Methoxychlor Effects on Hepatic Storage of Vitamin A in Rats

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TEGERIS *et al.* (1966) have described pathological changes in dogs and swine fed methoxychlor [1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane] at 1-4 g/kg of body weight/day. They also reported problems with maintaining food consumption at these high levels of methoxychlor. PHILLIPS (1963), PHILLIPS and HIDIROGLOU (1965), and TINSLEY (1969) observed that DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], an analog of methoxychlor, affected vitamin A metabolism as indicated by reduced hepatic storage of vitamin A in rats and cattle.

PHILLIPS and HATINA (1972) and CECIL *et al.* (1973) reported that methoxychlor fed at a single level of 100 ppm in the diet did not affect hepatic storage of vitamin A in rats. We report effects of methoxychlor on hepatic storage of vitamin A in rats when methoxychlor was fed over a range of levels. The report of TEGERIS (1966) guided us in designing the experiment.

Methods

Sprague-Dawley rats (23 days old) were fed diets containing technical methoxychlor[1] at 0, 10, 100, 1,000, and 10,000 ppm for 16 weeks. The basic diet was a ground, commercial chow fortified by the manufacturer[2] with 33 IU of vitamin A/gram of diet. The acetone used for dispersing the methoxychlor in the diet evaporated during mixing.

Twelve rats, caged individually, were used for each level of methoxychlor fed. One-half of the rats were fed *ad libitum*, and one-half were fed according to a restricted regimen. The restricted regimen proceeded as follows: during the first 4 days, the rats

[1] Sigma Grade II, Sigma Chemical Co., St. Louis, Mo. (Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.)

[2] Ralston Purina Co., St. Louis, Mo.

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were all given 6 g of food/day, and during the next 3 days they were all given 8 g/day; thereafter, rats fed diets containing 10,000 ppm of methoxychlor were fed ad libitum, and rats fed diets containing 0-1,000 ppm of methoxychlor were fed daily an amount of food equal to the average daily consumption for the previous week of rats fed diets containing 10,000 ppm of methoxychlor.

Body weights were recorded at the end of weeks 1 and 2 and at biweekly intervals thereafter. At 16 weeks, the rats were killed by decapitation. The livers were removed and weighed, and hepatic vitamin A was determined colorimetrically (DAVIES 1933). The data were analyzed by analysis of variance, and significant differences among individual means were determined by Scheffé's test (SNEDECOR and COCHRAN 1967).

Results and Discussion

Four rats fed diets containing 10,000 ppm of methoxychlor died; the first died on the 8th day of methoxychlor feeding.

Feed consumption and body weight are shown in Figure 1. Under ad libitum feeding, rats fed 10,000 ppm of methoxychlor ate less feed than rats fed 1,000 ppm ($P < 0.01$), and rats fed 1,000 ppm of methoxychlor ate less feed than control rats or rats fed 10 and 100 ppm of methoxychlor ($P < 0.01$). Ad libitum-fed control rats and rats fed 10 or 100 ppm of methoxychlor ate similar amounts of feed.

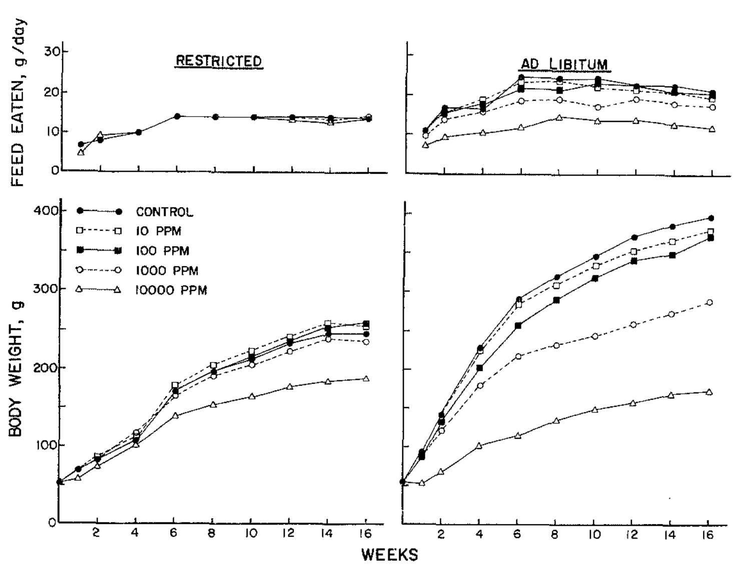

Figure 1. Food consumption and body weight of rats fed methoxychlor at 0, 10, 100, 1,000, and 10,000 ppm under restricted- or ad-libitum-feeding regimens.

TABLE I

Liver weight and total vitamin A per liver of rats fed methoxychlor[1]

Feeding regimen and level of methoxychlor	Liver wt. (g)	Liver wt/ body weight (mg/g)	Vitamin A/ liver (mg)
Rats fed ad libitum:			
Control	14.4 ± 0.53	36.2 ± 0.95	3.69 ± 0.18[a]
10 ppm	13.6 ± 0.30	35.8 ± 0.77	3.43 ± 0.19[ab]
100 ppm	13.5 ± 0.72	36.1 ± 0.80	3.23 ± 0.24[b]
1,000 ppm	10.4 ± 0.44[a]	35.8 ± 0.50	2.32 ± 0.13[c]
10,000 ppm	7.7 ± 0.28[a]	44.7 ± 1.34[a]	1.18 ± 0.09[d]
Restricted feeding:			
Control	6.5 ± 0.13	26.4 ± 0.28	2.45 ± 0.06[a]
10 ppm	6.8 ± 0.32	26.6 ± 0.67	2.12 ± 0.11[ab]
100 ppm	7.4 ± 0.44	28.4 ± 0.87	1.86 ± 0.25[b]
1,000 ppm	7.4 ± 0.60	29.1 ± 1.93	1.87 ± 0.09[b]
10,000 ppm	8.3 ± 0.29	43.7 ± 1.28[a]	1.22 ± 0.07[c]

[1]Data are means ± standard errors of the means. Means followed by different superscript letters differ significantly, $P < 0.01$.

When feed was offered *ad libitum*, gain in body weight for rats fed 10,000 ppm of methoxychlor was less than that for rats fed 1,000 ppm of methoxychlor ($P < 0.01$), and gain in body weight for rats fed 1,000 ppm of methoxychlor was less than that for control rats or rats fed 10 and 100 ppm of methoxychlor ($P < 0.01$). When food intake was restricted to that of rats fed 10,000 ppm of methoxychlor, gain in body weight of only those rats fed 10,000 ppm of methoxychlor was lower than that of the corresponding controls ($P < 0.01$). Thus, some factor other than reduced food intake was contributing to reduced rate of gain when 10,000 ppm of methoxychlor was fed.

Weight and vitamin A content of livers are given in Table 1. Total liver weights of *ad libitum*-fed rats receiving 1,000 ppm and 10,000 ppm of methoxychlor were lighter than liver weights of rats fed up to 100 ppm of methoxychlor. However, when liver weight was considered relative to body weight, livers of rats fed 10,000 ppm of methoxychlor were heavier than livers of rats on all other treatments regardless of feeding regimen. Within feeding regimens, there were no significant differences in liver-to-body weight ratios of rats fed up to 1,000 ppm of methoxychlor. Thus, the reduced total liver weight of rats fed 1,000 ppm of methoxychlor *ad libitum* was related to the reduced body weight of these rats.

Total liver vitamin A generally diminished with increased amount of methoxychlor fed, regardless of feeding regimen.

In experiments in which *p,p'*-DDT was fed to rats in amounts from 0-100 ppm of the diet for periods up to 72 days, PHILLIPS (1963) observed decreased hepatic storage of vitamin A beginning at 10 ppm of DDT. Total hepatic storage of vitamin A was reduced when the diets were supplemented with either carotene or vitamin A, and liver enlargement was also observed (PHILLIPS 1963). We observed liver enlargement relative to body weight only in rats fed 10,000 ppm of methoxychlor (an amount of methoxychlor that was lethal to some rats) and detected decreased hepatic storage of vitamin A when 100 ppm or more of methoxychlor was fed. However, from the data it appears that the effects of methoxychlor on decreased hepatic storage of vitamin A was continuous, with a slight (7-13%) reduction in storage occurring when 10 ppm of methoxychlor was fed.

Summary

Sixty Sprague-Dawley rats were fed diets containing 0, 10, 100, 1,000, and 10,000 ppm of methoxychlor for 16 weeks under *ad libitum*- and restricted-feeding regimens. Methoxychlor at 10,000 ppm was lethal to some rats, reduced food consumption and growth, and increased liver weight relative to body weight. Methoxychlor at 1,000 ppm reduced food consumption and growth of rats fed *ad libitum* but did not reduce growth of restricted-fed rats. Reduced hepatic storage of vitamin A was detectable when methoxychlor was fed at levels of 100 ppm or higher.

References

CECIL, H. C., S. J. HARRIS, and J. BITMAN: Bull. Environ. Contamin. Toxicol. 11, 496 (1973).
DAVIES, A. W.: Biochem. J. 27, 1770 (1933).
PHILLIPS, W.E.J.: Can. J. Biochem. Physiol. 41, 1793 (1963).
PHILLIPS, W.E.J., and G. HATINA: Nutri. Reports Internat. 5, 357 (1972).
PHILLIPS, W.E.J., and M. HIDIROGLOU: J. Agr. Food Chem. 13, 254 (1965).
SNEDECOR, G. W., and W. G. COCHRAN: Statistical Methods, 6 ed., Ames: Iowa State University Press, p. 271, 1967.
TEGERIS, A. S., F. L. EARL, H. E. SMALLEY, JR., and J. M. CURTIS: Arch. Environ. Health 13, 776 (1966).
TINSLEY, I. J.: J. Nutr. 98, 319 (1969).