Effect of the insecticide Sumithion (Fenitrothion) on embryonic development in a frog

K. R. Pawar and M. Katdare[1]

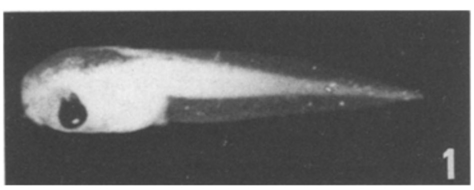
Department of Zoology, University of Poona, Pune-411 007 (India), May 27, 1982

Summary. The toxic effects of Sumithion on developing embryos of a frog, *Microhyla ornata*, were investigated for a period of 96 h. Abnormalities observed were loss of balance, abnormal behavior, curvature of the body axis, poor pigmentation, feeble circulation in the tail, blisters on the body, distension of body cavities and retarded growth.

The organophosphorous insecticides are highly effective for the control of mosquitoes and agricultural pests in many parts of the world[2,3]. Since these insecticides are nonpersistent, they are used repeatedly, and the danger to nontarget organisms such as fish and amphibians, which are the natural enemies of the insect larvae, becomes greater. The highly toxic effects of some organophosphorous insecticides on fish have already been shown[4,5]. The aim of this investigation was to study the toxic effects of the insecticide Sumithion, which is much used locally on the embryonic development of the frog, *Microhyla ornata* (Dumeril and Bibron).

Materials and methods. Naturally-fertilized eggs of *M. ornata* were collected from natural ponds and de-jellied, keeping the vitelline membrane intact. Groups of 20 embryos at the yolk plug stage were transferred to 10 cm diameter glass dishes containing 100 ml aged tap water at 25 °C. A stock solution of technical Fenitrothion 97% purity (0,0-dimethyl-0-(3methyl-4-nitrophenyl) phosphorothioate) which was supplied by Rallis India Ltd, was prepared in acetone. Appropriate amounts were taken from the stock solution to prepare 1–13 ppm concentrations of the insecticide in aged tap water. The treatment was carried out for a period of 96 h. During this test period the concentrations were renewed every 24 h to maintain relatively constant exposure to the given concentration. In all 140–150 embryos were exposed to each concentration. Embryos in aged untreated tap water served as master controls, and embryos in aged tap water containing the amount of acetone used for the higher concentrations of insecticide served as solvent controls. All the embryos were observed at 24-h intervals, and the percentage of abnormalities and the mortality was calculated. The gross morphological changes in the embryos were photographed.

Results. Embryos of *Microhyla* growing in the master control medium showed muscular movement in about 24 h.



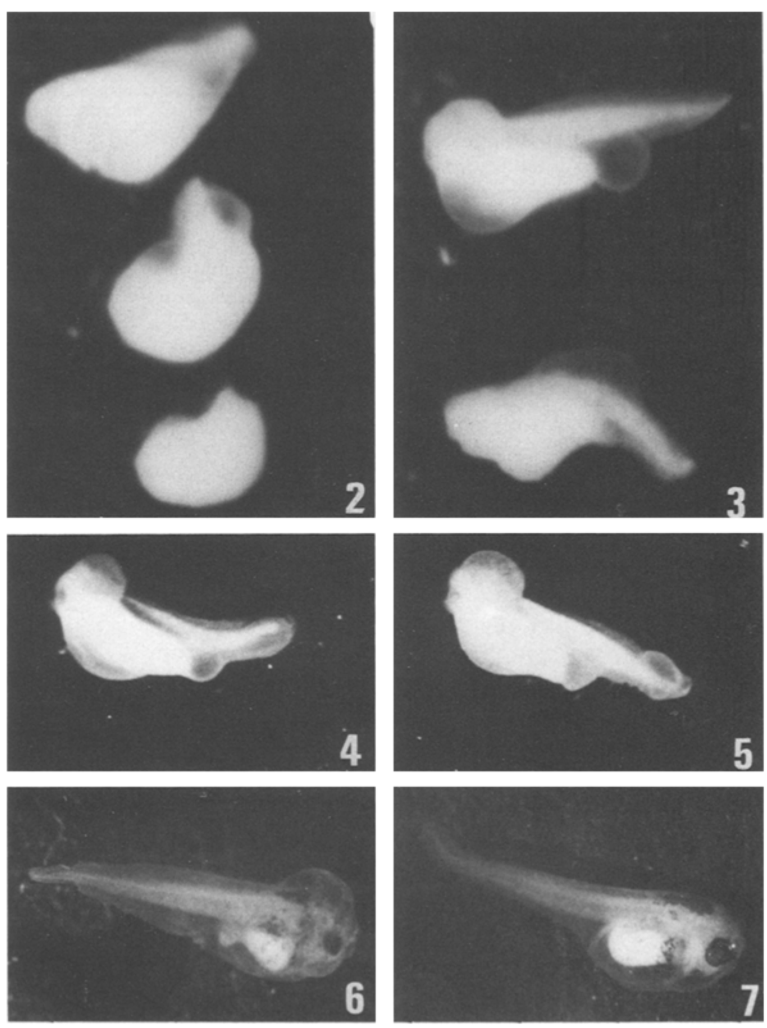


Figure 1. Control tadpole at 96 h showing well developed eyes, head, tail and pigmentation.

Figure 2. Experimental embryos exposed to 9, 11 and 13 ppm concentrations. Note the total lack of differentiation of the embryos.

Figure 3. Experimental tadpoles (7 ppm) showing blisters, curvature of the body axis and lack of pigmentation.

Figures 4 and 5. Experimental tadpoles (5 ppm). Note the blisters on the head and curved tail.

Figures 6 and 7. Experimental tadpoles (5 ppm) showing blisters and abnormal distension of body cavities, uneven distribution of pigmentation and curved tail.

The effect of Sumithion on the development of the frog *Microhyla ornata* at yolk plug stage

Concentration (ppm)*	Duration of the treatment 24 h A/S**	%	48 h A/S	%	72 h A/S	%	96 h A/S	%	Total dead	% dead after 96 h
0	1/150	0.66	1/150	0.66	1/150	0.66	3/148	2.62	2/150	1.33
1	1/137	0.72	0/134	0.00	2/134	1.49	5/134	3.73	6/140	4.28
3	87/139	62.58	94/138	68.11	98/138	71.01	87/120	72.50	20/140	14.28
5	119/148	80.37	120/125	96.00	101/103	98.05	43/43	100.00	107/150	71.33
7	147/147	100.00	118/118	100.00	81/81	100.00	18/18	100.00	130/150	86.66
9	150/150	100.00	84/84	100.00	51/51	100.00	15/15	100.00	136/150	90.06
11	149/149	100.00	110/110	100.00	17/17	100.00	5/5	100.00	145/150	96.66
13	150/150	100.00	-	-	-	-	-	-	150/150	100.00

*Number treated at each concentration 150 except at concentrations 1 and 3 ppm where it was 140. **Number of abnormal/number of surviving embryos and percent abnormal. Abnormalities include head, trunk and tail defects, abnormal behavior, poor pigmentation and retarded growth.

All embryos hatched as tadpoles in about 48 h with well developed suckers, eyes, tail and pigmentation. By the end of 96 h tadpoles appeared as shown in figure 1. No abnormal development was observed in 150 embryos, apart from 3 which failed to develop, and disintegrated. Embryos from solvent controls showed normal development.

The insecticide-treated embryos showed various abnormalities and mortality rates (figs 2-7 and table) which were dose-dependent.

At a concentration of 1 ppm, abnormalities and mortality were very low, but at a concentration of 13 ppm there was almost total mortality of the embryos within about 24 h with severe defects, and most of the embryos failed to hatch. The most common abnormality observed was the presence of blisters on the body, which appeared at concentrations of 3 ppm and above, and remained up to 96 h. The strongly affected tadpoles with 2-3 blisters showed abnormal behavior and loss of balance (figs 2-5). The abnormalities like curvature of body axis, poor body pigmentation, feeble blood circulation and retarded growth were prominent at concentrations of 5 ppm and above (figs 4 and 5). Some apparently normal tadpoles (about 30%, table) exposed to a concentration of 5 ppm showed loss of balance and circular swimming. Presence of blisters and distended body cavities, poor pigmentation and feeble blood circulation was also observed in these surviving tadpoles (figs 6 and 7). Surviving highly abnormal tadpoles subjected to concentrations of 5 ppm and above failed to revive when returned to normal aged tap water.

Discussion. Some of the organophosphorous insecticides have been shown to cause developmental abnormalities in fish, reptiles, birds and mammals[4-8]. The present investigation indicated that Sumithion is also teratogenic and embryotoxic for developing frog embryos. The loss of balance, abnormal behavior and curvature of the body axis may have resulted from neurotoxic effects[9,10]. Our finding that embryos exposed to 1 and 3 ppm showed loss of balance and abnormal behavior, though they were morphologically normal, could indicate that the insecticide has a neurotoxic effect.

The loss of balance and abnormal behavior observed in embryos exposed to 5 ppm and above could be a cumulative effect of neurotoxicity, curved body axis and poor development. The blisters and distension of body cavities observed might be due to a disturbance in the osmoregulatory mechanism[10,11].

It has been reported that organophosphorous insecticides induced inhibition of the ion-transporting enzyme ATPase in fishes[12]; this might be one of the reasons for the observed interference with development, and the fluid imbalance in frog embryos exposed to insecticides. This could be the reason for the overall poor development of the embryos exposed to higher concentrations. The lack of pigmentation or delay in the development of pigment indicated that this inhibition is partial; in the case of embryos treated with lower concentrations, pigmentation was delayed as compared to controls. In higher concentrations, total absence of pigment may be due to inhibition of melanogenesis[9,13]. Weis and Weis[14] have reported that insecticides cause developmental arrest prior to the initiation of heart-beat, resulting in circulatory failure which could be the reason for poor blood circulation in the tadpoles.

With the present environmental contamination by pesticides, other synthetic compounds, chemicals and natural products, many of which are teratogenic, carcinogenic or mutagenic[15], it is desirable to understand the exact mechanism of action of these chemicals (since there exists a wide variation in the sensivity and resistance of different species to different pesticides) in order to safeguard public health.

1 We wish to thank Dr V. Agnihothrudu and Mr P.R. Borde Rallis India Ltd for the gift of insecticide. KRP gratefully acknowledges UGC New Delhi for the award of 'Teacher Fellowship'. Reprint requests to M.K.
2 Muirhead-Thomson, R.C., Pesticides and fresh water fauna. Academic Press, New York 1971.
3 Duke, T.W., in: Pesticides in aquatic environments, p. 1. Ed. M.A.Q. Khan. Plenum Press, New York 1977.
4 Kaur, K., and Toor, H.S., Indian J. exp. Biol. *15* (1977) 193.
5 Paflitschek, R., Z. angew. Zool. *60* (1979) 143.
6 Mitchell, J., and Yntema, C.L., Anat. Rec. *175* (1973) 390.
7 Meiniel, R., and Autissier-Navarro, C., Acta Embryol. Morph. exp. *1* (1980) 33.
8 Robens, J.F., Toxic. appl. Pharmac. *15* (1969) 152.
9 Bancroft, R., and Prahlad, K.V., Teratology *7* (1973) 143.
10 Ghate, H.V., and Mulherkar, Leela, Indian J. exp. Biol. *18* (1980) 1094.
11 Chang, L.W., Reuhl, K.W., and Dudley, Jr, A.W., Envir. Res. *8* (1974) 82.
12 Mukhopadhyay, P.K., and Dehadrai, P.V., Indian J. exp. Biol. *18* (1980) 348.
13 Eppig, J.J., J. Embryol. exp. Morph. *24* (1970) 447.
14 Weis, P., and Weis, J.S., Teratology *10* (1974) 263.
15 Fishbein, L., in: Insecticide biochemistry and physiology, p. 555. Ed. C.F. Wilkinson. Plenum Press, New York 1976.

0014-4754/83/030297-02$1.50 + 0.20/0