Bull. Environ. Contam. Toxicol. 31, 170–176 (1983)

Effect of Malathion on Embryonic Development of the Frog *Microhyla ornata* (Dumeril and Bibron)

Kishore R. Pawar, H. V. Ghate, and Meena Katdare*

Department of Zoology, University of Poona, Pune-411007, India

The organophosphorous insecticides are employed against mosquito and agricultural pests in many parts of the world (Muirhead-Thomson 1971, Duke 1977). Since these insecticides are non-persistant, their repeated use becomes dangerous to non-target organisms like fish and amphibians, which are the natural enemies of insect larvae. Toxic and teratogenic effects of some organophosphorous insecticides on the embryonic development of fishes have already been shown (Kaur and Toor 1977, Paflitschek 1979). Anuran embryos and tadpoles are being used with increasing frequency in toxicological tests because of their sensitivity to a variety of chemicals (Cooke 1972, Bancroft and Prahlad 1973, Anderson and Prahlad 1976, Greenhouse 1976, Dutta and Mohanty-Hejmadi 1978, Ghate and Mulherkar 1980, Cooke 1981, Pawar and Katdare 1983). However, studies on toxic and teratogenic effects of organophosphorous insecticides on amphibian embryos are relatively few. Therefore the aim of this investigation was to study the toxic and teratogenic effects of the highly used insecticide malathion on the embryos of the frog Microhyla ornata.

MATERIALS AND METHODS

Naturally fertilized eggs of M. ornata were collected from a natural pond and dejellied keeping the vitelline membrane intact. Twenty embryos of the yolk-plug stage were transferred to each 10 cm diameter glass petri dish containing 100 ml of aged tap water at 25° C. Different concentrations 1 to 20 ppm of malathion (50 E. C.), O,O-Dimethyl-S-(1,2-Dicarbethoxyethyl) phosphorodithioate (Indiclay, Bombay) were prepared by adding appropriate quantity of the insecticide to the aged tap water. Instead of technical grade malathion, we specifically used formulated spray preparation of malathion, since the formulated material is always used in the fields. Aged tap water alone served as a control medium. All media were changed every twenty-four hours and the experiment was carried out for a total of ninety-six hours. In all, 100 embryos were exposed to each concentration of malathion. All the embryos were observed every 24 hours and the percent of adnormalities and mortality were recorded. The gross morphological changes in the embryos were observed under a dissecting binocular microscope. Representative embryos from control and experimental

*To whom correspondence should be addressed.

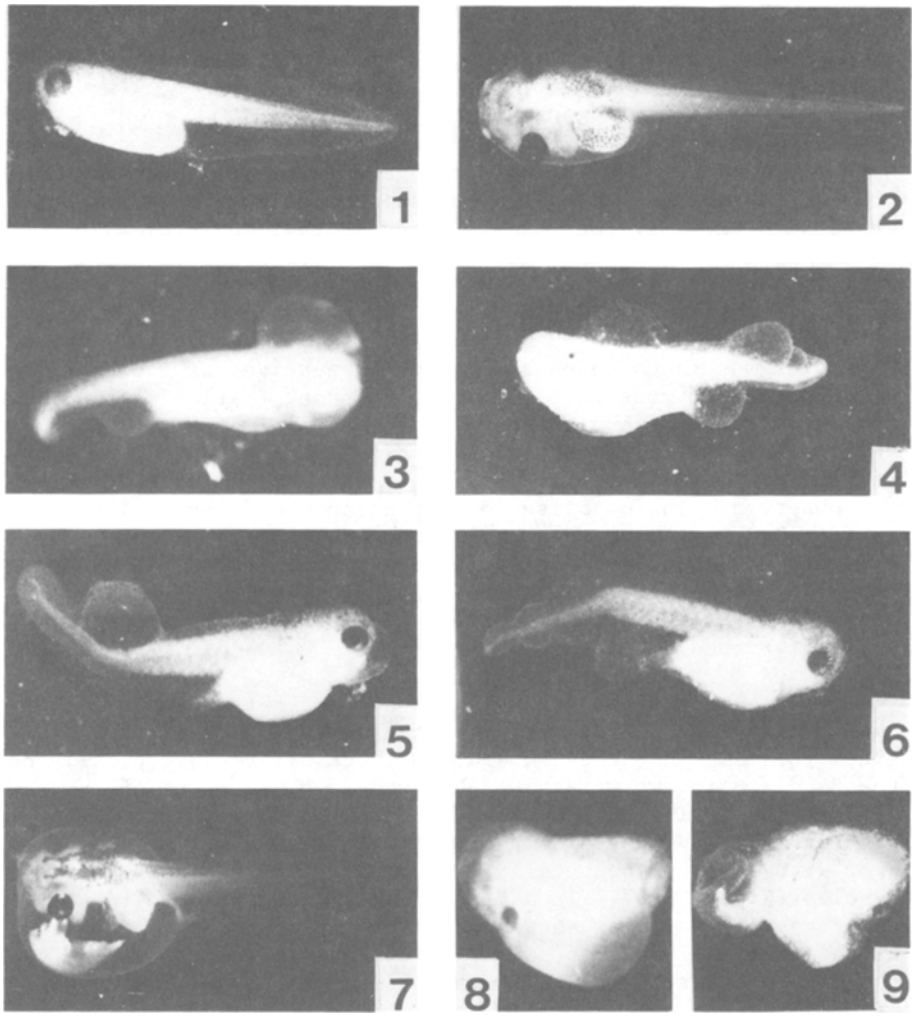


Figure 1 Control tadpole at 48 h.
Figure 2 Control tadpole at 96 h showing normal development.
Figures 3 & 4 Experimental tadpoles at 48 h exposed to 5 ppm malathion. Note the blisters on head and body.
Figures 5 & 6 Experimental tadpoles at 96 h treated with 5 ppm malathion showing upward and downward curvature with blisters.
Figure 7 Experimental tadpole at 96 h exposed to 5 ppm. Note the distended head.
Figures 8 & 9 Experimental tadpoles at 48 h exposed to 10 ppm. Note the stunted growth and no differentiation.

group were fixed in Bouin's fluid and processed for routine histology. Sections were taken at 7um and stained with Harris haematoxylin and eosin.

RESULTS AND DISCUSSION

Embryos of M. ornata growing in the control medium showed muscular movement in about 24 h. All embryos hatched as tadpoles in about 48 h. with well developed suckers, eyes, tail and typical pigmentation (Fig. 1). By the end of 96 h. tadpoles were actively swimming in the water and they appear as shown in Fig. 2. Abnormal development was not observed in the control embryos.

Malathion was found to be affecting the survival of the embryos and the rate of mortality was dose dependant (Table 1). The lower concentrations which were not immediately embryotoxic, induced various abnormalities in the developing embryos. At a concentration of 1 ppm no mortality or gross morphological abnormality was observed. In few cases loss of balance and abnormal behavior of tadpoles was observed. These tadpoles were swimming either in a circle or showed abnormal twitching of the tail during swimming. Their swimming activity was considerably reduced as compared to controls and some of them remained at the bottom of the petri dish until disturbed.

Embryos treated with 5 ppm malathion showed retardation of development. After 24 h exposure, while the embryos were still in the vitelline membrane, they showed prominent blisters, typically on the head. Blisters were also observed on other parts of the body (Fig. 3 & 4). The blisters enlarge in size during further period of treatment and they affect normal morphogenesis. The tadpoles grow with kinky or bent tails with disarray myomeres of the tail muscles (Fig. 5 & 6). In addition, the growth was considerably retarded with tadpoles showed distinct microcephaly as compared to the controls. Due to upward or downward curvature of the tail, the tadpoles were forced to remain at the bottom of the petri dish. They showed twitching muscular activity and attempted erratic swimming when prodded with a needle. In a few cases, the entire head was distended and fluid-filled after 96 h continuous treatment with 5 ppm concentration (Fig. 7).

Malathion at a concentration of 10 ppm had drastic effect on embryos. Embryos were highly stunted in growth and grossly malformed. At 48 h, they did not show any visible differentiation into head and tail regions (Fig. 8 & 9). These embryos were just a mass of cells often with a blister. Even at 96 h they were highly abnormal and retarded, showing muscular twitching when disturbed.

Concentrations beyond 15 ppm were rapidly embryotoxic, usually bringing about 100% mortality within 24 to 48 h. The embryos were highly malformed and stunted at the time of death. Apart from this malathion also delayed hatching usually by about 24 to 48 h. The pigmentation of the tadpoles was often reduced or sometimes absent, especially beyond 10 ppm concentration. Heart

beat rate was also reduced and there was poor tail circulation as it could be observed through the transparent tail.

Histology of the 96 h control tadpole revealed normal development (Fig. 10) of the different organs like the neural tube; the notochord surrounded by muscles. Well-developed mesonephros are seen to the lateral sides of muscles. The coelomic cavity is filled by coils of digestive system and liver. Whereas, the histology of treated tadpoles (5 ppm) of the same age of development showed stunted growth, poor differentiation of various organs, displacement of mesonephros and general edema (Fig. 11). Instead of gut coils only, a mass of the yolky cells was observed in the coelomic cavity. Poorly developed neural tube notochord and muscles were also noticable. In general, histological analysis showed that malathion affected the differentiation of the embryonic cells. In all the sections observed there was no pycnotic cell seen.

The results of the present investigation clearly showed that malathion is teratogenic to the embryos of M. ornata in the range of 5 to 10 ppm. Beyond 10 ppm it is highly embryotoxic killing embryos within 24 to 48 h. The abnormalities observed in the frog embryos like curvature body axis, blister formation, poor development and abnormal behaviour appeared to be similar to those of fish embryos treated with an organophosphorous insecticide (Kaur and Toor 1977). The retarded growth and delayed development in microhyla is probably related to the interference of malathion and its metaboltes in DNA and protein synthesis as reported by Wilson et al (1973). Our histological observations show the decreased cell number but no pycnosis.

The malathion-treated embryos showed prominent blisters which often induced abnormalities such as curvature of tail and distention of body cavities. The occurrence of blisters and distention of body cavities suggests, disturbance in osmoregulatory mechanism. This may be caused by inhibition of the vital enzyme ATPase which is often affected by malathion (Mukhopadhya and Dehadrai 1980). Malathion also affected development of pigment or delayed melanogenesis which again suggests interference in enzymatic synthesis of melanin. Inhibition of pigment synthesis in frog embryos due to dithiocarbamate pesticides has also been reported (Bancroft and Prahalad 1973, Ghate and Mulherkar 1980).

The tadpoles that had hatched from embryos treated with 5 and 10 ppm malathion showed abnormal behaviour and abnormal muscular twitching. Such an abnormality may be related to inhibition of acetylcholinesterase system of which malathion is a potent inhibitor (Coppage et al 1975). We did not observe cardiac abnormalities in tadpoles, but heart beating was distinctly reduced and there was poor tail circulation. Solomon and Weis (1979) however reported cardiac abnormalities and heart malfunctioning in fish embryos treated with carbamate and organophosphorous pesticides. They also reported failure of hatching in fish embryos however, in the present study hatching usually delayed by 24 to 48 h.

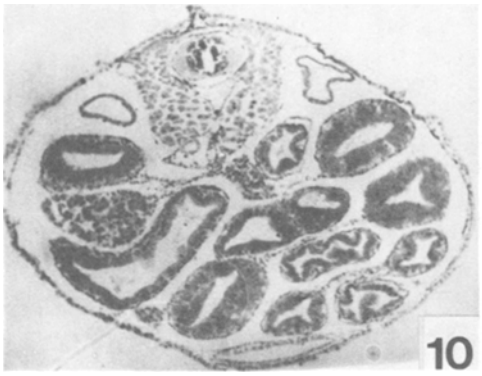
Table 1. The effect of malathion on the development of the frog, Microhyla ornata at yolk plug stage.

ppm	Duration of treatment								% mortality after 96 h.
	24 h		48 h		72 h		96 h		
	A/S**	%	A/S	%	A/S	%	A/S	%	
0	0/100	0.00	0/100	0.00	0/100	0.00	0/100	0.00	0.00
1	0/100	0.00	5/100	5.00	13/100	13.00	23/100	23.00	0.00
5	100/100	100.00	100/100	100.00	100/100	100.00	100/100	100.00	0.00
10	100/100	100.00	91/91	100.00	73/73	100.00	65/65	100.00	35.00
15	100/100	100.00	43/43	100.00	0/0	-	-	-	100.00
20	100/100	100.00	0/0	-	-	-	-	-	100.00

*Number of embryos treated at each concentration was 100.

**Number of abnormal/number of surviving embryos and percent abnormals.

Abnormalities include head, trunk and tail defects, abnormal behaviour, loss of balance, poor pigmentation and retarded growth.



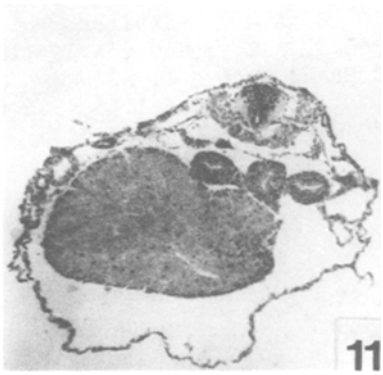


Figure 10 T.S. of 96 h control tadpole. Note the various organ systems formed. H. & E. 75 X.

Figure 11 T.S. of 96 h experimental tadpole (5 ppm). Note the distended body cavity, unutilized yolk mass, displacement of mesonephros, occlusion of the neural tube and general edema. H. & E. 75 X.

The histological observations in the treated tadpoles showed stunted growth, poor differentiation of the various organs and mass of the yolky cells in the coelomic cavity. Similar histological analyses has also been reported in the frog embryos exposed to heavy metals and pesticides (Imberti et al 1974, Vailati 1980).

Malathion has also been reported as a teratogen in other vertebrates like birds and reptiles (Meiniel and Austissier - Navarro 1980, Mitchell and Yntema 1973). According to the present understanding, the teratogenesis due to malathion is related to massive cell death and/or effect on early embryonic enzyme systems that are essential in morphogenesis. In our observations, (5 ppm), we did not see any cell death. In case of birds,acetylcholenesterase inhibition has been suggested to be a major cause leading to teratogenesis (Meiniel et al 1970). It is possible that in frogs also, similar inhibition of enzyme may be responsible for the teratogenesis.

Acknowledgements. We wish to thank B. S. Gokhale for photographic work. One of the authors (KRP) gratefully acknowledges the University Grants Commission for the award of a "Teacher Fellowship."

REFERENCES

ANDERSON, R.J. and K.V. PRAHLAD : Arch. Environ. Contam. Toxicol. 4, 312 (1976).

BANCROFT, R. and K. V. PRAHLAD : Teratology 7, 143 (1973).
COOKE, A.S. : Environ. Pollut. 3, 51 (1972).
COOKE, A.S. : Environ. Pollut. (Series A) 25, 123 (1981).
COPPAGE, D.L., E. MATHEWS, G. H. COOK and J. KNIGHT : Pest. Biochem. Physiol. 5, 536 (1975).
DUKE, T.W. : In Pesticides in Aquatic Environment. pp. 1, M.A.Q. Khan (Ed.), New York: Plenum Press (1977).
DUTTA, S. K. and MOHANTY-HEJMADI : Indian J. exp. Biol. 16, 727 (1977).
GHATE, H.V. and L. MULHERKAR : Indian J. exp. Biol. 18, 1094 (1980).
GREENHOUSE, G. : Environ. Pollut. 11, 303 (1976).
IMBERTI, N.R., G. VAILATI and S.S. RANZI : Acad. nat. lin. LVI, 635 (1974).
KAUR, K. and H.S. TOOR : Indian J. exp. Biol. 15, 193 (1977).
MEINIEL, R. Y. LUTZ-OSTERTAG and H. LUTZ : Arch. Anat. Microsc. Morphol. Exp. 59, 167 (1970)
MEINIEL, R. and C. AUSTISSIER-NAVARRO : Acta Embryol. Morphol. Exper. n.s. 1, 33 (1980).
MITCHELL, J. and C.L. YNTEMA : Anat. Rec. 175, 390 (1973).
MUIRHEAD-THOMSON, R.C. : Pesticides and Fresh water Fauna. New York: Academic Press (1971).
MUKHOPADHAY, P. K. and P.V. DEHADRAI : Indian J. exp. Biol. 18, 348 (1980).
PAFLITSCHEK, R. : Z. angew. Zool. 60, 143 (1979).
PAWAR, K. R. and M. KATDARE: Experientia, in press (1983).
SOLOMON, H.M. and J.S. WEIS : Teratology 19, 51 (1979).
VAILATI, G. : Riv. Museo. Sci. nat. 1, 36 (1980).
WILSON, B.W., H. O. SINNETT, D.M. FRY and P.S. NIEBERG : Arch. Environ. Health 26, 96 (1973).
Accepted March 2, 1983