Heptachlor Induced Changes in Fenitrothion Metabolism

by M. MESTITZOVA, J. KOVAC and K. DURCEK
Research Institute of Industrial Hygiene and Occupational Medicine
Research Institute of Agrochemical Technology Institute of Hygiene
Bratislava, Czechoslovakia

Previous work in our laboratory established that the long term burden of chlorinated hydrocarbon in the animal body stimulates the metabolism of orally administered fenitrothion [0,0-dimethyl-0-(3-methyl-4-nitrophenyl)-thiophosphate] (1). It was concluded that this effect, enhanced symptoms of toxicity, changed mortality rates and earlier onset of p-nitro-m-kresol elimination, is due to the stimulated activity of microsomal enzymatic systems of the liver. As the stimulation was manifested by augmented toxicity, we assumed that the cause may be the enhanced and accelerated production of the oxon analogue. Induction and Inhibition of the oxidising systems of the liver has been demonstrated to play an important role in pesticide toxicity (2-22).

The effect of low levels of heptachlor on liver and kidney functions was recently investigated (23) and it was found that feeding as little as 6 mg/kg body-weight in the diet for 3 months resulted in no significant changes in functional and morphological regards. Since many humans have measurable concentrations of several halogenated hydrocarbon insecticides in fat, it is possible that such individuals may have elevated levels of liver microsomal enzymes that oxidatively influence the toxicity of organophosphorous insecticides. The doses of heptachlor, used in chronic intoxication, do not approach the cumulation values (24), but they are still below the amount necessary to produce clinical, functional or histological alterations.

This paper investigated the effect of heptachlor on the liver microsomal metabolism of fenitrothion, especially the conversion to fenitrooxon by means of labelled ^{32}P-fenitrithion and using paperchromatography.

Materials and Methods

Chemicals: ^{32}P-labelled fenitrothion, specific activity 0,0698 mc/mM, was purchased from the Radiochemical Centre, Amersham, Bucks., England. The purity of the compound was determined by paper chromatography. Heptachlor, 1,4,5,6,7,10,10-heptachloro-4,7,8,9-tetrahydro-4, 7-methyleneindene, was provided by the Research Institute of Agrochemical Technology, Bratislava. Administration of the insecticides to animals and method of

analysis: Young adult female Wistar rats were used. One group, fed on ordinary Larson diet, served as control group, the experimental group was chronically poisoned with heptachlor at a dose of 5 mg/kg body weight/day for 3 months. Both groups fasted for 18 hours and recieved 74 mg ^{32}P-fenitrothion/kg dissolved in oil by stomach tube. After treatment, food and water were supplied ad libitum. At that dose the animals developed only slight symptoms of poisoning; however, they were more pronounced in the groups fed heptachlor.

For each series of experiments, assays were performed on control rats that were handled in a manner equivalent to that of the experimental groups, except that heptachlor was not given in the diet. Values shown in the table and the figure are the mean values from four or more experiments for each experimental condition.

At selected intervals after fenitrothion application, the animals were sacrificed, exsanguinated, blood volumes measured and livers weighed.

Radiometric measurements were made of the total activities of the livers and of the degradation products in blood and livers. Extraction of the materials: Both the materials were homogenized with anhydrous sodiumsulfate w/w 1:3. Three times 0,1 g of liver homogenate was taken for determining the total activity. The homogenates of blood and liver were extracted with 40 ml chloroform by shaking on a mechanical shaker for 20 min. After discarding the chloroform layer the extraction was repeated with further 40 ml chloroform. After filtering and rinsing the filter cake with chloroform both the chloroform extracts were shaked in a separatory funnel with the same volume of water. After evaporating the chloroform layer under reduced pressure in a rotary evaporator at 40°C the concentrate was redissolved in 10 ml of petroleum ether 40-60°C. Petroleum ether extract was reextracted twice with the same volume of acetonitrile. After discarding the petroleum ether the combined acetonitrile extracts were shaken again with fresh petroleum ether and the layers allowed to separate. Acetonitrile was made up to a volume of 20 ml and an aliquot volume was after evaporation dissolved in ethylether. These concentrates were quantitatively applied to Whatman paper Nr 1, and the chromatograms were develped by the descending technique to a hight of 20 cm in a solvent system of 25% formamid aceton/petroleum ether described by Bátora (25). The chromatograms were evaluated radiometrically by strip counting.

Results

After application of ^{32}P-fenitrothion to both groups the activities were estimated at 30,60,120,240 min. and 24 hours intervals. The mean values of the total activity of the livers are given in Table 1. The data show that the heptachlor groups had a

markedly greater activity at each time interval. The difference between the groups was significant ($P<0,010$). From this table it is clear that the total activity of fenitrothion and its metabolites in the liver reaches its maximum in the experimental groups up to 4 hours and then it decreases to the 30 min. level. In the control groups the values are almost 50% lower and diminishe gradually beginning from 30 minutes.

TABLE 1

The ^{32}P activity of rat livers after oral application of ^{32}P-fenitrothion as % of applied dose/g tissue

Time after dose	heptachlor groups		control groups	
	imp/min/g	% recov.	imp/min/g	% recov.
30'	44,888	3,2	36,481	1,8
60'	40,472	2,6	29,011	1,3
120'	54,498	3,0	31,803	1,4
240'	81,435	4,3	25,363	0,9
24h	44,311	2,7	21,920	1,2

The liver weights per 100 g body weight were in the experimental groups 15% higher than in the controls ($P<0,10$). A similar difference showed the dry liver weight per 1 g fresh liver ($P<0,05$).

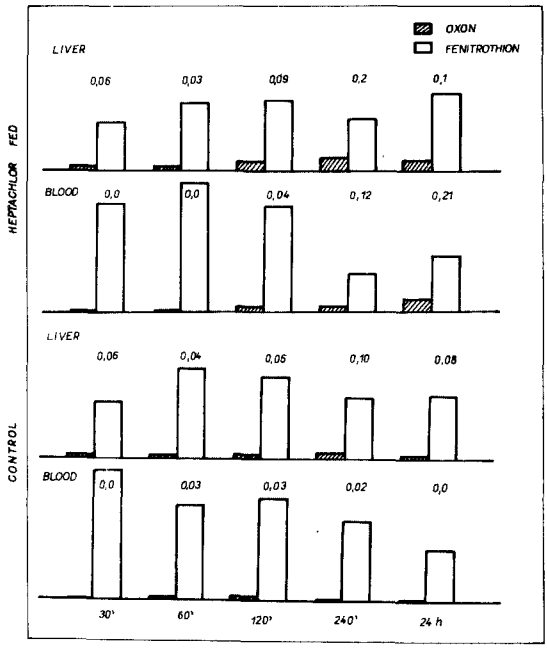
Further we measured the activity of the whole radiochromatogram and the activities of the peaks belonging to the oxon analogue and unmetabolized fenitrothion.

The summarized results of the ratio oxon/fenitrothion in liver and blood after 30,60,120,240 minutes and 24 hours intervals in both groups are reproduced in Fig. 1. These results indicate that the ratio was highest in the liver of the impregnated groups; it increased up to four hours after ^{32}P-fenitrothion application and then it decreases. In the blood the kinetics of oxon production are postponed and the absolute values are lower. In the control groups the ratio of oxon/fenitrothion in the liver is similar to that of the experimental groups, however, the absolute values were essentiallly lower. In the blood the oxon was hardly detectable.

Discussion

The present study was carried out to see whether the metabolism of fenitrothion in rats fed normal diets was changed

Fig. 1. Relation of fenitrothion to its oxon derivate in the blood and livers of normal and heptachlor fed rats.



Values above the columns indicate the ratio, oxon/fenitrothion.

by pretreatment of the animals with heptachlor in small amounts for prolonged time. The magnitude of the increased metabolism reported in a previous paper (1) has been demonstrated in two ways. First the total activity of the liver was increased by a factor of about two by heptachlor pretreatment. As an explanation for these activities differences one might presume that the prolonged influence of heptachlor can be related to a proliferation of SER/ smooth endoplasmatic reticulum/ and to an activation of ribosome biosynthesis, and so to a microsomal binding of fenitrothion and its metabolites. The augmented liver weight may confirm this assumption. The proliferation of SER was frequently pointed out following chlorinated hydrocarbon insecticides, drugs, and other chemicals application (5, 8, 20, 26-30). After interruption of drug application Seifert and Remmer (15) observed a regression of liver weight and a slowing down of the decrease of the specific radioactivity of ribosomes and of the radioactivity of total

liver RNA. The minimum dose of DDT required to stimulate the activity of oxidative enzymes in liver microsomals refer Conney et al. (31) to be 10-15 μg per g fat, a concentration approaching that reported to occur in the human population.

Secondly we could demonstrate the production of oxon derivates to predominate in the liver and blood of the groups pretreated with a chlorinated hydrocarbon insecticide from the beginning of the intoxication with an organothionophosphate. Our results are compatible with several above-mentioned reports regarding the induction of the enzymatic oxidative systems by halogenated hydrocarbons and its intracellular localization.

The stimulation of the fenitrothion metabolism is manifested by the rapid degradation to p-nitro-m-kresol elimination in the urine in the impregnated groups, as we could demonstrate in a previous paper (1). It seems that it may be attributed to the faster degradation of oxon derivates in comparison to the phosphorthioate itself which is in accordance with widely accepted results reported in the literature.

We brought the evidence that the interference of chlorinated hydrocarbon insecticides with organophosphorous metabolism takes place also in the case of prolonged application of small doses of the former.

Summary

Pretreatment of rats with heptachlor administered in the food for 3 months increases the metabolism of fenitrothion. Comparison of oxon analogue levels in the blood and livers of pretreated and control animals suggests that the conversion of fenitrothion to its oxon analogue is enhanced and accelerated. This was studied by tracer methods, using P-fenitrothion.

References

1. M. MESTITZOVÁ, J. KOVÁC, K. DURCEK and A. HLADKÁ, Prac.Lék. (in press)

2 J. P. FRAWLEY, E. C. HAGAN, O. G. FITZHUGH, H. N. FUYAT and W. I. JONES, J. Pharmacol. Exp. Ther. 119, 147 (1957)

3. A. H. CONNEY, C. DAVIDSON, R. GASTEL and J. BURNS, J. Pharmacol. Exp. Ther. 130, 1 (1960)

4. A. H. CONNEY, Pharmacol. Rev. 19, 317 (1967)

5. R. L. CRAM, M. R. JUCHAU and J. R. FOUTS, J. Lab. Clin. Med. 66, 906 (1965)

6. R. L. CRAM and J. R. FOUTS, Biochem. Pharmacol. 16, 1001 (1967)

7. H. REMMER and H. J. MERKER, Klin. Wchschr. 41, 276 (1963)

8. J. R. FOUTS and L. A. ROGERS, J. Pharmacol. Exp. Ther. 147, 112 (1965)

9. H. UEHLEKE, Naunyn-Schmiedeberg's Arch. Pharmak. und exp. Path. 259, 66 (1967)

10. A. VARDANIS, Biochem. Pharmacol. 15, 749 (1966)

11. A. J. TRIOLO and J. M. COON, J. Pharmacol. Exp. Ther. 154, 613 (1966)

12. A. J. TRIOLO and J. M. COON, Federation Proc. 22, 189 (1963)

13. A. J. TRIOLO and J. M. COON, J. Agr. Food Chem. 14, 549 (1966)

14. J. C. STREET and A. D. BLAU, Toxicol. Appl. Pharmacol. 8, 497 (1966)

15. J. SEIFERT and H. REMMER, International Symposium on Microsomes and Drug Oxidations, Tübingen, Germany (1969)

16. W. L. BALL, J. SINCLAIR, M. CREVIER and K. KAY, Can. J. Biochem. Physiol. 32, 440 (1954)

17. S. BASS, A. TRIOLO, Toxicol. Appl. Pharmacol. 12, 289 (1968)

18. M. CREVIER, W. L. BALL and K. KAY, Arch. Industr. Hyg. Occup. Med. 9, 306 (1954)

19. J. GILLETT and T. CHAN, J. Agr. Food Chem. 16, 590 (1968)

20. L. HART, R. W. SHULTICE and J. R. FOUTS, Toxicol. Appl. Pharmacol. 5, 371 (1963)

21. T. NAKATSUGAWA, N. M. TOLMAN and P. A. DAHM, Biochem. Pharmacol. 17, 1517 (1968)

22. U. SCHWABA and L. WENDLING, Arzneimittel-Forschung, 17, 614 (1967)

23. M. MESTITZOVÁ and M. BENO, Prac. Lék. 18, 153 (1966)

24. M. de VLIEGER, J. ROBINSON, M. K. BALDWIN, A. N. CRABTREE and M. C. van DIJK, Arch. Environ. Health, 17, 759 (1968)

25. V. BATORA, Paper Chromatography, p 700 (1963), Czechoslov. Academy of Sciences, Prague

26. L. G. HART and J. R. FOUTS, Biochem Pharmacol. 14, 263 (1965)

27. L. G. HART and JR. FOUTS, Arch exper. Path. und Pharmakol. 249, 486 (1965)

28. L. G. HART and J. R. FOUTS, Proc. Soc. Exper. Biol. Med. 114, 388 (1963)

29. M. IKEDA, B. SEZESNY and M. BARNES, Fed. Proc. 25, 417 (1966)

30. H. REMMER and H. J. MERKER, Ann. New York Acad. Sc. 123, 79 (1965)

31. A. H. CONNEY, R. M. WELCH, R. KUNTZMAN and J. J. BURNS, Clin. Pharmacol. and Therapy, 8, 2 (1967)