Chemistry and Toxicology of Pesticide Chemicals V. Some Pharmacological Aspects of Leptophos Exposure in the Rat*

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INTRODUCTION

The insecticide Leptophos ; O-(4-bromo-2,5-dichlorophenyl)-O-methyl phosphonothioate is produced by Velsicol chemical corporation, USA, and marketed under the name "Phosvel". Leptophos possesses moderate toxicity to mammals and proved to be effective against pests attacking field crops. In the middle and far East, it found extensive application in controlling the cotton leaf worm "Spodoptera littoralis" (KAMEL and MITRI 1970) and has also been used on a number of agricultural commodities, including apples, pears, potatoes and tomatoes. Published toxicological and pharmacological studies on Leptophos are limited. HASSAN et al (1975) reported on the toxicity of Leptophos in the rat. Interst in the toxicology and pharmacology of Leptophos was stimulated following the observation that the chemical causes neurotoxicity in chicken (ABOU-DONIA 1974). The present investigation describes some pharmacological effects in the rat following subacute exposure to Leptophos.

MATERIALS AND METHODS

Male albino rats (120 - 150 g) were fed Leptophos in the diet at a concentration of 10 , 30, or 90 ppm, for a period of 12 weeks. At 3 week intervals, urine samples were collected and some animals sacrificed. Erythrocyte and plasma cholinesterase activity was determined according to MICHEL (1949). Brain acetylcholinesterase activity was determined after HESTRIN (1949). Other investigated parameters included assessment of the renal glomerular function and tubular phosphorus reabsorption (OSER 1965). For evaluation of hepatic function, serum Glutamic-pyruvic transaminase and alkaline phosphatase were determined (DAVIDSOHN and HENRY 1974). The urinary level of 3-methoxy-4-hydroxy

* This investigation was supported by SFCP, Environmental Protection Agency, USA, Contract No. 3-545-2.

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Bulletin of Environmental Contamination & Toxicology, Vol. 18, No. 5 © 1977 by Springer-Verlag New York Inc.

mandelic acid (VMA) was determined as described by SUNDERMAN et al (1960).

RESULTS

Cholinesterase activity : Table I shows the activity of erythrocyte cholinesterase in rats fed 10,30 or 90 ppm of dietary Leptophos, during 12 weeks. The extent of inhibition seems to parallel the concentration of the chemical. At 90 ppm, maximum inhibition (more than 60%) was reached after three weeks. Although intoxication was continued, the enzyme was slowly recovering during the succeeding weeks. The rate of recovery was faster, following cessation of treatment. At 10 and 30 ppm levels, the enzyme activity approached the baseline values ; even before withdrawal of the chemical.

TABLE I

Erythrocyte Cholinesterase activity

Weeks	Enzyme activity - Δ pH/hr at different Leptophos concentrations			
	0 ppm	10 ppm	30 ppm	90 ppm
0	0.23	-	-	-
3	0.21	0.21	0.14	0.07
6	0.22	0.18	0.12	0.08
9	0.24	0.20	0.16	0.10
12	0.20	-	0.18	0.11
15	0.20	-	0.18	0.16
18	0.22	-	0.19	0.18

Data represent means of 4-6 animals
Treatment stopped after 12 weeks

The pattern of inhibition of the plasma enzyme is shown in table II. The magnitude of inhibition was much smaller than that of the RBC enzyme. The 10 ppm level did not cause any inhibition, and the 30 ppm level resulted in a mild inhibition. Maximum depression of the plasma enzyme was shown to occur at the 90 ppm concentration, after six weeks. Recovery followed the same pattern

as the erythrocyte enzyme.

TABLE II

Plasma cholinesterase activity

Weeks	Enzyme activity - ΔpH/hr at different Leptophos concentrations			
	0 ppm	10 ppm	30 ppm	90 ppm
0	0.36	-	-	-
3	0.36	0.34	0.31	0.27
6	0.36	0.35	0.31	0.25
9	0.34	0.36	0.30	0.29
12	0.38	0.37	0.35	0.33
15	0.35	-	0.34	-
18	0.42	-	0.39	0.40

Data represent means of 4-6 animals
Treatment stopped after 12 weeks

Table III shows the inhibition of brain acetylcholinesterase at different dietary levels of Leptophos. The lowest concentration of the chemical inhibited the enzyme only mildly. The 30 and 90 ppm levels resulted in a significant depression. Following cessation of treatment, the enzyme activity was restored slowly to approach the control values after 18 weeks.

TABLE III

Brain cholinesterase activity

Weeks	Enzyme activity - O.D. at different Leptophos concentrations			
	0 ppm	10 ppm	30 ppm	90 ppm
0	0.28	-	-	-
3	0.28	0.24	0.19	0.17
6	0.30	0.26	0.19	0.17
9	0.28	0.27	0.22	0.16
12	0.27	-	0.23	0.17
15	0.27	-	0.24	0.20
18	0.25	-	0.22	0.24

Data represent means of 4-6 animals
Treatment stopped after 12 weeks

Effect of Leptophos exposure on SAP and SGPT :

Table IV shows SAP and SGPT activities after feeding dietary Leptophos for 12 weeks. Alkaline phosphatase activity increased after 3 weeks and decreased below control levels, during the succeeding weeks. The enzyme activity was restored to normal after 18 weeks. Serum Glutamic-Pyruvic transaminase activity also increased after three weeks and remained high during the intoxication period and through the 15th week. A maximum increase of 2.5 - 3.0 fold was reached after 12 weeks at the 90 ppm level. The activity approached the control values on the 18th week.

Effect of Leptophos exposure on TPR, BUN and urinary VMA :

The tubular phosphosus reabsorption was determined to serve as an index for the integrity of the renal tubules. Table V shows that the TPR remained unaffected during and after intoxication. Blood urea nitrogen was determined to reflect changes in the glomerular filteration. Elevated BUN values were observed after 12 and 15 weeks and control values were restored 6 weeks after cessation of treatment (Table V). Urinary VMA levels apparently remained within normal values (Table V).

Hemograms, urine and other parameters :

The hemograms of control animals and rats fed the median and high concentrations of Leptophos were examined during and after the intoxication period. No significant changes were observed in total erythrocyte count, total and differential leukocyte count, hemoglobin concentration or hematocrit. Fasting blood glucose level remained essentially unaltered. Qualitative urine analysis and urine microscopic examination did not reveal any abnormalities. Body weights and organ weights (liver, kidney, spleen, heart and brain) of intoxicated rats did not show any significant differences ; as compared with the corresponding controls.

DISCUSSION

The cholinesterase data indicate that the erythrocyte enzyme may serve as a suitable index for Leptophos exposure. A previous study (HASSAN et al 1975) reported similar findings in the rat. HASSAN et al (1976a) reporting on humans exposed to Leptophos under field conditions and on intoxicated humans (1976b) reached the same conclusions. In general, the plasma enzyme was much less sensitive. A consistent feature of long-term subacute exposure to Leptophos is the recovery of the plasma and erythrocyte enzymes, inspite of intoxication. This may suggest inhibition of the intoxication mechanism"s" (such as P = S→P = O conversion) and/or activation of the degradation mechanism"s"

TABLE IV

Effect of Leptophos exposure on serum alkaline phosphatase and serum Glutamic-Pyruvic transaminase

Week	S A P Activity ± S.D. (King-Armstrong units)			S G P T Activity ± S.D. (Karmen units)		
	0 ppm	30 ppm	90 ppm	0 ppm	30 ppm	90 ppm
0	22.5±2.9	-	-	12.0±1.8	-	-
3	-	29.2±4.0	37.2±5.1	-	19.3±2.2	23.2±3.0
6	18.3±1.7	9.0±2.0	8.3±1.1	10.2±1.9	20.7±2.9	22.4±3.8
9	-	11.9±2.9	9.8±2.0	-	18.9±3.1	25.0±3.1
12	17.2±2.3	7.4±1.5	8.7±1.5	9.3±1.2	23.7±3.6	30.0±4.8
15	-	4.8±1.0	9.1±1.3	-	25.4±3.6	23.2±2.2
18	20.0±2.8	20.5±3.1	26.4±4.1	10.5±2.0	9.8±1.8	8.5±1.7

Data represent means of 4-6 animals
Treatment stopped after 12 weeks

TABLE V

Effect of Leptophos exposure on TPR, BUN and urinary VMA

Weeks	TPR±S.D.			BUN±S.D. mg/100 ml			VMA±S.D. ug/mg creatinine	
	0 ppm	30 ppm	90 ppm	0 ppm	30 ppm	90 ppm	0 ppm	90 ppm
0	0.92±0.04	-	-	32±4	-	-	1.3±0.5	-
3	0.95±0.02	0.87±0.03	0.94±0.04	-	27±3	33±3	2.7±1.1	1.6±0.5
6	0.90±0.04	0.89±0.07	0.92±0.07	30±3	38±3	35±2	1.3±0.7	1.7±0.9
9	0.83±0.03	0.90±0.05	0.95±0.03	-	36±4	37±4	1.6±0.5	2.4±0.8
12	-	0.88±0.04	0.91±0.04	34±3	46±4	50±4	2.4±1.3	2.8±1.6
15	0.97±0.02	0.97±0.04	0.98±0.02	-	37±4	46±5	2.5±1.0	1.3±0.6
18	0.93±0.04	-	0.91±0.02	30-4	34±2	33±3	1.6±0.7	1.3±0.4

Data represent means of 4-6 animals

Treatment stopped after 12 weeks

(such as hydrolysis). Other parameters may also contribute to this phenomenon. These include the elimination rate, lipid storage and formation and dissociation of the E-I complex (HASSAN et al 1976c). The depression of brain acetylcholinesterase remained fairly constant throughout the whole intoxication period (at 90 ppm).

SGPT and SAP are known to be valuable in studing exposure to hepatotoxic chemicals. Activity of both enzymes may be elevated with the early recognition of toxic hepatitis (DAVIDSOHN and HENRY 1974). Elevated SGPT values observed in this study are suggestive of a mild degree of hepatocellular injury. Increased SAP values were observed only after 3 weeks and the activity dropped below normal levels during the succeeding weeks. This may be attributed to inhibition of the circulating enzyme by Leptophos and/or its metabolites. In this connection, it is worth mentioning that elevated SGPT and SAP activities were observed in Leptophos-exposed humans (HASSAN and ABOU-ZEID 1976).

The reabsorption of phosphorus remained unaffected indicating that the renal tubules did not suffer any measurable changes which may be related to Leptophos exposure. However, the urea level in the blood was increased after 12 weeks of exposure and returned to the control values six weeks after cessation of treatment ; suggesting a temporary reversible interference with the glomerular filteration. Studies on humans exposed to Leptophos revealed that the TPR was not affected and that glomerular filteration was temporarily interfered with (HASSAN and ABOU-ZEID 1976).

Urinary vanillyl mandelic acid was determined to reflect possible changes in sympathoadrenergic activity. Exposure to pesticide chemicals may increase this activity (HASSAN and CUETO 1970) (HASSAN 1971). However, exposure to Leptophos did not seem to have interfered with the adrenergic mechanisms.

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Acknowledgement : The authors wish to acknowledge Mr. M. El-Fahl for his skilful technical assistance.