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Evaluation of Fenitrothion Toxicity to *Rana temporaria* L.

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It has been demonstrated in investigations on quails that fenitrothion causes important changes in the blood morphology of these birds, appearing much earlier in males than in females (Gromysz-Kałkowska and Szubartowska 1991). According to the authors, this difference is connected with the high endogenous testosterone level in males, causing a quicker rate of fenitrothion transformation to more toxic metabolites. It may, therefore, be supposed that animals with a low metabolic rate may be resistant to the toxic influence of fenitrothion. It seemed, therefore, useful to determine the toxicity of this substance in frogs by establishing the LD_{50} value. The question arises, moreover, whether animals in which sexual activity is limited to a relatively short period of seasonal reproduction, show different reactions to the poison independent of sex. For solving these problems it was decided to evaluate the influence of fenitrothion on the blood picture of the frog Rana temporaria L. males and females in the autumn-winter season.

MATERIALS AND METHODS

The experiments comprised 132 Rana temporaria L. frogs (66 males and 66 females) weighing 21-56 g. Animals of each sex were divided into 6 groups of 11 individuals, one control and 5 experimental ones. Owadofos liquid 50 was used as the sourcce of fenitrothion. Fenitrothion, as an aqueous suspension, was administered to frogs at 100, 500, 1000, 2000, and 3000 mg/kg of b.w. via the dorsal lymphatic heart.

The LD_{50} was determined using the meethod of Trevan and that of Reed and Muench (Jeske 1955).

Cholinesterase activity (μM/ml/hr) was determineed according to Hestrin's method as modified by Juszkiewicz (Juszkiewicz 1966).

Three hours after administration of the pesticide the

erythrocyte (RBC) and leucocyte (WBC) counts were determined by the chamber method in Natt-Herrick's diluting solution (Natt and Herrick 1952), the haemoglobin level (Hb) by the cyanmethaemoglobin method of Drabkin and haematocrit (Hct) by the micromethod. The erythrocyte indices were calculated: mean RBC volume (MCV), mean haemoglobin content in the erythrocyte (MCH), and mean haemoglobin concentration in the erythrocyte (MCHC) after Wintrobe (1956). Reticulocytes (R) were counted per 1000 red blood cells on smears. These smears were prepared from the fresh blood samples stained immediately after collection with cresyl brilliant blue (Pinkiewicz 1971). The percentage composition of the WBC was determined on smears stained by Pappenheim's method. The number of erythroblasts (Et) per 1000 red blood cells was determined on the same preparations. On the basis of the leucocyte count and their percentage composition the absolute values of the particular forms of white blood cells were calculated. The leucocyte index (WBCI) was calculated from the formula of Stankiewicz (1973).

The results were subjected to statistical analysis by Student's t test for independent data. A statistically significant difference was assumed $p < 0.05$ (Oktaba and Niedokos 1980).

RESULTS AND DISCUSSION

According to Trevan's method the LD_{50} of fenitrothion in male and female frogs was 2400 and 2220 mg/kg b.w., respectively. The LD_{50} according to the method of Reed and Muench was 2342 and 2182 mg/kg b.w., respectively.

The LD_{50} values indicate that fenitrothion is less toxic to frogs than to quail (55.6 mg/kg b.w., Grün et al.1982), and mammals (242-740 mg/kg b.w., Gaines 1969, Rusiecki 1973, Byrdy et al. 1976, Kagan et al. 1977).

Edery and Schatzberg-Porath attribute the low susceptibility of frogs to organophosphorus insecticides to the very rapid detoxication. The cause of the resistivity of frogs to poisoning may be the lower affinity of cholinesterase to the organophosphorus inhibitors (Kossakowski 1974). It should also be noted in frogs hypoxia, characteristic for organophosphorus pesticide poisoning is probably less intensive because of their skin respiration. Moreover, the toxicity of pesticides depends on the rate of their metabolic transformation and the kind of metabolites formed (Przeździecki 1976). One may, therefore, suppose that the metabolic processes in frogs are slow and the metabolites formed are of low toxicity.

The activity of cholinesterase was lower in frogs after doses of the fenitrothion (Table 1).

Table 1. The activity of cholinesterase in the frogs after poisoning with fenitrothion

Doses of fenitrothion in mg/kg b.w.	♂ $\bar{x} \pm$ SE	♀ $\bar{x} \pm$ SE
Control	54.87 ± 1.50	54.69 ± 1.97
100	48.79 ± 1.04[c]	52.32 ± 1.12
500	44.47 ± 0.63[d]	44.66 ± 0.76[d]
1000	36.32 ± 1.02[d]	39.13 ± 0.60[d]
2000	32.34 ± 0.80[d]	34.73 ± 0.74[d]

[c] $p < 0.01$; [d] $p < 0.001$

Similar changes were observed in frogs after acute poisoning with Foschlor. In frogs after repeated contacts with a therapeutic dose of this pesticide no changes were noted in the ChE level (Szubartowska et al. 1983).

The administration of fenitrothion resulted in a dose-dependent fall in erythrocyte counts in both male and female frogs (Table 2, Fig.1).

Similar results were obtained with fenitrothion in the pigeon (Mandal and Lahiri 1985) and in the quail (Gromysz-Kałkowska and Szubartowska 1991). According to the former authors, the fall in red cell count is caused by haemorrhaging produced by lesions in the vascular wall. The fall in red cell count in the frog was also attributed to vascular haemorrhaging.

The haematocrit and haemoglobin values were lower in treated than control animals (Table 2, Fig.1). The decrease is believed to be result of haemorrhaging and compensatory dilution (make up of fluid volume) from body tissues.

The content of reticulocytes and erythroblasts increased after the 100 mg/kg b.w. dose and decreased after higher doses (Table 2, Fig.2). At the 100 mg/kg b.w. dose the increase offset the loss of red blood cells produced by haemorrhaging, but the haemopoietic system was unable to compensate for these losses at higher doses of fenitrothion.

A decrease in erythrocyte volume, MCV, at doses above 100 mg/kg b.w. occurred and is believed to be defence reaction to the toxic agent. The reduction in volume results in a more rounded cell, thus limiting the area exposed to the agent. Similar changes were noted in frogs after application of trichlorfon and Ekatin (Gromysz-Kałkowska and Szubartowska 1986, Szubartowska 1990).

Table 2. Values of the red blood cell system in frogs after poisoning with fenitrothion

Parameters	Sex	Doses of fenitrothion in mg/kg b.w.				
		Control	100	500	1000	2000
		$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
RBC in mln/mm³	♂	0.579 ± 0.013	0.599 ± 0.018	0.434 ± 0.039^{c}	0.377 ± 0.042^{c}	0.431 ± 0.048^{b}
	♀	0.609 ± 0.028	0.609 ± 0.020	0.446 ± 0.033^{b}	0.382 ± 0.035^{c}	0.437 ± 0.028^{c}
Hct in %	♂	38.32 ± 0.383	30.04 ± 1.306^{c}	26.18 ± 1.419^{c}	30.00 ± 2.102^{c}	29.09 ± 2.934^{b}
	♀	35.91 ± 0.858	31.09 ± 1.386^{b}	29.82 ± 1.320^{c}	29.18 ± 1.439^{c}	29.45 ± 1.894^{b}
Hb in g%	♂	9.60 ± 0.392	7.68 ± 0.495^{b}	5.50 ± 0.544^{c}	7.05 ± 0.597^{c}	7.04 ± 0.747^{b}
	♀	8.47 ± 0.493	7.61 ± 0.439	7.51 ± 0.751	7.62 ± 0.558	7.16 ± 0.495
MCV in μ³	♂	663.75 ± 10.40	502.54 ± 19.12^{c}	623.11 ± 35.43	838.35 ± 52.06^{b}	684.33 ± 15.60$_{a}$
	♀	601.31 ± 29.69	512.67 ± 21.12^{a}	691.52 ± 42.78	838.51 ± 103.5^{a}	675.83 ± 10.20^{a}
MCH in pg	♂	167.25 ± 8.720	128.03 ± 6.792^{b}	126.77 ± 7.594^{b}	196.80 ± 16.31	164.44 ± 1.817
	♀	140.10 ± 7.566	125.31 ± 6.383	172.20 ± 16.95	217.62 ± 28.01^{d}	163.46 ± 1.659^{b}
MCHC in %	♂	25.12 ± 1.111	25.35 ± 0.342	20.67 ± 1.155^{d}	23.34 ± 0.826	24.10 ± 0.322
	♀	23.61 ± 1.247	24.43 ± 0.818	24.70 ± 1.676	25.85 ± 0.735	24.22 ± 0.282
Rt in ‰	♂	168.00 ± 2.800	180.00 ± 5.000^{a}	102.00 ± 3.400^{c}	124.00 ± 2.600^{c}	155.00 ± 7.600
	♀	174.00 ± 3.400	184.00 ± 5.000	97.00 ± 3.300^{c}	119.00 ± 4.400^{c}	163.00 ± 3.100$_{a}$
Et in ‰	♂	7.82 ± 0.569	23.27 ± 0.895^{c}	15.00 ± 0.588^{c}	7.82 ± 0.464	4.64 ± 0.432^{c}
	♀	8.09 ± 0.579	22.00 ± 0.915^{c}	13.82 ± 0.724^{c}	8.45 ± 0.455	4.09 ± 0.343^{c}

$^{a}p < 0.05$, $^{b}p < 0.02$, $^{c}p < 0.01$, $^{d}p < 0.001$

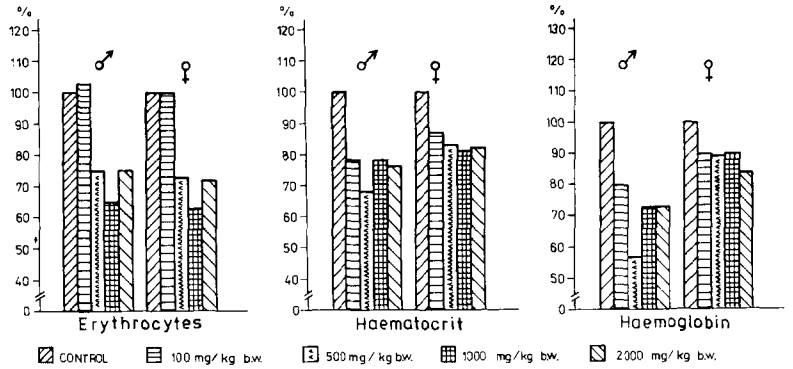

Figure 1. Effect of fenitrothion on the erythrocyte number, haematocrit value and haemoglobin level in the frogs

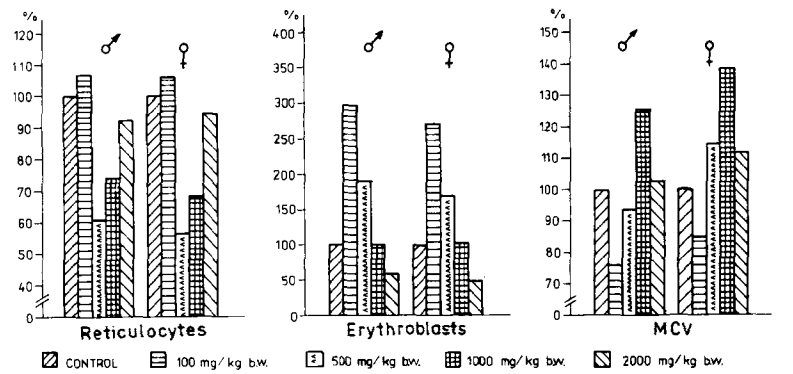

Figure 2. Effect of fenitrothion on the reticulocytes and erythroblasts content and the MCV in the frogs

The enlarged erythrocyte volume after the 1000 mg/kg b.w. dose appeared to be a result of the rounding process giving rise to erythrocyte swelling. The swollen cells are believe to be eliminated after bursting as bare nuclei were observed in the blood smears of this group. Similar effects were observed in frogs poisoned with other organophosphorus agents such as trichlorfon and Ekatin (Gromysz-Kałkowska and Szubartowska 1986, Szubartowska 1990).

After the two lower doses (100 and 500 mg/kg b.w.), the leucocyte count was depressed and lymphopenia and neutrophilic granulocytosis were observed (Table 3, Fig.3).

These changes are the result of thee lytic influence of the pesticide on the cell membrane. Lymphocytes seem to be particularly susceptible to fenitrothion resulting

Table 3. Values of the white blood cell system in the frogs after poisoning with fenitrothion

Parameters in thous/mm³	Sex	Doses of fenitrothion in mg/kg b.w. Control $\bar{x} \pm SE$	100 $\bar{x} \pm SE$	500 $\bar{x} \pm SE$	1000 $\bar{x} \pm SE$	2000 $\bar{x} \pm SE$
Leucocytes	♂	12.83 ± 0.943	10.17 ± 0.327^{d}	12.21 ± 1.253	13.51 ± 0.911	15.69 ± 1.224^{a}
	♀	12.35 ± 0.990	9.93 ± 0.456^{a}	12.17 ± 1.016	14.37 ± 1.209	16.23 ± 1.258^{a}
Neutrophils	♂	3.56 ± 0.389	4.53 ± 0.243^{a}	5.78 ± 0.682^{d}	2.74 ± 0.320	3.60 ± 0.434
	♀	2.69 ± 0.182	4.63 ± 0.217^{c}	5.40 ± 0.419^{c}	2.95 ± 0.333	3.62 ± 0.348^{a}
Eosinophils	♂	2.61 ± 0.241	2.06 ± 0.190	2.89 ± 0.378	3.60 ± 0.253^{d}	3.79 ± 0.522
	♀	2.54 ± 0.263	1.93 ± 0.170	3.30 ± 0.479	4.30 ± 0.476^{b}	4.12 ± 0.467^{b}
Basophils	♂	0.358 ± 0.062	0.057 ± 0.022^{c}	0.121 ± 0.042^{b}	0.188 ± 0.047^{a}	0.089 ± 0.040^{b}
	♀	0.295 ± 0.105	0.067 ± 0.021^{a}	0.095 ± 0.034	0.138 ± 0.039	0.095 ± 0.021
Lymphocytes	♂	6.07 ± 0.594	3.01 ± 0.173^{c}	3.01 ± 0.365^{c}	6.63 ± 0.468	7.79 ± 0.620
	♀	6.59 ± 0.713	2.88 ± 0.146^{c}	3.08 ± 0.291^{c}	6.71 ± 0.530	7.89 ± 0.715
Monocytes	♂	0.249 ± 0.066	0.530 ± 0.070^{b}	0.398 ± 0.048	0.320 ± 0.093	0.427 ± 0.108
	♀	0.248 ± 0.031	0.407 ± 0.039^{b}	0.293 ± 0.067	0.264 ± 0.058	0.505 ± 0.128
WBCI	♂	1.071 ± 0.078	1.938 ± 0.133^{c}	2.687 ± 0.185^{c}	0.950 ± 0.056	0.938 ± 0.067
	♀	0.848 ± 0.052	2.028 ± 0.084^{c}	2.754 ± 0.276^{c}	1.068 ± 0.067^{d}	0.993 ± 0.076

$^{a}p < 0.05$, $^{b}p < 0.02$, $^{c}p < 0.01$, $^{d}p < 0.001$

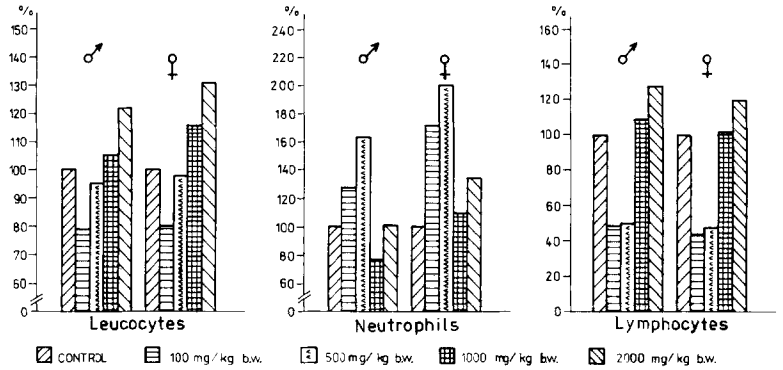

Figure 3. Effect of fenitrothion on the leucocytes, neutrophils and lymphocytes number in the frogs

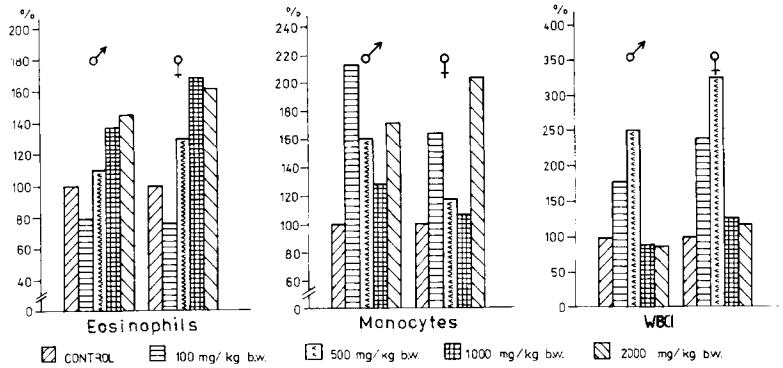

Figure 4. Effect of fenitrothion on the eosinophils and monocytes number and the white blood cell index value in the frogs

in significantly diminished numbers. This mechanism was described in the pigeon by Mandal and lahiri (1985). The increased neutrophil count, on the other hand, is probably the result of intensive granulopoiesis stimulated by the disintegration products of the blood cells (Bogdanik 1963, Stankiewicz 1973, Gromysz-Kałkowska et al. 1981).

After the two highest doses of fenitrothion (1000 and 2000 mg/kg b.w.) an increase in leucocyte count was observed with slight neutrophilic granulocytosis, marked lymphocytosis and eosinophilia (Table 3, Figs 3 and 4).

These changes are seemingly connected with the stressful action of fenitrothion.

Marked monocytosis was noted after all pesticide doses

(Table 3, Fig.4). It is probably the result of enhanced activity of reticuloendothelial system in response to the pathomorphological changes in the tissues (Stankiewicz 1973).

Fenitrothion in 100 and 500 mg/kg b.w. doses caused a marked increase in the leucocyte index, whereas in 1000 and 2000 mg/kg b.w. ddoses it only slightly affected this value (Table 3, Fig.4).

It may be affirmed on the basis of the changes in the leucocyte system that the reactions characteristic for stress are in frogs less pronounced than in birds intoxicated with the same pesticide.

Similar values of LD_{50} and almost the same changes in ChE activity and morphology of the blood of males and females indicate, that in autumn-winter season reaction of the frogs to poison is independent of sex.

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