

# INVESTIGATIONS UPON THE EFFECTS OF SOME PESTICIDES ON CARP (*CYPRINUS CARPIO*)

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The investigations were made upon toxic effects of 12 pesticides, commonly used in Poland, on one-year-old carp (*Cyprinus carpio*). It was showed that Euparen, Gamakarbatox and Melipax were particularly toxic to fish and could be the cause of some losses in fish cultures.

In the last 20 years numerous cases of fish mortality were attributed to pesticide poisonings. It was found that pesticides reached our waters mainly as the result of washing these compounds out of the ground after heavy rain and at the time of snow melting, sometimes also during the spraying of plants (3, 4, 7).

In the United States agricultural chemicals, which include chlorinated hydrocarbon pesticides, were responsible for 32 per cent of all known sources of fish killed in 1960, 21 per cent in 1961, and 18 per cent in 1962 (6). Also, in Poland, the relationship between fish mortality and pesticide application in the vicinity of ponds was documented (2, 4). Some of the

symptoms and pathological changes noted in fish in the last decade had not been observed before (15). The pathological changes were most visible in the gills, swimbladder and vertebral column. Abnormal carp behaviour, accompanied sometimes by high mortality, usually followed the inflow of muddy water from fields to nearby ponds shortly after heavy rain. The presumptions were that, at least in some cases, the cause of these disturbances could be attributed to chemical compounds widely used in agriculture including the pesticides. Better knowledge of symptoms and pathology of fish intoxications caused by the chemicals is needed for the preliminary diagnosis of the fish kills and as a direction to further specific toxicological investigations.

### Material and Methods

Twelve pesticides commonly used for plant pest control were examined: Benlate (50 per cent benomyl), Cynkotox (65 per cent zineb), Dithane M-45 (80 per cent mancozeb), Gesaprim (50 per cent atrazin), Gramoxone (20 per cent paraquat), Metasystox (50 per cent demeton — S methyl), Patoran (50 per cent metobrom), Tribunil (70 per cent methabenzthiazuron), Wofatox (50 per cent methyl parathion) at concentrations of 0.1, 2.0, 3.0, 4.0, 5.0 mg/l, Euparen (50 per cent dichlofluanid) at concentrations of 0.05, 0.15, 0.25, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6 mg/l, Gamakarbatox (40 per cent carbaryl and 10 per cent lindane) at concentrations of 0.5, 0.75, 1.0, 1.25, 1.5, 2.0 mg/l, and Melipax (60 per cent chlorinated camphens) at concentrations of 0.005, 0.01, 0.015, 0.02, 0.025 mg/l.

Fifty, one-year-old carp, weighing 32—65 g, free of parasites and acclimatized to the aquarium environment were tested at each concentration of the compounds. The experiments were carried out with dechlorinated tap water at pH between 6.24 and 7.48, average total hardness 168 mg/l, calcium hardness 120 mg/l. The temperature was maintained at  $16^{\circ}\text{C} \pm 1$ , oxygen concentration 7.72—8.57 mg/l, ammonium concentration up to 0.125 mg/l. The fish mass/solution volume ratio never exceeded 1.0 g of fish per liter of water according to Kenneth (5).

In the course of 6-day exposure, fish were transferred after each 48 h to a fresh dilution of the pesticide. At the end of bioassays the survivors were placed in an aquarium with well aerated, free of pesticide, dechlorinated tap water where the feeding tests were performed for the next 15 days. The control groups were kept in an aquarium with pesticide-free water.

The carp placed in concentrations of 0.005, 0.01 and 0.02 mg/l of Melipax were additionally subjected to forced swimming and rapid increase in the temperature up to  $21^{\circ}\text{C}$ . The toxicological effects of the preparations were measured in terms of  $\text{CL}_0$  — the highest concentration at which no signs of intoxication were detected, and  $\text{CL}_m$  — the lowest concentration that caused death or irreversible pathological changes (9). In the cases of pathological lesions in the gills, the gill filaments were subjected to histological examinations; the preparations were stained with hematoxylin and eosin.

### Results

The carp placed in solutions containing up to 5 mg/l of Benlate, Cynkotox, Dithane M-45, Gesaprim, Gramoxone, Metasystox, Patoran, Tribunil and Wofatox showed no signs of intoxication during exposure and also in 15 days postexposure when they were fed normally.

Clinical symptoms and pathological lesions of intoxication were observed only in fish exposed to Euparen, Gamakarbatox and Melipax. The data concerning  $\text{CL}_0$  and  $\text{CL}_m$  of these pesticides are presented in Table 1.

Table 1

The toxicity of Gamakarbatox, Euparen and Melipax for one-year carp

	CL <sub>50</sub> mg/l	CL <sub>m</sub> mg/l
Gamakarbatox	0.5	1.5
Euparen	0.05	0.5
Melipax	0.005	0.03

Euparen. — At concentrations between 0.15 and 0.45 mg/l increased excitability of the carp, manifested by unusually rapid response to disturbances, was noted. Excessive slime secretion on the skin and on the gill sheets of these fish was also observed. In the water the slime showed filament-like appearance. When the fish were transferred to aquariums filled with pesticide-free water, the signs of intoxication gradually disappeared.

At concentrations between 0.5 and 0.6 mg/l death appeared within the time of exposure and when the survivors were transferred to the uncontaminated water. The first cases of fish death were noted 24 h after their exposure to the pesticide.

Twenty-eight per cent of the fish subjected to concentrations between 0.5 and 0.6 mg/l recovered after the experiment. In heavily intoxicated fish the distal ends of the gill sheets became pale because of ischaemia of the peripheral portions of the gill. The ischaemic areas were usually larger in the fish placed in the higher concentration of the pesticide (0.6 mg/l). In some cases they occupied 90 per cent of the gill. Also, deformities of the gill sheets, such as fusions between some of the sheets accompanied by gaps between the others, were noted. Deformities appeared mainly at the ends of the gill sheets, where the thick slime layer was observed.

Gamakarbatox. — At concentrations between 0.75 and 1.25 mg/l carp displayed unusual characteristic nervous symptoms i.e. swimming sidewise in a circle, or laying on the bottom of the aquarium. The carp exposed to 0.75 and 1.25 mg/l had already shown their first nervous symptoms after 8 h of exposure. Fish placed in pesticide-free water gradually recovered; neither deaths nor pathological changes were detected.

Signs of acute intoxication of the central nervous system, accompanied by death of the fish, were observed shortly after placing them at concentrations of 1.5 and 2 mg/l of the tested compound. The fish died within the exposure time as well as after transferring them to pesticide-free water. Totally 86 per cent of the tested fish died during the experiments.

Melipax. — The carp placed at concentrations between 0.01 and 0.025 mg/l of the tested compound only exhibited increased excitability, expressed by unnatural rapid swimming when they were disturbed. After the forced swimming and rapid increase in the temperature (up to 21°C) the tested animals showed the same signs of intoxication as did those subjected to higher concentrations of the pesticide.

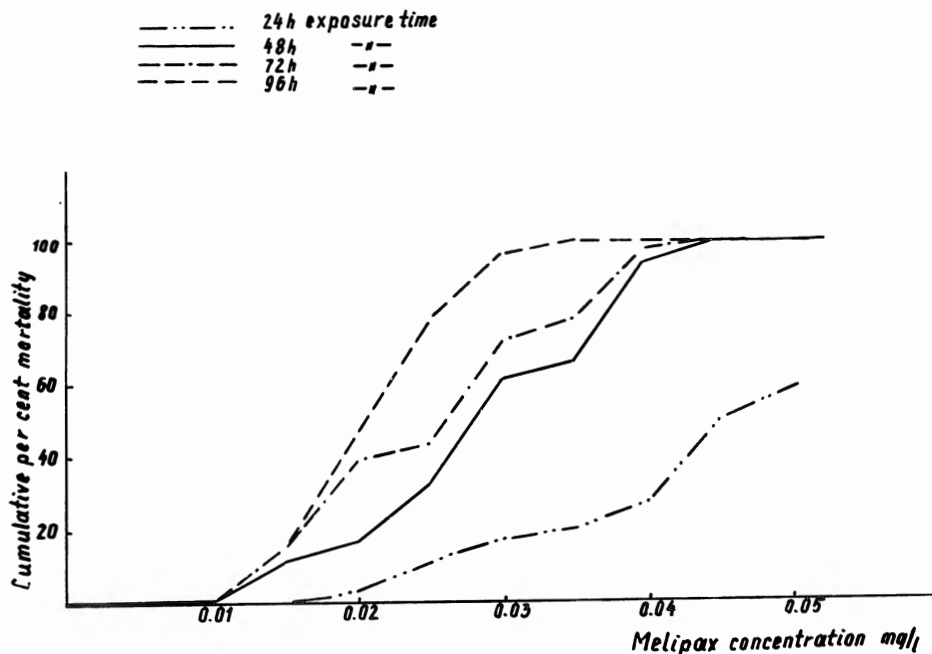


Fig. 1. — The effect of Melipax on one-year-old carp (*Cyprinus carpio*)

At concentrations between 0.04 and 0.03 mg/l of the pesticide, the first symptoms of intoxication were noted after 24-h exposure. These concentrations disturbed function of the swimbladder, digestive tract and central nervous system. The fish were swimming at the surface water, and the dorsal fin was visible out the water. The carp had difficulties in swimming to the deeper part of the aquariums; when the fish were disturbed they swam to the bottom but soon returned to their previous position.

After 48 h of exposure, fish placed in concentrations of 0.035 and 0.04 mg/l showed considerable enlargement of the body cavity (Fig. 2). The fish thus afflicted swam head down with tail fin and adjacent part of the body protruding out of the water (Fig. 3). The anatomopathological examinations showed slight enlargement of the swimbladder chambers and distension of the digestive tract which contained a very visible gas bubble. In some carp, swelling of the hepatopancreas was also observed (Fig. 4). The effect of Melipax on carp with respect to cumulative fish mortality was presented in Fig. 1.

### Discussion

There are no available data concerning the effects of Benlate, Cynko-tox, Dithane M-45, Euparen, Gamakarbatox, Gesaprim, Gramoxone, Meta-systox, Patoran, Tribunil or Wofatox on fish. Our experiments showed that only Gamakarbatox, Euparen and Melipax induced significant toxic

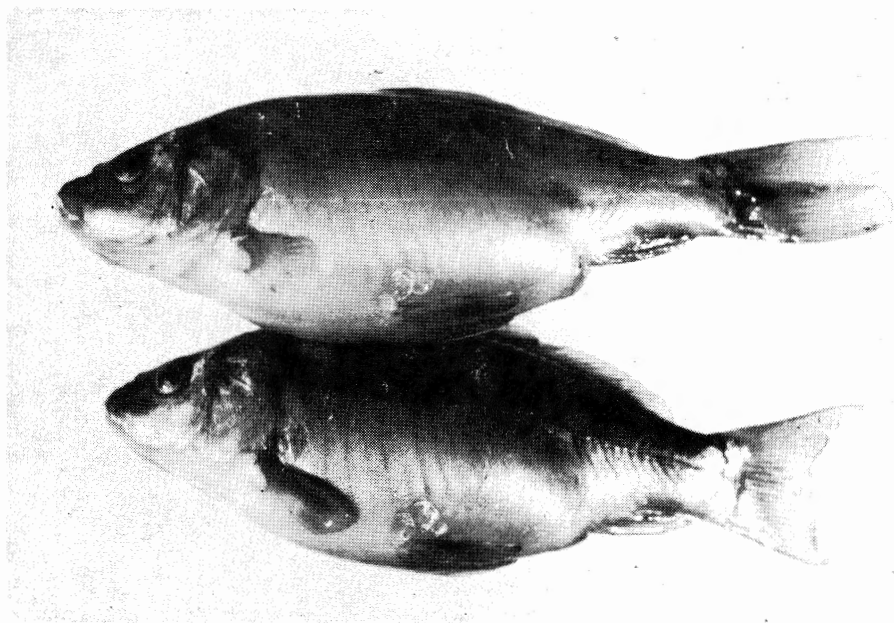


Fig. 2. — The enlargement of the body cavity

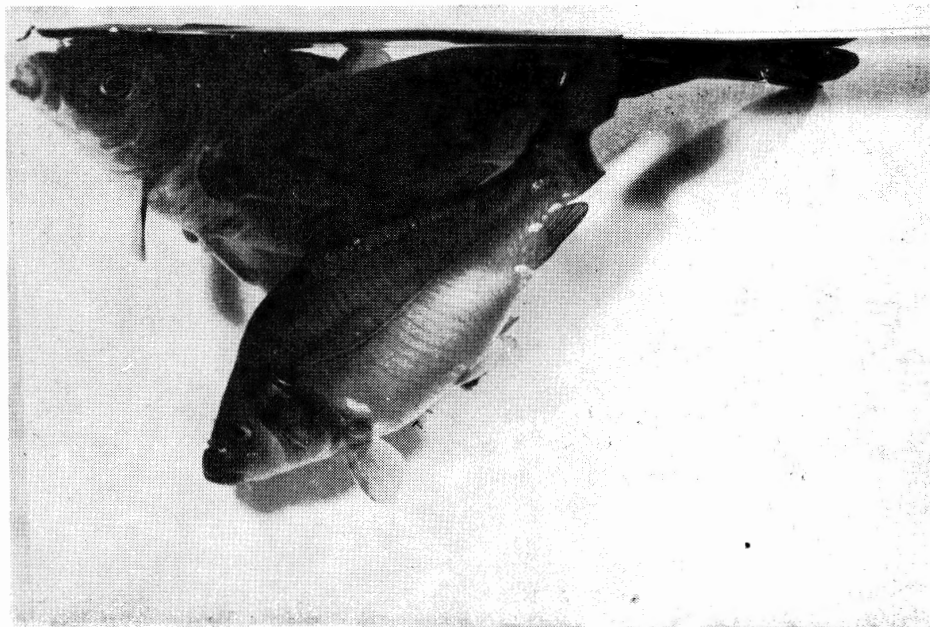


Fig. 3. — The swimming disturbance caused by the distension of swimbladder chambers

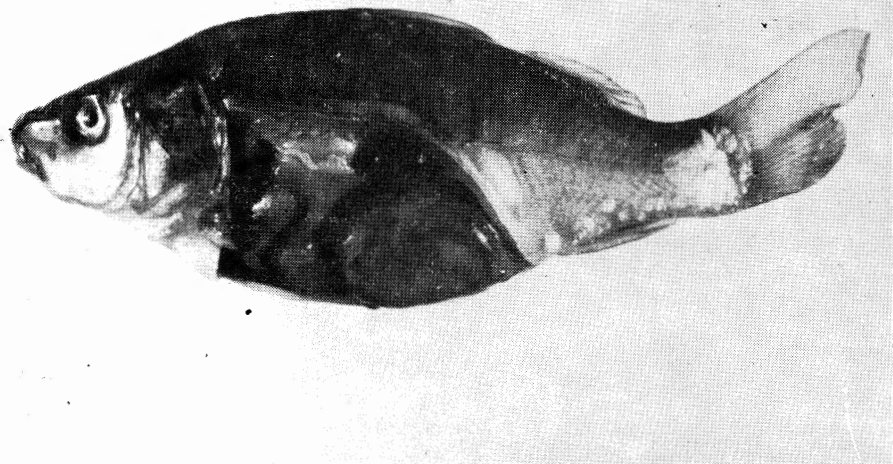


Fig. 4. — The swelling of the hepatopancreas and the distension of the intestine.

effects in carp. These effects varied distinctly. Gamakarbatox caused disturbance of the labyrinth function; Euparen caused intoxication of the central nervous and cardiovascular systems; Melipax caused damage to the hepatopancreas and digestive tract and the disturbance of the swim-bladder function.

The high toxicity of Melipax is very well known (1, 2, 10, 11, 12, 14). The 96-h  $CL_{50}$  of toxaphene (the active component of Melipax) to carp is 0.003—0.005 mg/l (5). Our experiment, conducted with one-year-old carp, did not confirm such a high toxicity of this compound. According to our findings, the lowest toxaphene concentration that caused death or irreversible lesions in experimental fish, within the same period of time (96 h), was 0.018 mg/l, although the first slightest signs of intoxication were observed at a concentration of 0.003 mg/l.

Additional experiments showed that when the fish placed in the above-mentioned concentration of toxaphene (0.003 mg/l) were subjected to stress, for instance forced swimming and rapid change of temperature, deaths appeared. Properly conducted acclimatization in aquariums before bioassays, which was the case in our experiment, elevated undoubtedly the threshold of toxaphene toxicity in experimental fish. This explanation seems to be supported by the fact that goldfish (*Carassius auratus*) closely related to the common carp (*Cyprinus carpio*) when acclimatized to the aquarium environment died at a concentration of 0.025 mg/l of toxaphene after 10 days (1).

The experiments concerning the effect of toxaphene on various fishes demonstrated that this compound caused vertebral damage connected with a decrease in the backbone collagen (10, 11) and alterations of the hepa-

tic cells (13). However, there are no available reports on the toxic action of toxaphene on the digestive tract or swimbladder function.

The results of our study are not sufficient to draw conclusions whether there is any relationship between gill necrosis or swimbladder inflammation and some of the pesticides used in our experiments although, some of the symptoms induced by the pesticides appeared to be similar to the symptoms observed in carp at fish farms. The swelling of the swimbladder and the presence of gas bubbles in the digestive tract induced by DDT were also reported in fry of lake trout (*Salvelinus namaycush*) (7). It should be noted that the first severe cases of swimbladder inflammation in the Lublin region were found when the pesticides were applied in masses for plant protection. The results of our experiments and observations pointed out that in the study on the etiology of swimbladder inflammation not only the infectious agents but also some of the chemical compounds should be taken into account (15).

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