# Effect of Several Dietary Levels of Technical Methoxychlor on Reproduction in Rats

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Technical methoxychlor (MeOCl) was compared with estrogen to determine direct effects upon mating and reproduction of adult rats as well as indirect latent effects, vaginal opening time, and reproductive performance of offspring. Mature female rats were fed MeOCl before mating and throughout pregnancy and lactation. One thousand parts per million of MeOCl had no effect on reproduction. Higher levels (2500 and 5000 ppm) reduced the number that mated, and only one rat littered. If these adult rats were fed uncontaminated feed, normal reproduction returned. Female pups from mothers fed 1000 ppm had early vaginal openings and reduced reproduction when they attained maturity and were mated. Males from mothers fed 1000 ppm of MeOCl also had impaired reproductive behavior. This impairment indicates that methoxychlor transmitted through the placenta or mother's milk can affect reproduction adversely. This study showed that technical MeOCl at very high dosages adversely affects reproduction in adult rats and can affect the reproduction of the young in the next generation.

Methoxychlor, a degradable organochlorine, is being used as a substitute for the nonbiodegradable organochlorine DDT to control such pests as flies, fleas, and lice on animals (Hodge *et al.*, 1950). Dairy and beef cattle are sprayed, dipped, or directly dusted with methoxychlor. Methoxychlor is also used to control plant pests. Consequently, feedstuffs for animals could be contaminated with methoxychlor residues.

Tullner (1961) reported that technical methoxychlor (MeOCl) stimulated uterine growth in mice and rats. MeOCl also causes atrophy and abnormal development of testes of young male rats (Hodge *et al.*, 1950; Tullner and Edgcomb, 1962). Recently, more evidence of the action of MeOCl on reproductive tissues has been found. Welch *et al.* (1969) found that MeOCl interfered with the *in vivo* uptake of estradiol by the rat uterus. Bitman and Cecil (1970) found that MeOCl was four times more estrogenic than pure *p,p'*-methoxychlor in immature female rats.

The present study assesses the influences of feeding technical methoxychlor on reproduction in rats. Because the estrogenic influences of MeOCl could reduce fertility, MeOCl was compared with estrogen to determine direct effects upon mating and reproduction as well as indirect latent effects such as age of female offspring at the time of vaginal opening and subsequent reproductive performance of male and female offspring.

## ANIMALS AND TREATMENTS

In preliminary experiments, rats fed chow containing 2500 and 5000 ppm of technical methoxychlor consumed only 16 g/rat per day whereas controls consumed 20–22 g/rat per day. Consequently, food intake of adult rats was limited to 16 g/rat per day for all groups.

**Treatment 1: Comparative Effects of Methoxychlor and Estradiol on Reproduction.** Mature female albino rats (Sprague Dawley), 60–90 days old, were fed 0, 1000, 2500, or 5000 ppm of MeOCl (90% *p,p'*-methoxychlor, grade II, Sigma) and 0, 0.25, 0.62, or 1.25 ppm of estradiol benzoate (EB) mixed in chow meal diets. We have shown that 0.1 μg of diethylstilbestrol has the same estrogenic activity as 1 mg of technical methoxychlor (Bitman and Cecil, 1970). Since EB and diethylstilbestrol have the same estrogenic activity we estimated these amounts of EB to have 2.5 × the estrogenic activity of 1000, 2500, and 5000 ppm of MeOCl, respectively (Bitman and Cecil, 1970). After 6 weeks feeding, the females were mated with control males of the same age and strain. Mating was determined by checking for vaginal copulation plugs every morning for 2 weeks. Rats that had plugs but did not litter were killed 21 days after mating and their uteri checked for implantation or resorption sites. The pregnant females were allowed to litter, and the young were kept with their mothers. No adjustments were made in the numbers of young per litter. After weaning, the pups were maintained on the treatment of their mothers. Sex, age at vaginal opening, and body weight of pups were recorded. Organ weights, liver lipid, vitamin A, and pesticide residues were determined in virgins of each treatment at the time of mating and in the mothers after weaning.

**Treatment 2: Comparative Effects of Methoxychlor and Estradiol on Estrous Cycle Length.** Estrous cycle length of mature female rats was determined by microscopic examination of vaginal smears taken by lavage each afternoon for 3 weeks (Long and Evans, 1922). Thirty-six rats with 4-day estrous cycles were divided into three groups—control, 5000 ppm of MeOCl, and 1.25 ppm of EB. The estrous cycles were followed for 12 more weeks. After 5 weeks treatment, the rats were mated with vasectomized males and checked for vaginal copulation plugs every morning for the next 6 weeks.

**Treatment 3: Prenatal and Early Natal Influences of Methoxychlor.** This experiment was designed to determine whether the presence of methoxychlor in the mother's diet would exert adverse influences on subsequent reproduction in the young. Weanling female pups (21 days old) from mothers fed control feed or feed containing 1000 ppm of MeOCl were pair fed either control feed or feed containing 1000 ppm of MeOCl. Six weeks after weaning, females were mated for a 2-week period with control males or with males fed 1000 ppm of MeOCl from the time of weaning. The males were housed with the females (for mating groups see Table II), and reproductive parameters were determined as in treatment 1.

## ANALYTICAL TECHNIQUES

After decapitation, body and organs (uterus, ovaries, adrenals, kidneys, spleen, liver) were weighed. Liver lipids were determined gravimetrically on dried acetone–ethanol extracts of homogenized tissues. Liver vitamin A was determined by the antimony trichloride colorimetric procedure (Carr and Price, 1926) after KOH digestion and ethanol–ethyl ether extraction.

For whole body methoxychlor analysis, the entire animal, including the alimentary tract and its contents, was homogenized in a Waring Blendor with 100 ml of distilled water and 200 g of ice. Residues were determined in 1-g

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**Table I. Reproduction in Adult Female Rats Fed Various Levels of Technical Methoxychlor (MeOCl) and Estradiol Benzoate (EB) for 6 Weeks**

| Treatment | Level in feed, ppm | $n$ | Mated, % | Littered as % of Total | Littered as % of Mated | Litter size[e] | Age at vaginal opening, days |
|---|---|---|---|---|---|---|---|
| Control | 0 | 17 | 82 | 53 | 64 | 10.6 | 39 |
| MeOCl | 1000 | 17 | 76 | 59 | 77 | 8.9 | 23[d] |
| MeOCl | 2500 | 20 | 45[c] | 5[c] | 11[c] | 9.0 | 19[d] |
| MeOCl | 5000 | 25 | 48[c] | 0[c] | 0[c] | | |
| EB | 0.25 | 17 | 59[b] | 41 | 70 | 7.5[b] | 34[d] |
| EB | 0.62 | 18 | 78 | 67 | 86 | 11.0 | 27[d] |
| EB | 1.25 | 16 | 75 | 75 | 100 | 8.9[a] | 27[d] |

[a-d] Statistical comparisons of treated *vs.* control; chi-square test: (*a*) $P < 0.050$; (*b*) $P < 0.025$; (*c*) $P < 0.005$; student "*t*" test; (*d*) $P < 0.001$. [e] Mean litter size of rats that littered. Rats that were not pregnant were not included.

aliquots. Residues were also determined on 300 mg of abdominal fat. The samples were dried with sodium sulfate and extracted with petroleum ether. Lipid content was determined gravimetrically on a dried aliquot of the petroleum ether. Another aliquot of the petroleum ether was used directly for florisil cleanup. After elution from a florisil column with 500 ml of 3% ethyl ether in petroleum ether, residues were determined by gas–liquid chromatography (Fries *et al.*, 1973).

Statistical comparisons were made by using either the students "*t*" test with correction for unequal group size or by the chi-square analysis.

## RESULTS

**Reproduction (Treatment 1).** Feeding 1000 ppm of MeOCl to adult female rats did not affect reproduction (Table I). However, feeding 2500 or 5000 ppm impaired reproduction; fewer of these rats mated and many that did mate did not litter, did not have implantation sites, or did not have reabsorbed embryos. In the 2500-ppm MeOCl group, nine rats (45%) mated, but only one of these rats littered; no rats littered or had implantation sites in the 5000-ppm MeOCl group. Even though there was a reduced conception rate in the rats fed 2500 or 5000 ppm of MeOCl, the one 2500-ppm rat that did conceive had a normal-size litter. The effects of MeOCl on reproduction were reversible. Three out of four rats fed 5000 ppm of MeOCl for 9 weeks and then put on control feed for 4 weeks regained the ability to conceive, and on the 14th day of pregnancy had 11 to 15 embryos per rat. The lowest level of EB fed (0.25 ppm) reduced the number of rats that mated and also the litter size; higher levels of EB (0.62 and 1.25 ppm) did not reduce the number of rats that mated nor the litter size (Table I). The number of rats littering was also comparable with that of controls.

Vaginal opening time is an indicator of the presence of functional ovaries and the onset of sexual maturity. Exogenous estrogen is known to decrease the vaginal opening age of young female rats. In our experiment, female pups from rats fed a control diet had a mean vaginal opening age of 39 days (Table I), and MeOCl treatment significantly hastened the onset of vaginal openings. Pups from mothers fed 1000 and 2500 ppm of MeOCl had vaginal openings at 23 and 19 days, respectively. Pups from rats fed 0.25, 0.65, and 1.25 ppm of EB had mean vaginal opening ages of 34, 27, and 27 days, respectively.

**Estrous Cycle Length (Treatment 2).** Feeding 5000 ppm of MeOCl increased the incidence of estrus. Control or EB-fed rats had smears 1-day proestrous, 2-days estrous, and 1-day diestrous in a typical estrous cycle; MeOCl-fed rats had an increased number of estrous smears in each cycle. After 3 weeks of feeding, the average numbers of rats per day with smears typical of estrous

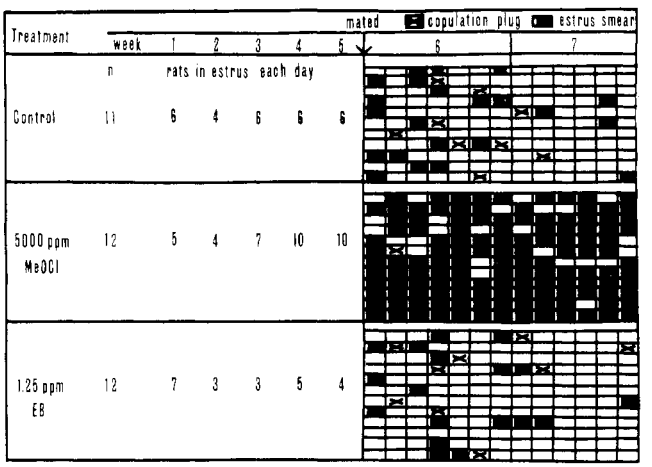


**Figure 1.** Effects of MeOCl and estrogen feeding on estrous cycle and mating of rats.

were 10, 5, and 6 for 5000-ppm MeOCl-fed, 1.25-ppm EB-fed, and control rats, respectively (Figure 1). After 5 weeks of treatment, 5000-ppm MeOCl-fed rats had vaginal smears showing constant estrus, where control and EB-fed rats still had normal estrous cycles. When these females were mated with vasectomized males, fewer of the MeOCl-fed females mated. Only 8% of the 5000-ppm MeOCl-fed female rats mated during a 2-week mating period (6th and 7th weeks of feeding). During this same time period, 82% of the controls and 66% of the EB-fed rats mated. The females that did mate became pseudopregnant and did not mate again for 19 to 22 days.

**Prenatal and Early Natal Effects of MeOCl (Treatment 3).** Rats exposed to MeOCl prenatally (from conception) were compared with rats fed MeOCl from weaning (22 days of age). Both prenatal and post-weaning exposure of females to MeOCl reducing mating. Prenatal and early natal exposures were more deleterious because fewer of these females littered (Table II, groups 3 and 4) than those fed MeOCl from weaning (Table II, groups 2 and 7). Of the 15 females in group 4 exposed prenatally to MeOCl and then fed MeOCl from weaning, only one rat (7%) littered. Prenatal influences of MeOCl could not be completely reversed by control feed at weaning (group 3), and only 50% of the mated rats in this group littered. Exposure of males to MeOCl both prenatally and from weaning (group 6) decreased their reproductive performance; there were fewer litters from control females mated with these males than from females mated with males fed MeOCl only from weaning (group 5). Feeding males 1000 ppm of MeOCl from weaning did not affect their reproductive

**Table II. Effect of Maternal Diet and Postweaning Feeding of 1000 ppm of Technical Methoxychlor on Reproduction in Rats**

| Group | Rat matings, MeOCl treatment, ppm; Female: Prenatal + early natal[e] | Female: Post-weaning[f] | Male: Prenatal + early natal[e] | Male: Post-weaning[f] | Females tested: n | Females tested: % mated | Littered as % of: Total | Littered as % of: Mated | Litter size | Age at vaginal opening, days |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 13 | 84 | 77 | 91 | 9.4 | 41 |
| 2 | 0 | 1000 | 0 | 0 | 14 | 64[a] | 50[b] | 77 | 5.2 | 22[d] |
| 3 | 1000 | 0 | 0 | 0 | 19 | 63[c] | 32[c] | 50[c] | 9.3 | 41 |
| 4 | 1000 | 1000 | 0 | 0 | 15 | 73 | 7[c] | 9[c] | 6.0[g] | |
| 5 | 0 | 0 | 0 | 1000 | 13 | 77 | 69 | 90 | 9.0 | 40 |
| 6 | 0 | 0 | 1000 | 1000 | 28 | 53[c] | 28[c] | 53[c] | 9.3 | 44 |
| 7 | 0 | 1000 | 0 | 1000 | 14 | 43[c] | 28[c] | 66[a] | 4.8 | 23[d] |

[a-d] Statistical comparisons of treated *vs.* control; chi-square test: (*a*) $P < 0.050$; (*b*) $P < 0.025$; (*c*) $P < 0.005$; student "*t*" test; (*d*) $P < 0.001$. [e] Diet fed to dams 6 weeks before pregnancy and throughout pregnancy and lactation. [f] Diet fed to pups from weaning and throughout experiment. [g] Determined by number of implantation sites; mother ate pups at birth.

**Table III. Uterine and Ovarian Weights of Adult Female Rats Fed Technical Methoxychlor or Estradiol Benzoate (Treatment 1)**

| Treatment | Level in feed, ppm | Wt at time of mating: n | Uterus, mg | Ovaries, mg | mg of ovary/100 g BW | Wt at time of weaning: n | Uterus, mg | Ovary, mg | mg of ovary/100 g BW |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | 5 | 249 | 76.9 | 35.5 | 4 | 410 | 79.8 | 29.7 |
| MeOCl | 1000 | 5 | 329 | 50.6[d] | 23.3[c] | 7 | 369 | 46.1[e] | 16.3[d] |
| MeOCl | 2500 | 5 | 372[a] | 25.2[e] | 11.5[e] | 1 | 420 | 37.6 | 14.7 |
| MeOCl | 5000 | 6 | 300[a] | 26.3[e] | 13.0[e] | | No pregnancies | | |
| Control | 0 | 5 | 360 | 94.0 | 35.0 | 6 | 430 | 80.3 | 25.9 |
| EB | 0.25 | 4 | 400 | 71.1[b] | 27.3[a] | 4 | 410 | 78.0 | 24.9 |
| EB | 0.62 | 5 | 340 | 73.2 | 26.4[b] | 6 | 310 | 79.1 | 25.1 |
| EB | 1.25 | 5 | 280 | 76.4 | 25.3[a] | 6 | 370 | 62.2 | 23.6 |

[a-e] Statistical comparisons of treated *vs.* control using the student "*t*" test: (*a*) $P < 0.050$; (*b*) $P < 0.025$; (*c*) $P < 0.010$; (*d*) $P < 0.005$; (*e*) $P < 0.001$.

performance (group 5). The average litter size was 9.2, except in the females fed 1000 ppm of MeOCl from weaning. These had a mean litter size of 5.2 (Table II, group 2) or 4.8 (Table II, group 7). The one rat that littered in group 4 also had a smaller litter.

Offspring of rats whose only MeOCl influence was prenatal feeding to their mothers (group 3) had normal vaginal opening times. However, as before, vaginal openings occurred earlier in female pups from mothers ($F_1$) fed MeOCl post-weaning and during pregnancy and lactation (groups 2 and 7).

**Organ Weights.** Feeding 2500 and 5000 ppm of MeOCl to virgin adult female rats increased uterine weight (Table III). Although EB-fed rats had uterine weights comparable with those of MeOCl-fed rats, estrogen did not increase uterine weights when compared with those of its own controls. The difference in control uterine weight is probably due to the differences in body weights (BW) (217 g BW for MeOCl controls and 274 g BW for EB controls). However, at the time of weaning, the uterine weights of treatment groups were similar to those of comparable control mothers. Ovarian weights of virgin rats decreased 33% with the 1000-ppm MeOCl treatment and 66% with the 2500- and 5000-ppm treatments. At the time of weaning, ovarian weights of the treated mothers were still 50 and 66% of the ovarian weights of control rats. Although body weights of the 2500-ppm MeOCl-fed rats were less than those of controls, the ovarian weights per unit body weight of the MeOCl-fed rats were also less than those of the controls. Because the weight of a rat ovary is mainly a function of the number of corpora lutea, inhibition of ovulation would result in no corpora lutea, and, consequently, in ovaries that weighed less. All of the EB treatments decreased ovarian weight by 33%. None of the treatments had any effect on spleen, adrenal, and kidney weights.

**MeOCl Residues in Body Fat.** After rats were fed MeOCl for 6 weeks, the *p,p'*-methoxychlor present in the abdominal fat of virgins at the time of mating was 21, 68, and 61 μg/g of fat for 1000-, 2500-, and 5000-ppm MeOCl diets, respectively (Table IV). Residues of virgin rats did not further increase. After rats were fed 5000 ppm of MeOCl for 9 weeks, they were fed uncontaminated feed. The *p,p'*-methoxychlor residues were depleted rapidly, and after the rats had been fed uncontaminated feed for 3 weeks, the *p,p'*-methoxychlor level had fallen to 1.2 μg of MeOCl/g of body fat. Kunze *et al.* (1950) also found that the methoxychlor residue levels returned to normal 2 weeks after the rats were returned to control feed. The *p,p'*-methoxychlor content in the abdominal fat of weaning mother rats was 1.5 to 2 times that of virgin rats although total body burdens were similar (Table IV). This could be due to the lower total body lipid content of weaning mothers.

**Liver Weight, Lipid, and Vitamin A.** Feeding 1000, 2500, or 5000 ppm of MeOCl to virgin rats had no effect on liver lipid but increased liver weight and decreased liver vitamin A concentration. The decreased vitamin A concentration appears to be due to dilution by more liver

**Table IV. *p,p'*-Methoxychlor Concentration in Abdominal Fat and Total Body of Female Rats Fed Technical Methoxychlor for 6 to 12 Weeks**

| Time of sample | MeOCl, ppm | Body wt, g | Total body lipid, % | *p, p'*-Methoxychlor | | | |
|---|---|---|---|---|---|---|---|
| | | | | *n* | μg/g of abdominal fat | *n* | μg/total body |
| Mating | 0 | 207 | 7.1 | 12 | N.D.[a] | 15 | 177 |
| | 1000 | 217 | 7.2 | 12 | 21 | 7 | 1092 |
| | 2500 | 219 | 4.3 | 9 | 68 | 7 | 2028 |
| | 5000 | 280 | 4.7 | 7 | 61 | 11 | 2324 |
| Weaning | 0 | 295 | 6.4 | 5 | N.D.[a] | 7 | 244 |
| | 1000 | 282 | 4.1 | 11 | 34 | 6 | 1713 |
| | 2500 | 255 | 2.0 | 1 | 140 | 1 | 2884 |
| | 5000 | | | No pregnancies | | | |

[a] N.D., not detectable.

**Table V. Liver Vitamin A and Lipid Content of Adult Female Rats (Treatment 1)**

| Time of sample | Treatment | Level in feed, ppm | *n* | Liver wt, g | g liver wt./ 100 g BW | Liver lipid, % | μg of vitamin A/ 100 mg of liver | μg of vitamin A/ total liver |
|---|---|---|---|---|---|---|---|---|
| Mating | Control | 0 | 11 | 5.90 | 2.85 | 4.3 | 143 | 7547 |
| | MeOCl | 1000 | 5 | 7.37[c] | 3.38[d] | 4.9 | 119[c] | 8765 |
| | MeOCl | 2500 | 5 | 7.26[b] | 3.30[c] | 5.1 | 124[b] | 9017 |
| | MeOCl | 5000 | 6 | 7.45[d] | 3.46[d] | 4.0 | 85[b] | 6325 |
| PBS[e] | Control | 0 | 2 | 9.04 | 3.37 | 5.8 | 135 | 8534 |
| | MeOCl | 1000 | 3 | 8.32 | 3.28 | 6.5 | 148 | 9097 |
| | MeOCl | 2500 | 0 | | | | No pregnancies | |
| | MeOCl | 5000 | 0 | | | | No pregnancies | |
| Weaning | Control | 0 | 4 | 11.28 | 3.82 | 5.9 | 127 | 15004 |
| | MeOCl | 1000 | 6 | 13.62 | 4.82[c] | 6.0 | 115 | 15527 |
| | MeOCl | 2500 | 1 | 12.77 | 5.01 | 6.1 | 137 | 17454 |
| | MeOCl | 5000 | 0 | | | | No pregnancies | |
| Mating | Control | 0 | 5 | 6.65 | 2.44 | 5.4 | 299 | 20308 |
| | EB | 0.25 | 4 | 6.38 | 2.38 | 5.9 | 257 | 16326 |
| | EB | 0.62 | 5 | 6.74 | 2.43 | 5.9 | 188 | 12452 |
| | EB | 1.25 | 5 | 9.34[d] | 3.12[b] | 5.5 | 162[a] | 15016 |

[a-d] Statistical comparison of treated *vs.* control; student "*t*" test: (*a*) $P < 0.050$; (*b*) $P < 0.010$; (*c*) $P < 0.005$; (*d*) $P < 0.001$. [e] PBS, placental blood sign, vaginal clot that occurs approximately 7 days before littering.

mass because the total liver vitamin A content of all treatments was similar to that of the controls. Rats fed 5000 ppm had a liver vitamin A concentration approximately one-half that of controls (Table V) but a total liver vitamin A content similar to that of controls. In an earlier study (Cecil *et al.*, 1973), we found that low levels of MeOCl had no effect on liver constituents.

Virgins and weaning mothers had the same liver vitamin A concentration, but as a result of the increased liver size, the vitamin A per total liver was higher in the mothers than in virgin female rats. The liver vitamin A levels in the 1.25-ppm EB-fed rats decreased to 54% of those of control; this result agreed with the 59% reduction in the levels of rats fed 5000 ppm of MeOCl.

## DISCUSSION

We concluded that 1000 ppm of MeOCl fed to adult female rats did not impair reproduction. When compared with other organochlorine pesticides, higher levels of MeOCl in the feed are necessary to impair reproduction. Decreased reproductive success in mice has been found after feeding either 1 ppm of telodrin (Ware and Good, 1967), 5 ppm of kepone (Good *et al.*, 1965), 40 ppm of kepone (Huber, 1965), 5 ppm of Mirex or 7 ppm DDT (Ware and Good, 1967), 250 ppm of DDT, 10 ppm of chlordane, or 10 ppm of dieldrin (Deichmann and Keplinger, 1966), and 6 mg of heptaclor/kg body weight (Mestitzova, 1966). Lundberg and Kihlström (1973) found that a single intraperitoneal injection of *p,p'*-DDT to mice, if administered 12 hr after mating, interfered with ova implantation. Continuous feeding of *p,p'*-DDT (equivalent to 10 ppm of the diet) also interfered with ova implantation in mice (Lundberg, 1973). Aldrin (10 or 20 ppm) decreased the frequency of estrus in rats (Ball *et al.*, 1953).

Feeding high levels of technical MeOCl completely inhibited reproduction in adult rats, and they had persistent estrous smears and failed to mate. The deleterious effects on reproduction were reversible when the rat was exposed to MeOCl after weaning.

The prenatal and early natal effects on female pups could not be completely overcome by withdrawal of the MeOCl at weaning, and the effects of the pesticide persisted into adult life, with MeOCl-treated females giving birth to fewer litters than the controls. Neonatal treatment of female rats with estrogen is known to result in reduced mating behavior and sterilization, which can persist throughout adult life (Harris, 1964). In our experiments, neonatal effects of MeOCl cannot be differentiated

from *in utero* effects because the mothers were fed MeOCl throughout both pregnancy and lactation. Because organochlorine pesticides have been shown to pass the placental barrier (Woolley and Talens, 1971), these pups would have been exposed to the estrogenic effects of MeOCl *in utero* and by ingestion of the pesticide-containing milk of the mother. Furthermore, young rats commonly ingest their mother's feed during the latter parts of the lactation period.

The effects on reproduction that we have demonstrated with MeOCl may be the result of adverse actions on a number of biological systems.

We have not been able to ascribe all of the adverse characteristics in adult rats to the estrogenic properties of MeOCl because of the great inconsistency between technical methoxychlor and estradiol benzoate in their effects on the estrous cycle. We found that methoxychlor elicited some of the classical endocrinological estrogenic effects such as earlier vaginal opening time, constant estrus, larger uteri, and smaller ovaries, resulting in fewer matings and consequently decreased reproductive performance. However, we have also found that estrogen itself did not elicit some of these estrogenic effects, a finding which agrees with other studies in the literature. Female rats fed 1.25 ppm of EB (intake = 20 $\mu$g of EB per day) did not exhibit constant estrus although ovarian weights were decreased. Austin and Bruce (1956) found that 22 $\mu$g of diethylstilbestrol did not consistently result in constant estrus in rats although ovulation was completely inhibited.

The estrogenic activity of technical DDT and similar organochlorine compounds has been attributed to the presence of the ortho, para′ isomer, which contains one ring that can readily undergo hydroxylation (Bitman, 1969; Bitman and Cecil, 1970). Recently, Heinrichs *et al.* (1971) found that *o,p′*-DDT injected into neonatal female rats advanced vaginal openings and first estrus and that all rats showed signs of persistent estrus at 10 days of age. Wrenn *et al.* (1970) found 50 $\mu$g of *o,p′*-DDT administered daily to 18-day-old female rats hastened the time of vaginal opening. Hydroxy and methoxy metabolites of *o,p′*-DDT have recently been found by Feil *et al.* (1971, 1973).

Technical methoxychlor contains about 90% *p,p′*-MeOCl and a number of minor constituents. Until recently, there has been little literature on the metabolism of MeOCl. In a preliminary account three urinary metabolites were found after MeOCl was fed to rabbits (Prickett and Laug, 1953). Weikel (1957) found most of the radioactivity of intravenously administered C-ring-labeled MeOCl was excreted as polar, water-soluble metabolites in rat feces. Kapoor *et al.* (1970) conducted an elegant, complete study of the metabolism of [$^{14}$C]-*p,p′*-methoxychlor after single oral doses to mice. Recovery of almost all of the radioactivity was excellent, 90% in the feces and 10% in the urine. MeOCl was O-demethylated to phenolic products, and five major metabolites were isolated and identified. The fact that hydroxy compounds were the major metabolites of *p,p′*-methoxychlor lends support to the existence of compounds with greater estrogenicity in MeOCl. Possibly the effects of MeOCl on reproduction and its estrogenic activity may be due to the presence of an ortho,para′ isomer or to the formation of a metabolite with estrogenic activity.

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