

and transformed the bacilli into L forms. The L type cultures obtained are similar in appearance and morphology to those isolated previously from a Gram positive spore-bearing bacillus. They differ from the L forms of Gram negative bacilli inasmuch as they grow

better in the absence rather than the presence of animal serum and have a tendency to form large spreading colonies increasing in size for several months.

Received August 18, 1950. P.S.E.B.M., 1950, v75.

The Storage of Methoxychlor in the Fat of the Rat. (18215)

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(Introduced by A. J. Lehman)

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Methoxychlor, p,p' dimethoxy diphenyl trichloroethane has been shown to have a considerably lower order of toxicity than DDT(1,2). There is some indication, as in the case of certain isomers of benzene hexachloride(3), that a low order of toxicity may be associated with a reduced tendency for storage in the fat. A study of the storage of methoxychlor has therefore been made to see whether a similar correlation exists.

Method. Three groups of 24 weanling 21-day-old rats, equally divided as to sex were placed on diets containing methoxychlor at concentrations of 25, 100 and 500 p.p.m. After 4-, 9-, 13- and 18-week exposures, 2 males and 2 females from each group were sacrificed and abdominal fat analyzed for methoxychlor. Equal numbers of control animals were treated similarly. At the end of 18 weeks, exposure to methoxychlor was terminated and the survivors placed on control diet. Following 2 and 4 weeks on control diet the remaining animals were sacrificed and the tissues also analyzed for methoxychlor. The basic diet was a ground commercial rat biscuit with a fat content of 5%. Analysis for methoxychlor gave negative re-

sults. The experimental diets were prepared by mixing appropriate amounts of corn oil solution of methoxychlor with the ground rat biscuit. The methoxychlor used was the p,p' isomer and was of technical grade. The experimental animals were kept in separate cages and food consumption and weight gains recorded weekly. The control animals were housed in groups, and their weights recorded at approximately four-week intervals. Tissues were prepared for analysis by successive extraction of the fat with ethyl ether. Methoxychlor was determined by a method developed in this laboratory(4). This method consists essentially of saponification of the fats by ethanolic KOH, with simultaneous conversion of the methoxychlor to the dehydrochloride. Separation of the dehydrochloride of methoxychlor from the saponified fats is effected by extraction with petroleum ether. The dehydrochloride is then nitrated by the standard Schechter procedure. Upon treatment with sodium methylate in benzene-methanol solution a stable red color is produced having an absorption maximum at 530 m μ . The sensitivity is sufficiently great to quantitate 5 μ g of methoxychlor when contained in a maximum of 5 g of rat fat (1 p.p.m.).

Results. The incorporation of methoxychlor at concentrations of 100 and 500 p.p.m. in the diet produced a retardation of growth in both male and female rats. After 14

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2. Smith, M. I., Bauer, H., Stohlman, E. F., and Lillie, R. D., *J. Pharm. and Exp. Therap.*, 1946, v88, 359.

3. Davidow, B., Frawley, J. P., Fitzhugh, O. G., and Woodward, G., unpublished observations.

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TABLE I. Storage of Methoxychlor in the Perirenal Fat of the Rat.

P.P.M. methoxychlor in diet	P.P.M. methoxychlor* retained in the perirenal fat									
	During exposure								Cessation of exposure	
	4 wk		9 wk		13 wk		18 wk		2 wk	
	M	F	M	F	M	F	M	F	M	F
0							0	0		
25	0	0	0	0	0	0	0	0	0	0
100	0	0	4	7	3	4	1	1	0	0
500	36	17	26	20	19	14	16	14	0	0

* Each figure represents the avg of 2 animals with the exception of the control (0.0 p.p.m. methoxychlor in diet) where average represents 8 animals.

weeks. retardation at the 100 p.p.m. level was slight, but definite; at the 500 p.p.m. level retardation was marked averaging 60 g for the males and 40 g for the females. No effect was noted at the 25 p.p.m. level. None of the animals, on the 100 and 500 p.p.m. diets, however, showed any toxic symptoms. This is consistent with unpublished observations(6) from this laboratory on adult animals fed diets containing as much as 1000 p.p.m. methoxychlor. Since the effect on growth was unexpected, the food consumption of the 500 p.p.m. group was examined. It was found that the animals on the 500 p.p.m. methoxychlor diet consumed on the average about 25% less food than the animals on the 25 p.p.m. level. Thus, lower acceptability of the high methoxychlor diet may be the explanation. Further evidence in this direction is the observation that a number of methoxychlor animals, upon being put on control diet rapidly resumed normal food consumption and weight within a period of several weeks.

Table I shows the results of analyses of perirenal fat for methoxychlor. It is evident that no storage of methoxychlor occurs at the 25 p.p.m. level, very slight at the 100 p.p.m. level, and although measurable, only moderate even at the 500 p.p.m. level. In contrast to DDT(5) there is no tendency for the female to store more methoxychlor than the male, in fact, the reverse seems indi-

cated. However, due to the small number of animals, this cannot be statistically defined. It is interesting to note also, that a trend toward lower storage values during the exposure period seems indicated. Here again, and for the same reason, statistical significance does not obtain. Finally, it can be seen that upon cessation of exposure the loss of stored methoxychlor is complete within two weeks.

Discussion. From the data presented it would appear that the storage of methoxychlor in the fat of the rat occurs to a very limited extent and then only at relatively high doses. This is in sharp contrast to the storage of DDT. Thus a diet containing only 10 p.p.m. of this insecticide will cause the accumulation of 80 to 160 p.p.m. DDT depending on the sex of the animal(5). The finding of a low order of methoxychlor storage in fat is consonant with the low order of toxicity for the compound, and may be a partial explanation. However, in view of the fact that the oil solubility of methoxychlor is nearly identical with that of DDT, extensive storage in fat could be expected. It is therefore also possible that lack of storage of methoxychlor is a reflection of the rapid destruction(1) of the compound before storage can occur.

Summary. 1. Diets containing 100 and 500 p.p.m. methoxychlor reduce the growth rate and food consumption of young rats. 2. Storage of methoxychlor in the fat of the rat is minimal and can be demonstrated only at relatively high dietary levels.

6. Woodward, G., and Nelson, A. A., unpublished observations.

5. Laug, E. P., Nelson, A. A., Fitzhugh, O. G., and Kunze, F. M., *J. Pharm. and Exp. Therap.*, 1950, v98, 268.