

Effects of an Organophosphorous Insecticide on the Cholinesteratic Activities of *Bufo arenarum* (H)

J. A. Guzman and T. Guardia

Laboratory of Pharmacology, University of San Luis, San Luis, Argentine

Several studies have been done about the toxic effects of Parathion; these effects were provoked by injections or by adding the insecticide to the diet of laboratory animals.

Research work with animals that are directly or indirectly in contact with vegetables and soils treated with those compounds are less frequent, (OBERHEU et al. 1970, DEUBERT & GRAY 1976). We are interested in the study of the effect of organophosphorous insecticide (Parathion) on the local fauna of S. Luis, Argentine.

The first part of this research involves; survival and modification of the cholinesteratic activity of B. arenarum in a Parathion fumigated medium.

MATERIAL AND METHODS

The brain, liver, erythrocyte and plasma cholinesteratic activity were studied (AUGUSTINSSON 1957) in adult toads of both sexes during hibernation (April-June, winter) and during the mating season (November-December, summer). Ninety-six animals grouped in three lots were used for each period. Lot I: Toads kept constantly throughout the experiment (45 days) in "habitat" fumigated with the insecticide. Lot II: Two hours on the fumigated medium and transferred to a normal medium. Control Lot: Animals kept under normal conditions. All animals were placed in a previously prepared 2 x 1 m. "habitat" and kept at $16 \pm 2^{\circ}\text{C}$ during hibernation and at $22 \pm 3^{\circ}\text{C}$ during mating season. At the same time the cholinesteratic activity was determined in the tissues mentioned above of four animals of each lot. They were kept in the respective "habitats" during 2, 6, 10, 18, 27, 36, 40 and 45 days.

RESULTS

Toads in Lots I and II did not show changes in color, motility, or visible traces of intoxication. The greatest decrease in cholinesteratic activity was observed in Lots I and II (2 hrs. or 6 days), (see Tables 1 to 4). In both periods, the plasma cholinesteratic activity was decreased the most (less than 86% of the normal value).

T A B L E 1
CHOLINESTERASE ACTIVITY OF PLASMA AND ERYTHROCYTES OF B. ARENARUM (H)
DURING HIBERNATION, IN "HABITAT" WITH PARATHION (0,10 ml/m²)

T I M E	P L A S M A			E R Y T H R O C Y T E S		
	(μ mol.Ach. hydrol./ml/min./25°C			(μ mol.Ach. hydrol./ml./min./25°C)		
	CONTROL ^a	LOT I ^b	LOT II ^c	CONTROL ^a	LOT I ^b	LOT II ^c
2 hours	1,60 ⁺ -0,046	0,22 ⁺ -0,032	0,36 ⁺ -0,06	2,35 ⁺ -0,10	1,80 ⁺ -0,18	1,60 ⁺ -0,10
6 days	1,12 ⁺ -0,060	0,29 ⁺ -0,060	1,72 ⁺ -0,08	2,25 ⁺ -0,12	1,25 ⁺ -0,17	1,50 ⁺ -0,11
10 "	1,13 ⁺ -0,050	0,21 ⁺ -0,030	1,07 ⁺ -0,07	1,90 ⁺ -0,22	1,91 ⁺ -0,04	1,50 ⁺ -0,16
18 "	1,17 ⁺ -0,041	0,41 ⁺ -0,07	0,78 ⁺ -0,04	2,20 ⁺ -0,21	2,50 ⁺ -0,22	1,56 ⁺ -0,03
24 "	1,11 ⁺ -0,011	0,42 ⁺ -0,037	0,80 ⁺ -0,03	2,10 ⁺ -0,08	2,25 ⁺ -0,14	1,85 ⁺ -0,04
36 "	1,11 ⁺ -0,011	0,45 ⁺ -0,030	0,85 ⁺ -0,07	2,00 ⁺ -0,00	1,90 ⁺ -0,11	1,91 ⁺ -0,04
40 "	1,16 ⁺ -0,040	0,48 ⁺ -0,32	0,80 ⁺ -0,05	2,10 ⁺ -0,08	1,80 ⁺ -0,07	1,85 ⁺ -0,07
45 "	1,06 ⁺ -0,040	0,48 ⁺ -0,40	0,80 ⁺ -0,08	1,90 ⁺ -0,08	1,90 ⁺ -0,08	1,80 ⁺ -0,05

a - Normal toads, in "habitat" without insecticide.

b - Until 45 days and c- 2 hours, in fumigated with Parathion.

- Each value represents the means of four animals ⁺ - SE

T A B L E 2

CHOLINESTERASIC ACTIVITY OF BRAIN AND LIVER OF B. ARENARUM (H) DURING
HIBERNATION, IN "HABITAT" WITH PARATHION (0,10 ml./m²)

T I M E	B R A I N			L I V E R		
	(μmol.Ach.hydrol.g.wet.wt./min./25°C)			(μmol.Ach.hydrol.g.wet.wt./min./25°C)		
	CONTROL ^a	LOT I ^b	LOT II ^c	CONTROL ^a	LOT I ^b	LOT II ^c
2 hours	7,18 ⁺ -0,24	3,93 ⁺ -0,28	4,12 ⁺ -0,18	4,62 ⁺ -0,28	4,00 ⁺ -0,19	3,87 ⁺ -0,13
6 days	6,68 ⁺ -0,25	6,00 ⁺ -0,15	8,87 ⁺ -0,18	4,50 ⁺ -0,45	3,18 ⁺ -0,32	5,41 ⁺ -0,37
10 "	6,56 ⁺ -0,22	6,38 ⁺ -0,25	8,18 ⁺ -0,40	3,56 ⁺ -0,13	5,12 ⁺ -0,32	3,62 ⁺ -0,18
18 "	6,62 ⁺ -0,18	6,12 ⁺ -0,18	7,68 ⁺ -0,33	4,00 ⁺ -0,26	3,31 ⁺ -0,05	4,25 ⁺ -0,26
24 "	7,18 ⁺ -0,10	7,06 ⁺ -0,31	7,93 ⁺ -0,22	4,81 ⁺ -0,55	4,93 ⁺ -0,28	4,87 ⁺ -0,31
36 "	7,18 ⁺ -0,20	6,31 ⁺ -0,29	6,75 ⁺ -0,19	4,75 ⁺ -0,19	4,37 ⁺ -0,10	5,12 ⁺ -0,31
40 "	7,31 ⁺ -0,10	6,75 ⁺ -0,31	7,00 ⁺ -0,20	4,62 ⁺ -0,22	4,25 ⁺ -0,12	4,75 ⁺ -0,22
45 "	7,25 ⁺ -0,37	7,25 ⁺ -0,45	7,15 ⁺ -0,19	4,63 ⁺ -0,33	4,43 ⁺ -0,32	4,70 ⁺ -0,18

a - Normal toads in "habitat" without insecticide.

b - Until 45 days and c-2 hours, in fumigate medium with Parathion.

Each value represents the means of four animals $\pm$ SE

T A B L E 3

CHOLINESTERASIC ACTIVITY OF BRAIN AND LIVER OF B. ARENARUM DURING MATING
SEASON (SUMMER), IN "HABITAT" WITH PARATHION (0,1 ml/m²)

T I M E	B R A I N			L I V E R		
	(μmol.Ach.hydrol. g.wet.wt./min./25°C) (% mol.Ach.hydrol.g.wet.wt./min./25°C)					
	CONTROL ^a	LOT I ^b	LOT II ^c	CONTROL ^a	LOT II ^b	LOT II ^c
2 hours	6,37 ⁺ -0,59	4,87 ⁺ -0,48	6,93 ⁺ -0,43	6,85 ⁺ -0,21	4,37 ⁺ -0,36	6,11 ⁺ -0,13
6 days	6,58 ⁺ -0,38	5,47 ⁺ -0,50	6,62 ⁺ -0,77	6,31 ⁺ -0,50	5,12 ⁺ -0,25	6,56 ⁺ -0,49
10 "	6,62 ⁺ -0,38	6,06 ⁺ -0,28	6,06 ⁺ -0,28	7,31 ⁺ -0,44	5,62 ⁺ -0,62	8,25 ⁺ -0,34
18 "	6,68 ⁺ -0,16	6,81 ⁺ -0,47	6,82 ⁺ -0,70	6,00 ⁺ -0,30	5,43 ⁺ -0,51	8,32 ⁺ -0,74
27 "	7,68 ⁺ -0,38	7,43 ⁺ -0,63	8,06 ⁺ -0,18	7,06 ⁺ -0,56	4,37 ⁺ -0,60	5,93 ⁺ -0,54
40 "	7,06 ⁺ -0,72	6,26 ⁺ -0,45	6,87 ⁺ -0,69	6,43 ⁺ -0,84	5,06 ⁺ -0,54	5,06 ⁺ -0,29
45 "	7,18 ⁺ -0,38	5,81 ⁺ -0,44	6,25 ⁺ -0,21	6,37 ⁺ -0,84	5,18 ⁺ -0,20	6,62 ⁺ -0,56

a - Normal toads in "habitat" without insecticide.

b - Until 45 days and c-2 hours, in fumigate medium with Parathion.

Each value represent the means of four animals ⁺ SE.

T A B L E 4

CHOLINESTERASE ACTIVITY OF PLASMA AND ERYTHROCYTES OF B. ARENARUM (H)
DURING MATING SEASON (SUMMER), IN "HABITAT" WITH PARATHION (0,1 ml/m²)

T I M E	P L A S M A			E R Y T H R O C Y T E S		
	(μmol. Ach. hydrol./ml./min./25°C)			(μmol. Ach. hydrol./ml./min./25°C)		
	CONTROL ^a	LOT I ^b	LOT II ^c	CONTROL ^a	LOT I ^b	LOT II ^c
2 hours	1,51 ⁺ -0,13	0,73 ⁺ -0,08	0,88 ⁺ -0,08	2,18 ⁺ -0,32	1,90 ⁺ -0,18	2,25 ⁺ -0,19
6 days	1,72 ⁺ -0,13	0,52 ⁺ -0,08	1,45 ⁺ -0,10	2,95 ⁺ -0,38	1,85 ⁺ -0,12	2,37 ⁺ -0,33
10 "	1,63 ⁺ -0,03	0,43 ⁺ -0,04	1,73 ⁺ -0,07	2,60 ⁺ -0,33	2,40 ⁺ -0,60	2,15 ⁺ -0,24
18 "	1,46 ⁺ -0,09	0,41 ⁺ -0,03	1,99 ⁺ -0,09	2,60 ⁺ -0,89	2,55 ⁺ -0,33	3,45 ⁺ -0,57
27 "	1,85 ⁺ -0,14	0,60 ⁺ -0,03	1,83 ⁺ -0,10	2,40 ⁺ -0,33	2,33 ⁺ -0,35	2,05 ⁺ -0,34
36 "	1,62 ⁺ -0,05	0,47 ⁺ -0,05	1,85 ⁺ -0,06	2,13 ⁺ -0,28	2,25 ⁺ -0,46	2,03 ⁺ -0,18
40 "	1,73 ⁺ -0,08	0,51 ⁺ -0,08	1,76 ⁺ -0,10	2,45 ⁺ -0,29	2,85 ⁺ -0,38	2,25 ⁺ -0,31
45 "	1,76 ⁺ -0,06	0,47 ⁺ -0,02	1,65 ⁺ -0,22	2,60 ⁺ -0,44	2,35 ⁺ -0,37	2,30 ⁺ -0,35

a - Normal toads in "habitat" without insecticide.

b - Until 45 days and c-2 hours, in fumigated medium with Parathion.

Each value represent the means of four animals ⁺ SE.

Cholinesteratic activity is quickly recovered when the animals are transferred to a Parathion-free medium. (Lot II) When toads are constantly subjected to insecticide-treated medium (Lot I), the plasma cholinesterase, both in winter and summer, remains significantly decreased throughout the experiment (45 days); on the other hand, that of the other tissues tends to reach normal values.

In summer the cholinesteratic activity is usually higher than in winter. The higher significant difference is observed in liver.

DISCUSSION

The toxic effects of organophosphorous anticholinesteratic insecticides are mainly caused by the inhibition of the true cholinesterase (HOBINGGER 1968). For intoxication traces to appear it is necessary that it be inhibited by more than 50% (GOODMAN & GILMAN 1970, AWAD 1972). This explains the absence of intoxication traces in the toad, since the greatest decrease of the brain cholinesteratic activity was 46% and 23.6% in winter and summer periods.

DIGGLES and GAGE(1951) have shown that the activity "in vivo" of Parathion is due to its active metabolite Paraoxon.

This transformation would also take place in the toad; however, there would also be the possibility of this transformation taking place in the fumigated medium and being absorbed through the skin directly as Paraoxon. According to GANELIN & CUETO(1964)the humidity and temperature of the environment favor this transformation.

Also, the encephalic cholinesterase would have little sensitivity to the insecticide (ZAHAVI et al. 1971). Regarding the marked and lasting decrease of the cholinesteratic activity in plasma, it may be attributed mainly to the fact that pseudocholinesterases are more sensitive to most phosphorous insecticides(HOBINGGER 1968) and that the half life of Parathion is 120 days,(GANELIN & CUETO 1964).

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