metaplasia and columnar cell metaplasia were also observed (Fig. 8). Some PAS-positive materials appeared inside the metaplastic epithelium. Moreover, pale-stained cells with relatively large ovoid nuclei, possessing 1-3 clear nucleoli, appeared scattering throughout the stroma (Fig. 9)

In (Mx)-exposed mice, the 0.1 mg methoxychlor doses exhibited glandular budding, branching and proliferation, as well, appearance of some cystic glandular units of the endometrium. The myometrium in vicinity to this cyst, was relatively thinner than elsewhere (Fig. 10). The 0.5 (MX)-dose, showed numerous cystic glands with epithelial focal metaplasia (Fig. 11). At the highest (Mx) dose, (1mg), extensive disorganization, abnormal proliferation and branching of the endometrial glands were observed. Increased PAS-positive materials, and metaplastic glandular epithelium were also induced. Some proliferated glandular units appeared located inside the inner circular layer of the myometrium (Fig. 12). The stromaa was infiltrated with pale stained cells.

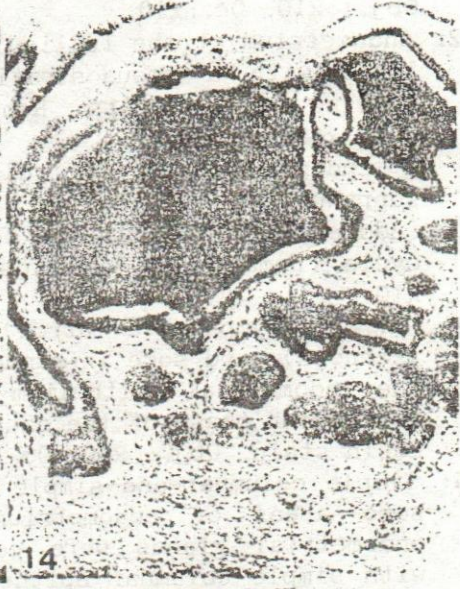


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LEGENDS

- Fig. 1: Uterus of adult (3 months-old) mouse at the luteal phase of estrous cycle. Hematoxylin and Eosin stain. Obj.: x10, Oc.:x10.
- Fig. 2: endometrium at the follicular phase of estrous cycle, lined with a pseudostratified columnar epithelium, showing several pits. Hematoxylin and Eosin stain. Obi: x10, Oc.: x10.
- Fig. 3: Endometrium of control mouse, showing numerous supranuclear vacuoles in the lining epithelium and endometrial edema at the follicular phase of estrous cycle. Hematoxylin and Eosin stain. obj.: x40, Oc.: x10.
- Fig. 4: Showing lymphocyte (arrow), epithelial cell division (2arrows), and some infranuclear vacuoles (3arrows) in the endometrial epithelium of control animal, at the follicular phase of the estrous cycle. Hematoxylin and Eosin stain. Obj.: x40, Oc.: x10.
- Fig. 5: Simple columnar-and simple cuboidal epithelium lining some areas of endometrium of control mouse. The endometrial glands were coiled, branched and lined with simple columnar epithelium. Hematoxylin and Eosin stain. obj.:x20, Oc.: x10.
- Fig. 6: Mitotic division within the columnar epithelium of the endometrial glands of control mouse. Hematoxylim and Eosin stain. Obj.: x100, Oc.: x10.
- Fig. 7: (E)-treated uterus, showing disorganization, proliferation and cystic formation in the endometrial glands. PAS-hematoxylin technique. obj.: x10, Oc.: x10.
- Fig. 8: (E)-treated uterus, showing proliferative, hyperplastic and metaplastic glandular epithelium, squamous metaplasia (S), columnar metaplasia (C). PAS-Hematoxylin technique. ob.: x20, Oc.: x10.
- Fig. 9: (E)-treated uterus, showing that the endometrial stroma infiltrated with pale-stained cells (arrows). PAS-Hematoxylin technique. Ob.: x40, Oc.: x10.
- Fig. 10: Uterus exposed to 0.1mg (Mx)-showing glandular buddings and proliferation, cystic gland, and myometrium. Pas-Hematoxylin technique. Ob.: x10, Oc.: x10.
- Fig. 11: Uterus exposed to 0.5mg (mx)-dose, exhibiting numerous cystic endometrial glands. PAS-Hematoxylin. Obj.: x10, Oc.: x10.

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Fig. 12: Uterus exposed to 1.0mg (mx)-dose, revealing extensive disorganization, hyperplasia and metaplasia of the endometrial glands, with increased PAS-positive materials. Some glandular units invaded the myometrium (arrow). PAS-Hematoxylin technique Obj.: x20, Oc.: x10.

Table 2: Body and reproductive tract only weights of three month-old mice treated neonatally with 14 daily ip injections of either sesame oil (C), estradiol (E), or technical grade methoxychlor (MX).

Group	Treatment	Body weight (g) X ± SE	Reporodyctive tract weight only (mg) X ± SE
C	Sesame oil (control)	26.3 ± 2.9 ^a	266.8 ± 90.4 ^a
E	10.0 ug	29.4 ± 4.2 ^a	215.4 ± 34.6 ^a
MX	0.1 mg	26.2 ± 2.7 ^a	182.9 ± 71.8 ^a
MX	0.5 mg	25.8 ± 2.6 ^a	115.6 ± 24.9 ^b
MX	1.0 mg	29.2 ± 2.4 ^a	155.7 ± 22.8 ^a

Different letters mean significant changes at $p > 0.05$.

DISCUSSION

The uterine wall of mice, simulating that of domestic and laboratory animals, was consisted of endometrium, myometrium and serosa or perimetrium (TRAUTMANN and FIEBIGER, 1957; DELLMANN and BROWN, 1987; and HAFEZ, 1970) The endometrial epithelium of mice was supranuclearly vacuolated, which may indicate sites of lipoidal materials. The lipid contents increased in the endometrial epitheelium during diestrous in rats (BOSHEIR, 1971). The latter author reported that the lipid content of the cells was elevated by progesterone and lowered by estradiol. Lipid might be utilized within the epithelium (BOSHEIR, 1971). However, MARINOV and LOVELL (1968), suggested that it might be used for nourishment of embryo or as a precursor for steroidal hormones.

The pesticide Methoxychlor exhibits low acute toxicity on laboratory animals (KAPOOR *et al.*, 1972). Although the highest (Mx)-dose significantly increased the weight of the reproductive tract of the neonatal mice (EROSCHENKO and COOK, 1990), the present finidings indicated that the exposure of neonatal mice to (T.g.Mx), significantly decreased the weight of reproductive tract of three months-aged mice.

In comparison to the uterus of control adult mice, the (E)-induced alterations were; disorganization, cystic formation of some endometrial glands, hyperplasia and metaplasia of the endometrial and glandular epithelium. Similar findings were reported in adult rodents, previously exposed (prenatally and neonatally) to estradiol or natural or synthetic estrogens including Diethylstilbestrol, Coumestrol and Zearalenone (WORDINGER and MORRIL, 1985; and MORI, 1986).

Moreover, there was a relative focal thinning of the myometrium of adult mice, neonatally treated with 0.1 or 0.5 mg (T.g.mx), wherever a cystic gland was located nearby the affected point of this myometrium, due to the presence of randomly proliferated glands within the myometrium. Concurrently, the (T.g.Mx) induced an adverse effects on uterus of mice, as claimed by GARY and CUMMING (1989) in rats.

The 1.0mg (T.g.Mx) and 10ug E-doses induced similar alterations in the mouse uterus. On the other side, the low (T.g.Mx)-doses, 0.1mg, exhibited a marked ductal buddings and proliferation of the endometrial glands, which simulate the influence of estrogen of the follicular phase of the estrous cycle, as mentioned by MAYER et al. (1976). The latter findings might indicate that the alterations in the uterus, might be attributed to the provoked estrogenic influence, following the neonatal exposure to the (T.g.Mx). Similarly, in vivo and in vitro studies showed that the (T.g.Mx), contain estrogenic contaminants and proestrogens, which were metabolized in the liver into potent estrogens (BULGER and KUPFER, 1985). Furthermore, permanent adult uterine abnormalities were induced, after administration of a minute amount of estrogen, during the neonatal period (IGUCHI et al., 1988).

The reproductive tract of newborn female mouse is developmentally immature and highly sensitive to estrogenic xenobiotics, when studied in ten days-old mice (EROSCHENKO and COOKE, 1990). The present study suggested a similar possibility for the neonatal administration of technical grade methoxychlor, as revealed in uterus of adult mice, and emphasizing the usefulness of neonatal mice as sensitive laboratory animal model, so as to figure out and study the estrogenicity of chemicals that may pollute the environment.

In conclusion, the findings from the adult three monthss-old female mice previously treated, during their neonatal period, with estradiol or technical grade methoxychlor, produced similar abnormal alterations in uterus histology, especially with 1.0mg (T.g.Mx)-dose. The exposure to such chemicals had the potential to exhibit an adult-related uterine abnormalities, which might interfere, or affect the

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reproduction and might produce infertility among rodents.

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