Long Term Exposure to Organophosphorus Pesticides and Lipid Metabolism in the Rat

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INTRODUCTION

The main public health problem raised by the ever-increasing use of organophosphorus pesticides is the possible toxicological effect of long term exposure to low concentrations mainly as residues in food.

In addition to the well known inhibition of cholinesterases, organophosphorus esters can also inhibit other hydrolases, some of which can play an important role in the lipid metabolism (DESNUELLE et al., 1960 ; SZENDZIKOWSKI et al., 1961 ; COLEY and JENSEN, 1973 ; PETERS et al., 1973).

Previous studies in our laboratory have demonstrated that mono- and diglyceridase activities from different rat tissues can be inhibited in vitro and in vivo by some organophosphorus pesticides or their active metabolites (BUCHET et al., 1970, 1971, 1974a). We have also found (unpublished results) that triglyceridase activities in particular hormone sensitive (HSL) and lipoprotein (LPL) lipases of rat adipose tissue are also inhibited in vitro by organophosphate esters. In order to assess if this inhibition could also be induced in vivo, two organophosphorus pesticides (Dursban, Triamiphos) were administered for one year to rats receiving a normal or a fat-enriched diet. At time of sacrifice HSL and LPL activities of adipose tissue were determined and some lipid components (total glycerol, fatty acids and cholesterol) were measured in the serum and the aortae. A preliminary study (BUCHET et al., 1974b) had indeed suggested that total cholesterol concentration could increase in the aorta of female rats receiving a diet enriched in triglyceride and containing 10 ppm of Triamiphos.

Bulletin of Environmental Contamination & Toxicology,
Vol. 17, No. 2 © 1977 by Springer-Verlag New York Inc.

EXPERIMENTAL

Animals

Male and female Sprague-Dawley rats (4 weeks old at the beginning of treatment) were used. They were kept in air-conditioned rooms with artificial light. They were weighed once a week and each month were bleeded at the tail for total blood cholinesterase determination.

Diet

Animals were fed a standard diet ad libitum (D03 flour from U.A.R., Villemoisson sur Orge, France) supplemented or not with corn oil (final concentration 20 %) and with an organophosphorus pesticide.

Two pesticides were tested : Dursban or 0,0-diethyl 0-(3,5,6 trichloro-2-pyridyl) phosphorothioate from Dow Chemical Company (Midland, Michigan, U.S.A.) and Triamiphos or 5-amino-1-(bis dimethylamido) phosphoryl 3-phenyl-1,2,4 triazole from Philips-Duphar (Amsterdam, The Netherlands). They were added to the diet to obtain a concentration of 100 ppm in the case of Dursban and 10 ppm in the case of Triamiphos. Both pesticides are indirect cholinesterase inhibitors, i.e. they must first be activated in the organism before exercising their inhibitory action.

Enzyme determination

Total blood cholinesterase activity was measured by the spectrophotometric method of Ellman slightly modified (LAUWERYS and BUCHET, 1971). Hormone sensitive lipase activity (HSL), the enzyme responsible for fatty acid mobilization from adipose tissue triglyceride, was measured in the whole adipose tissue homogenate as described by VAUGHAN et al. (1961) but in the presence of EDTA (1 mM) as recommended by HUTTUNEN et al. (1970).

The determination of the enzyme activity responsible for the uptake of circulating triglyceride fatty acids (i.e. lipopro-

tein lipase activity or L P L) was performed by incubating a portion of freshly homogenized adipose tissue in NH_3-NH_4Cl buffer according to BORENSZTAJN et al. (1973) with the artificial triglyceride emulsion described by SEZILLE et al (1973).

Lipid determination in aorta and serum

After sacrifice, the whole aorta from its origin to the bifurcation was removed and carefully dissected free of adventitious tissue at 2 - 4°C under a stereoscopic microscope Wild (Heerbrugg, Switzerland), blotted on a filter paper and then stored frozen at -20°C until further handling. Prior to lipid saponification the aortae were dissolved in 10 % aqueous tetramethylammonium hydroxyde by warming at 60°C in a water bath during 30 min.
Classical Folch mixture was used to extract the lipids from serum according to the simplified procedure of KATES (1972). Serum free fatty acids and tissue total fatty acids were determined by titration as described by DOLE and MEINERTZ (1960), glycerol by colorimetry according to KORN (1955), and cholesterol by the gaz chromatographic technique of VAN LIER and SMITH (1967) using stigmasterol as internal standard.

Protein determination

The protein content of aorta was determined on an aliquot of the tissue solution in the aqueous tetramethylammonium hydroxyde following the technique of LOWRY et al. (1951). The final concentration of $(CH_3)_4NOH$ was 0.03 % and did not interfere with the color development.

RESULTS AND DISCUSSION

I. One year 100 ppm Dursban, male rats

The increase in body weight during the observation period did not differ between control and exposed animals, neither in the normal diet group nor in the fat-enriched diet group ; however as expected a difference (80 g on the average) was observed between these two groups at the end of treatment. The total blood cholinesterase activity was reduced by 40 % in the exposed group with normal diet and by 60 % in that with fat-enriched regime.

The value of the other biochemical parameters are given in Table I. No statistical difference was observed, except the increase in the total fatty acid content of aortae when the diet is enriched with fat.

II. One year 10 ppm Triamiphos, male rats

Like for Dursban, no statistical difference was found between the body weight increase of treated and control animals : a decrease of 60 to 70 % of total blood cholinesterase activity was observed during the whole treatment period in the normal as well in the fat-enriched diet group. The results of Table II again show no statistical difference between the groups except the already reported increase in the total fatty acid content of aortae in the fat-enriched diet group.

TABLE I

Effect of Dursban (100 ppm - 1 Year) on male rats.
(Mean ± standard error)

Biochemical parameter	Normal diet		Fat-enriched diet	
	Control animals (19)⊠	Exposed animals (20)	Control animals (18)	Exposed animals (17)
Adipose tissue				
Hormone Sensitive Lipase µeq FA/mg prot. hour	0.953 ± 0.072	0.941 ± 0.066	0.542 ± 0.042	0.569 ± 0.039
Lipoprotein Lipase µeq FA/mg prot. hour	1.557 ± 0.088	1.635 ± 0.105	0.717 ± 0.057	0.704 ± 0.042
Serum				
Free fatty acids (µeq/ml)	0.881 ± 0.059	0.807 ± 0.056	1.16 ± 0.091	0.956 ± 0.05
Total fatty acids (µeq/ml)	8.33 ± 0.74	7.54 ± 0.65	6.95 ± 0.52	7.18 ± 0.64
Total glycerol (µmole/ml)	6.71 ± 0.31	6.42 ± 0.24	3.86 ± 0.16	4.13 ± 0.19
Total cholesterol (mg/ml)	1.71 ± 0.19	1.21 ± 0.10	1.42 ± 0.20	1.54 ± 0.23
Aorta				
Total fatty acids (neq/mg prot)	33.81 ± 2.11	33.57 ± 2.57	85.76 ± 3.52	88.02 ± 4.51
Total glycerol (nmole/mg prot)	26.28 ± 2.14	30.55 ± 1.48	27.38 ± 2.44	26.84 ± 3.43
Total cholesterol (µg/mg prot)	4.89 ± 0.24	4.66 ± 0.17	5.43 ± 0.29	5.64 ± 0.24

⊠ Number of animals

T A B L E II

Effect of Triamiphos (10 ppm - 1 Year) on male rats.
(Mean ± standard error)

Biochemical parameter	Normal diet		Fat-enriched diet	
	Control animals (9) ⊠	Exposed animals (10)	Control animals (8)	Exposed animals (10)
Adipose tissue				
Hormone Sensitive Lipase μeq FA/mg prot. hour	0.704 ± 0.048	0.845 ± 0.032	0.776 ± 0.063	0.786 ± 0.035
Lipoprotein Lipase μeq FA/mg prot. hour	1.599 ± 0.228	1.443 ± 0.173	0.908 ± 0.086	1.315 ± 0.197
Serum				
Free fatty acids (μeq/ml)	0.95 ± 0.11	1.19 ± 0.13	1.23 ± 0.10	1.30 ± 0.09
Total fatty acids (μeq/ml)	8.53 ± 1.49	8.28 ± 1.39	7.57 ± 0.47	7.38 ± 0.30
Total glycerol (μmole/ml)	2.64 ± 0.24	2.53 ± 0.17	2.68 ± 0.09	2.72 ± 0.11
Total cholesterol (mg/ml)	1.33 ± 0.24	1.47 ± 0.23	1.18 ± 0.08	1.14 ± 0.10
Aorta				
Total fatty acids (neq/ml prot)	47.82 ± 5.91	46.05 ± 4.51	95.05 ±12.50	93.48 ± 13.05
Total glycerol (nmole/mg prot)	53.34 ± 3.57	48.28 ± 2.83	58.51 ± 6.40	57.70 ± 4.94
Total cholesterol (μg/mg prot)	5.05 ± 0.15	4.88 ± 0.12	4.46 ± 0.23	4.41 ± 0.14

⊠ Number of animals

III. One year 10 ppm Triamiphos, female rats

A slightly more pronounced decrease (70 to 80 %) in total blood cholinesterase activity was observed among the females exposed to this pesticide in comparison with the males. Again a difference in body weight increase was ascribed to the presence or absence of fat in the diet and not to that of the pesticide. The results of the biochemical parameters are given in Table III. In contrast to the case with males, the total fatty acid content of aortae did not increase with the fat-enriched diet.

CONCLUSION

The present investigation was undertaken after a preliminary study (BUCHET et al., 1974b)had suggested that the exposure to the organophosphorus pesticide Triamiphos increases the cholesterol content of the rat aorta.
The repetition of the experiments with the same and another pesticide, as well as the extension to animals of both sexes, failed to confirm these preliminary results. At the doses and for the duration of exposure selected, an effect of Triamiphos and Dursban on fat metabolism in the rat cannot be evidenced. In this investigation total blood cholinesterase activity is the only biological parameter affected although this does not seem to impair the health of the animals.

ACKNOWLEDGMENTS

The technical assistance of Mrs. Hermans and Mr. Bauer is gratefully acknowledged.

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T A B L E III

Effect of Triamiphos (10 ppm - 1 Year) on female rats.
(Mean ± standard error)

Biochemical parameter	Normal diet		Fat-enriched diet	
	Control animals (14)⊠	Exposed animals (12)	Control animals (13)	Exposed animals (15)
Adipose tissue				
Hormone Sensitive Lipase μeq FA/mg prot. hour	0.951 ± 0.064	0.901 ± 0.110	0.910 ± 0.064	1.054 ± 0.056
Lipoprotein Lipase μeq FA/mg prot. hour	0.511 ± 0.062	0.453 ± 0.073	0.736 ± 0.129	0.661 ± 0.083
Serum				
Free fatty acids (μeq/ml)	1.05 ± 0.06	1.05 ± 0.08	1.32 ± 0.08	1.15 ± 0.06
Total fatty acids (μeq/ml)	6.16 ± 0.29	6.33 ± 0.19	8.00 ± 0.41	7.72 ± 0.31
Total glycerol (μmole/ml)	2.18 ± 0.06	2.25 ± 0.08	2.47 ± 0.16	2.41 ± 0.12
Total cholesterol (mg/ml)	0.81 ± 0.04	0.86 ± 0.03	0.99 ± 0.05	1.11 ± 0.06
Aorta				
Total fatty acids (neq/mg prot)	50.50 ± 9.64	44.44 ± 9.05	37.53 ± 5.66	33.76 ± 4.64
Total glycerol (nmole/mg prot)	39.29 ± 1.14	39.59 ± 1.36	48.76 ± 2.74	47.26 ± 2.04
Total cholesterol (μg/mg prot)	4.73 ± 0.07	4.84 ± 0.08	4.71 ± 0.09	4.42 ± 0.14

⊠ Number of animals

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