The Effect of Two Pesticides, Miedzian 50 and Gesagard 50, on the Development of Tadpoles of *Rana temporaria*

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Since the late sixties the problem of destroying amphibians by the use of pesticides for plant protection in the proximity of water bodies, small bogs, and wet meadows has become a matter of concern to herpetologists. In some countries, England for instance, attention was drawn to this problem comparatively early. SANDERS (1970) describes for 16 commonly used pesticides the toxic effect for tadpoles of various species of frog or toad. The greatest number of papers were devoted to the effect of DDT and related substances. COOKE (1972) was chiefly concerned with the effect of DDT and Dieldrin and investigated the action of these chemicals on the spawn, tadpoles, and juvenile forms of frogs, toads, and newts (COOKE, 1974; COOKE and ZORO, 1975).

The present paper is part of an investigation on the possible effects of other pesticides on amphibians.

MATERIAL AND METHODS

The experimental material was obtained during two spring seasons, 1974 and 1975, by artificial insemination from pairs of *Rana temporaria* caught in the wild. The particular experimental series and their controls were made on tadpoles originating from one pair. Two developmental stages were used for the experiments: so-called *younger tadpoles*, just after hatching, at a stage comparable to stage 20 according to SHUMWAY (1940) for *Rana pipiens*; and *older tadpoles*, after completion of the operculum, comparable to SHUMWAY stage 25. In selecting the animals, both stage and body length was taken into account. The experimental tadpoles and controls were kept in the same conditions (temperature, amount of water, form and size of vessels).

Two pesticides were chosen: (1) Medzian 50 [basic cupric chloride, 3 $Cu(OH)_2 \cdot CuCl_2$, produced by "Azot," Jaworzno], and (2) Gesagard 50 [Prometrine, Primaze; 2,4-bis(isopropyloamine)-6-(methylthio)-s-triazine, produced by Ciba-Geigy].

Since both substances are only slightly water-soluble, in order to obtain conditions approaching those when the chemicals

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are used for crop spraying in the field, the experiments were made only with aqueous suspensions.

The LD50/48 h for Xenopus tadpoles at stage 37/38 according to NIEUWKOOP and FABER (1956) was found to be for Miedzian 0.007-0.008%, and for Gesagard 0.0009-0.001% (MARYAŃSKA, unpublished).

The concentrations used and the time of treatment are summarized in the table:

	Younger Tadpoles		Older Tadpoles	
Time of treatment	3 days	5 days	3 days	5 days
Miedzian 50	0.05%	0.05%	0.01% 0.05%	0.01% 0.05%
Gesagard 50	0.05%	0.05%	0.01% 0.05%	0.01%* 0.05%*

*All tadpoles died

The liquids were changed every two days to keep within terms of pesticides validity. Each experiment was made on 500 tadpoles.

After treatment a part of the tadpoles were fixed in Bouin's fluid, paraffin-embedded, cut into 10 μm sections and stained with hematoxylin and eosin. The remaining animals were transferred to tap water for further observations and for making measurements.

RESULTS

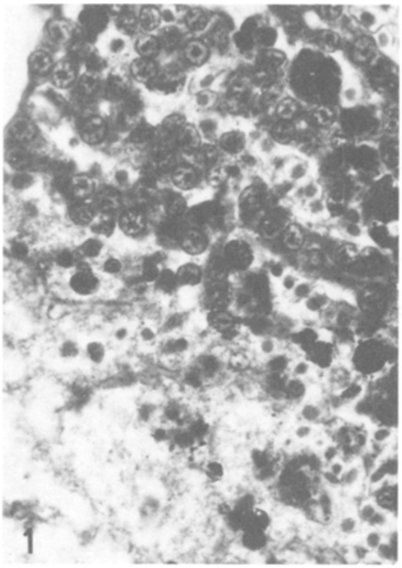
Miedzian 50 applied in 0.05% suspension during 3 days caused, in younger tadpoles, an inhibition of growth and numerous changes in the alimentary canal. In the liver and stomach as well as in the small and large intestines there appeared accumulations of pigment in the cells, and in the large intestine even partial cytolysis of the intestinal epithelium cells. In the liver parenchyma there appeared decay of the parenchymal cells as well as very strong pigmentation (Fig. 1), not found in control animals. These changes were accompanied by a strong hyperemia of the whole organ. Most of these tadpoles died, while the remainder, after being transferred into tap water, returned to normal within a few days.

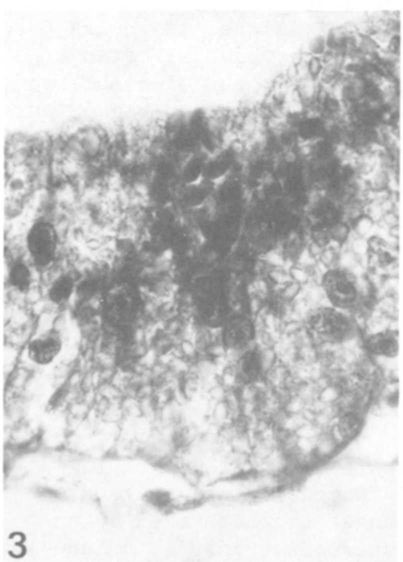
In the 0.05% suspension, after 5 days' contact, the tadpoles ceased to grow. Histologically, cytolysis occurred in the alimentary canal, preceded by a strong hyperemia of organs, especially in the liver. In the intestinal epithelium abnormal cell division was frequently seen (dispersal of chromosomes outside the spindle). In most of the animals the large intestine was usually hyperemic and abnormally distended; in many cells vacuolization, as well as eosin accumulations were visible. In numerous tadpoles--owing to inhibition of growth and development--the process of completion of the operculum was inhibited. The gills, therefore, protruded outwards and exhibited strong hyperemia. In the brain strong pigmentation of the ependymal coating of the ventricles was distinct; no changes in the eyes of the investigated tadpoles were found.

Miedzian applied to older tadpoles appeared to be more toxic. This was ascertained in the 3-day and 5-day groups in both 0.01% and 0.05% suspensions. In all animals of all four experimental groups an inhibition of growth occurred, usually leading to the death of the animals. As in the groups already described, the alimentary canal was most affected, especially the small and large intestines and the liver. In the intestinal cells there was a complete lack of cell divisions, the cells were tall and subject to vacuolization and cytolysis; often a complete disappearance of the epithelial layer was also observed. The internal organs were all hyperemic, and the liver pigmented, with partial decay of its parenchyma. The tail muscles were vacuolized, in some places decaying. In these animals there were also serious changes in the brain, consisting in vacuolization of the brain tissue, hyperemia of brain ventricles, and a partial cytolysis of the ependyma and the neural tissue. In the limb buds a complete lack of mitoses, as compared with controls, was observed. Very high mortality occurred in both 3-day and 5-day groups of animals. After transferring the remaining animals to tap water, a few tadpoles survived, stayed alone one or two days, but then died, as their growth and development seemed to be inhibited.

Gesagard 50 proved to be more toxic than Miedzian. In younger tadpoles, treated with 0.05% suspension of Gesagard for 3 days, there occurred intestinal changes characterized by an accumulation of pigment and strong hyperemia (Figs. 2, 3 and 4). In the brain there was also considerable hyperemia, especially in the ependyma of the IV ventricle. In some of the tadpoles of this group a strong swelling of the whole body occurred as a result of the exudation of water and body fluids under the skin; these animals died after a few hours.

Similar phenomena occurred in older tadpoles, after treatment with 0.01% and 0.05% suspensions in the 3-day groups. Such animals always died after 24-72 hours. The internal organs in these experimental groups were strongly hyperemic, especially in the alimentary canal. This was accompanied by partial cyto-





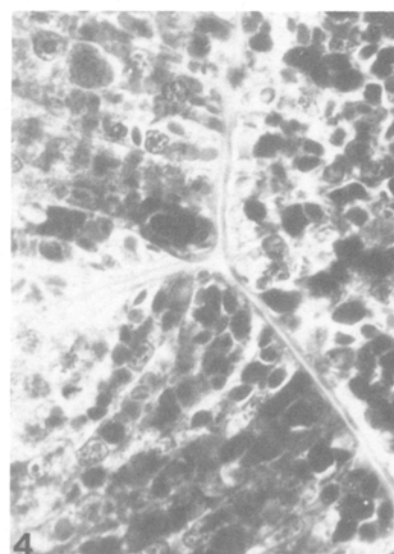


Fig. 1. Liver of a tadpole treated with 0.05% suspension of Miedzian 50 during 3 days. Note cytolysis and abnormal pigmentation. HE, X cz. 600.

Fig. 2. Cross-section through the alimentary canal of a control tadpole, HE, X ca. 600.

Figs 3 and 4. Cross-sections through the alimentary canal of tadpoles treated with 0.05% suspension of Geasgard 50 during 3 days. Note abnormal pigment accumulation and cytolysis. HE, X ca. 600.

lysis, distention of the intestine, vanishing of the boundaries between cells in the duodenum and in the large intestine, an intergrowth of connective tissue of the liver parenchyma, and partial cytolysis of its lobes. In some tadpoles, as an effect of inhibition of growth and development, the operculum was incomplete, the strongly hyperemic gills protruding outside. At The same time, in all control tadpoles the process of operculum formation was completed. Transferring such animals into tap water did not cause them to return to normal and they died.

DISCUSSION

In the present investigation it was found that younger stage tadpoles are more resistant to the action of pesticidal poisons. The authors explain this, particularly in the case of Gesagard, by its action on the central nervous system, which differentiates with development and becomes more and more sensitive to the toxic effect of this substance. Effects of this kind were observed also by other authors, when investigating the influence of 2,2-D on the eggs and embryos of the carp (MATLAK, 1972; KAMLER et al. 1974).

Frequently, similarly to the experiments of COOKE (1975), more or less reversible paralysis of the whole body of the tadpoles was observed after applying the investigated substances. Such paralysis lasted particularly long in the series of animals treated with Gesagard; these tadpoles did not react to stimuli, and after being transferred to tap water returned to normal only after a long time. Similar paralysis was obtained by other authors (ELLIS et al. 1944) in fishes and frogs as an effect of DDT.

It also seems interesting that in many cases the processes of severe poisoning may be reversible. Animals surviving the experiment and transferred to water often returned to normal. Similar data were obtained with other pesticides (KAMLER et al. 1974).

The changes in the alimentary canal caused by Miedzian and Gesagard arise from the fact that the animals living in an aqueous environment polluted by suspensions of the investigated pesticides took in particles of the toxic substances together with water and food. Hyperemia of the internal organs, cytolysis, abnormal pigmentation--these are symptoms of an overall poisoning, independently of the poisons used. Similar changes were also observed by KOKURICHEVA (1974) in fishes treated with various pesticides. It is known that in men engaged in crop-spraying with Miedzian, subacute poisoning is often found, which consists, among others, in hemolytic jaundice and pigmented cirrhosis of the liver. In the present investigation hyperemia, cytolysis, and pigmentation of the liver were especially visible in tadpoles treated with Miedzian.

In the present experiments comparatively low percentage suspensions of the investigated substances were used (0.01-0.05%). It should be remembered that for spraying in agricultural practice suspensions are used in concentration up to 15%.

SUMMARY

Tadpoles of the frog, Rana temporaria, in two developmental stages were subjected to the action of aqueous suspensions of two pesticides--Miedzian 50 and Gesagard 50.

The changes caused by these substances pertain chiefly to the alimentary canal, brain, and muscles, and are connected with the developmental stage of the animals.

Miedzian 50 caused a partial sytolysis in the cells of the intestinal epithelium and the parenchyma of the liver.

Gesagard 50 was more toxic; under its action strong degenerative changes were obtained in the alimentary canal and the brain. Also, disorders in the development of tadpoles were observed. These consisted in a partial inhibition of growth and a retardation of the process of completion of the operculum as compared with control animals.

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