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Histopathological Effects of Sublethal Exposure to Phenol on Two Variously Pre-Stressed Populations of Bullhead (*Cottus gobio* L.)

Franz Bucher and Rudolf Hofer

Institute of Zoology, University of Innsbruck, Austria

Phenol is a common pollutant of aquatic ecosystems, into which it can be introduced by industrial waste water of different kinds (from refineries, chemical plants and paper mills), communal sewage, or decaying vegetation (Swift 1978; Babich and Davis 1981). In fish a very broad spectrum of toxic effects of phenol has been reported, ranging from disturbances in behavior to impairment of growth and reproduction or even serious organic damage (Jones 1951; DeGraeve et al. 1980; Mitrovic et al. 1968; Reichenbach-Klinke 1965). It has also been observed that previous exposure to phenol confers a certain degree of resistance (Flerov 1971).

In the investigation reported here, the histopathological effects of phenol intoxication on bullhead (*Cottus gobio*) were studied, and the reactions of variously 'pre-exposed' populations compared.

MATERIAL AND METHODS

In summer 1990, using an electric fishing device (DC, 4.5 kW impulse), 50 adult male bullhead (length 8.8 ± 0.7 cm) were caught in each of two different sections of a water system in Upper Austria: group I from a location in the Lake Traunsee (area 24.4 km^2) where the only possible source of pollution was run-off water from roads, and group II from the River Traun (longitude 153 km) itself, below the site of entry of waste water from two paper mills (sum of the sewage data: volume of waste water 46341 m^3/d, chemical oxygen demand 2844 kd/d, hydrocarbons 1.0-2.2 mg/L, absorbable organic xenobiotics 125-212 µg/L, phenols 8-45 µg/L). The fish were transported to the laboratory and acclimated to laboratory conditions for 1 wk. Each population was divided into two subpopulations, placed into two 200-L glass aquaria. One subpopulation was exposed to 6 mg/L phenol for 35 d, the other (control) was kept in pure

Send reprint request to R. Hofer at the above address.

tap water (physicochemical properties: 13.5-15.0°C, pH 7.7-8.2, 150-180 mg/L $CaCO_3$, water flow 600 mL/min). Phenol p.a. (Merck, Darmstadt) dissolved in double distilled water (stock solution 4000 mg/L) was introduced into the tap water inflow by means of a peristaltic pump.
The phenol concentration in the experimental tanks was determined according to Merck (1990). At the end of the experiment bullhead were killed mechanically and pieces of the liver, kidney, gills and skin of 10 individuals per group were fixed in 5% formaldehyde solution for histological examination. After dehydration in an ethanol series, tissues were embedded in polyethylene glycol-methacrylate. Haematoxylin/ eosin, May-Grünwald/ Giemsa and periodic acid-Schiff stains were carried out on 3μm sections.

RESULTS AND DISCUSSION

The behavior of the fish changed in no way that could be attributed to phenol. During the daytime the fish in all of the experimental tanks behaved quite normally, remaining motionless between the stones provided as hiding places. However, both of the phenol-exposed groups produced great quantities of mucus which floated in the water like fine skin and coated the walls of the aquaria.

After 35 d no histopathological changes were found in the control fish of group I (bullhead from the Lake Traunsee) and II (bullhead from the River Traun).

The bullhead obtained from water containing hardly any industrial waste water (group I) showed severe histopathological changes in gills, lateral line organ and liver (Table 1) after phenol exposure. Gill damage was present in 70% of the fish, mostly in the form of massive hyperplasia of the gill epithelium. Necrotic foci were regularly found in this hyperplastic tissue, some of which had formed caseous nodules (Fig.2).
Exposure to phenol quite clearly left its mark on the bullhead liver also. This took the form of focal vacuolar degeneration in the hepatic parenchyma (Fig.4) and occurred in 50% of the fish.

Conspicuous pathological alterations were seen in the lateral line organ: its epithelium in most cases (60%) exhibited hyperplasia and hypertrophy, with leucocyte infiltration. In several cases (30%) a distended edematous space with diffusely distributed leucocytes had formed below the epithelium (Fig.6). Since neuromasts were only encountered in some of the sections, reliable conclusions regarding their pathological changes could not be drawn. In one case, however,

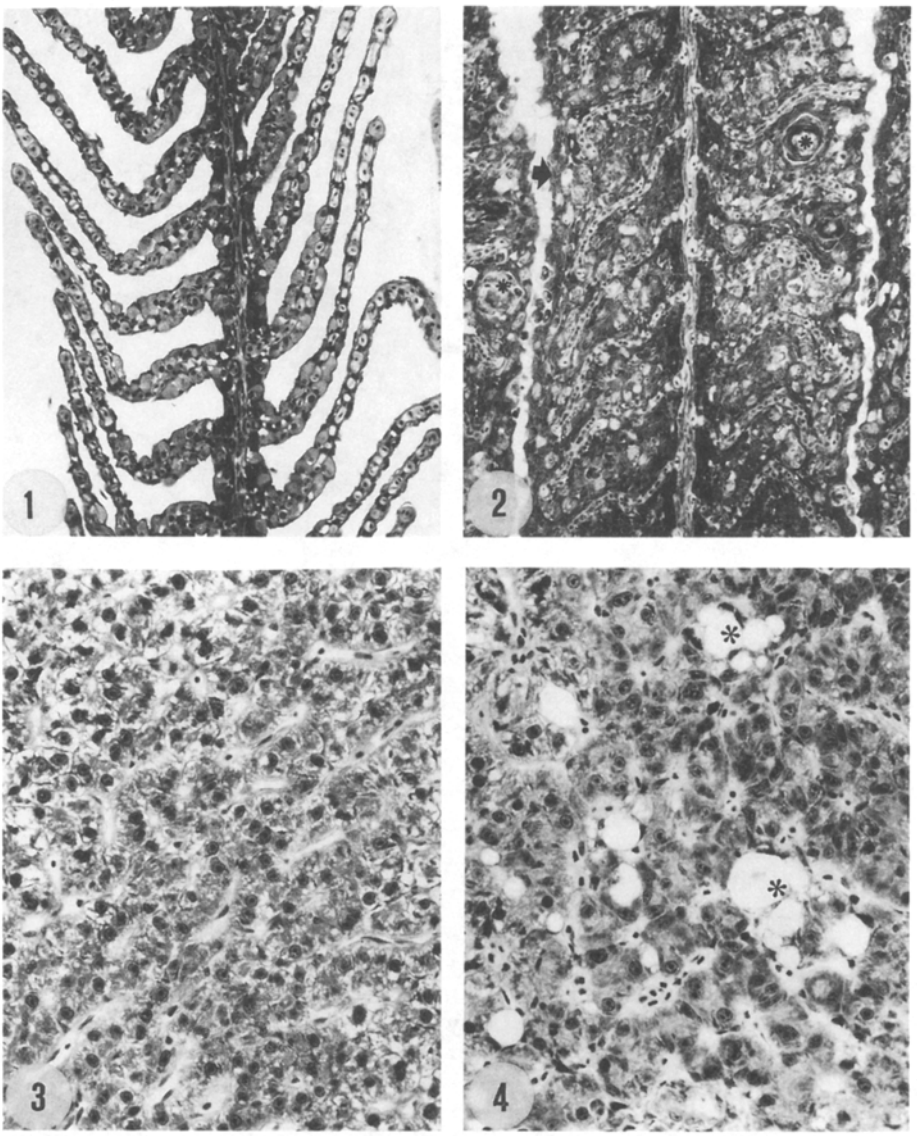

Figure 1. Gills of a control bullhead, x 200, MG/Giemsa.

Figure 2. Gills of a phenol-exposed bullhead of group I: severe hyperplasia of the gill epithelium (arrow), focal necroses, which have become caseous nodules (asterisks), x 200, MG/Giemsa.

Figure 3. Liver of a control bullhead, x 400, MG/Giemsa.

Figure 4. Vacuolar degeneration of the liver parenchyma (asterisks) of a phenol-exposed bullhead (group I), x 400, MG/Giemsa.

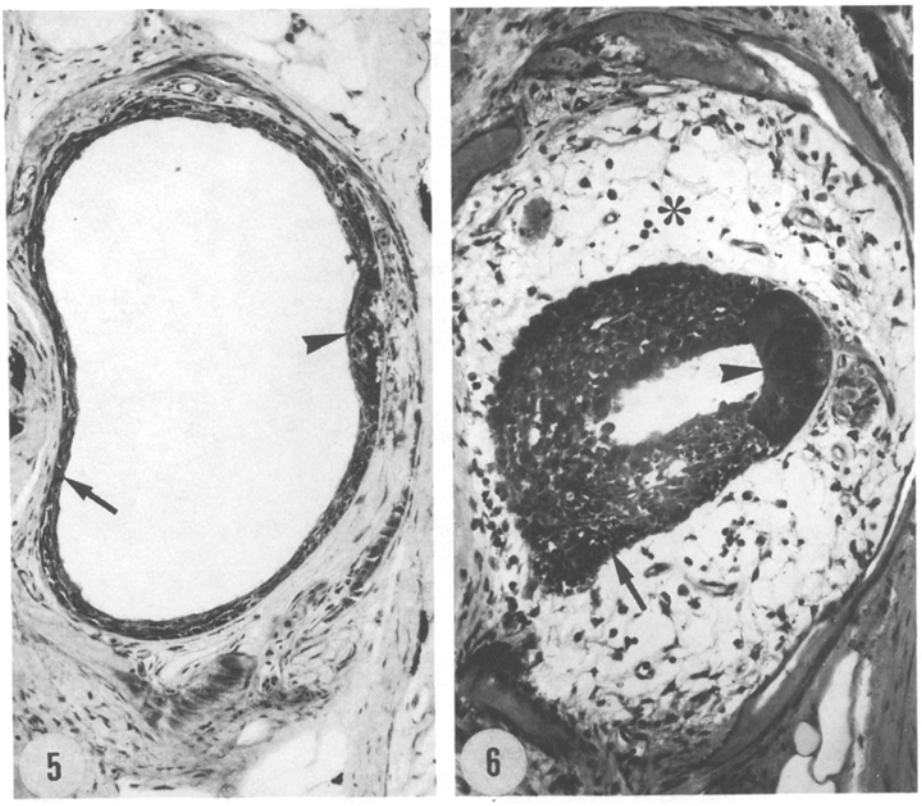

Figure 5. Lateral line canal of a control bullhead, arrow = epithelium of the canal, arrowhead = neuromast, x 250, MG/Giemsa.

Figure 6. Lateral line canal of a phenol-exposed bullhead (group I): severe hyperplasia of the canal epithelium showing leucocytic infiltration (arrow), subepithelial edema (asterisk), arrowhead=neuromast, x 250, MG/Giemsa.

vacuolar degeneration of the supporting cells was seen. Neither the kidney nor, surprisingly, the skin (which produced large amounts of mucus during the experiment) showed signs of pathological alteration.

The bullhead obtained from water within the sphere of influence of waste from paper mills (group II) exhibited no or considerably milder histopathological abnormalities in these organs after exposure to phenol (Table 1). The degree of gill damage was considerably less than in group I. In 30% of the fish, patchy adhesions of the secondary lamellae were recognizable, usually in the apical region of the primary lamellae, and were invariably accompanied by hyperplasia of the gill epithelium

with focal necrosis of the epithelial cells. The histological picture presented by the liver parenchyma was not different from that of the control animals.
Only one animal showed severe pathological changes of the lateral line canal: massive hyperplasia and leucocytic infiltration of the epithelium, and subepithelial edema. In two other individuals only mild pathological alterations were noted (Table 1).
Skin and kidney were completely normal in this group also.

Table 1: Incidence of the most important histopathological findings in *Cottus gobio* of group I and II. Number of specimens is 10.

	CONTROL		PHENOL EXPOSED	
	I	II	I	II
GILLS				
Hyperplasia, mild	-	-	4	3
severe	-	-	3	-
Necrotic foci	-	-	4	2
LATERAL LINE ORGAN				
Hyperplasia/Infiltration, mild	-	-	2	2
severe	-	-	3	1
Edema, mild	-	-	1	-
severe	-	-	2	1
LIVER				
Vacuolar degeneration, mild	-	-	2	-
moderate	-	-	3	-

Phenol is used by microorganisms as a carbon source and is therefore relatively rapidly broken down in aquatic ecosystems, so that running water is quite efficiently self-cleansing (Babich and Davis 1981). Nevertheless, since phenol is quickly and easily taken up by the intestinal tract and surface organs (gills, skin) (Babich and Davis 1981; Razani et al. 1986), it is of ecotoxicological importance and has various pathological effects. For example, unusual behavior patterns such as uncoordinated movements, disturbed balance, hyperactivity and gasping at the surface have been observed in various fish species (Jones 1951; De Graeve et al. 1980) following exposure to similar phenol concentrations, and it has been assumed to have negative effects on the nervous system (Lloyd and Swift 1976). Histopathological changes following phenol intoxication have been reported in skin, gills, musculature, heart, liver, kidney, spleen and ovaries (Reichenbach-Klinke 1965; Mitrovic et al. 1968; Razani et al. 1986).
During the experiment the phenol-exposed fish of both groups produced excessive quantities of mucus, demonstrating unequivocally the aggressive action of phenol on surface tissues. Increased mucus production in the gills and intestine of phenol-exposed fish was earlier reported by Reichenbach-Klinke (1965). Thus it is all

the more surprising that histological examination revealed no changes in the epidermis. Since the skin of the bullhead has no scales, the greater production of mucus obviously compensates for the lack of such protection. An impressive and apparently also highly efficient barrier to chemical toxicants is provided by very large and closely packed mucus cells.
In contrast to the skin, the epithelium of the lateral line canal - especially in group I - proved to be very sensitive to phenol and suffered severe damage. The fact that the mucus cells seen in this tissue were only small and scattered indicates a distinctly weaker protective potential than that of the skin. Similar histopathological changes in the lateral line system of *Salmo gairdneri* and *Fundulus heteroclitus* have also been reported to result from poisoning with mercury, silver, copper and methoxychlor (Gardner 1975).

The deleterious effect of phenol on the surface organs was also confirmed by the histological state of the gills. The most pronounced reactions were seen in the bullhead from the Lake Traunsee (group I), in some cases with a degree of tissue damage that, due to the resulting drastic reduction in respiratory surface, would have caused death within a very short time. Similar findings were reported by Lloyd and Swift (1976), who found serious damage to the respiratory epithelium due to phenol, with resulting asphyxiation of the animals.

Liver damage due to phenol was seen only in group I, in which vacuolar degeneration of the hepatic parenchyma was especially striking. Their finding of elevated blood levels of hepatic enzymes following exposure to phenol was attributed by Nemcsok and Benedeczky (1990) to liver necrosis. The same authors - in agreement with our own results - found vacuolar degeneration in the cytoplasm of hepatocytes (Benedeczky and Nemcsok 1990).

The mechanism of action of phenol is unknown. Within the organism some of the phenol is rapidly conjugated (to form phenyl sulphate or phenyl glucuronide) and thus detoxified (Von Oettingen 1949). The conjugates, and free phenol, are excreted via the urine and bile. The toxic effect is primarily attributed to the free substance (Deichman and Keplinger 1962).
The fish of group II proved to have a much higher resistance to phenol. Tissue damage to gills and lateral line system was much less pronounced than in group I, and liver damage attributable to phenol was entirely lacking. The differences in the pre-exposure conditions and development of resistance may be responsible for the different reactions of the two groups of bullhead. The fish of group II were taken from a river contaminated with paper-mill waste water and were therefore already

stressed. Physiological adaptation following pre-exposure to phenol has been reported in guppies (*Lebistes reticulatus*), bitterling (*Rhodeus sericeus amarus*) and perch (*Perca fluviatelis*) (Flerov 1971; EIFAC 1972). Guppies that had been exposed to phenol over three generations proved to be five times more resistent than non-adapted individuals (Flerov 1971). One possible reason for the higher phenol tolerance of our group II bullhead might be the presence of induced detoxification mechanisms and, as a consequence, more efficient detoxification. Free-living individuals of the river population from which group II originated have been found to have an elevated activity of hepatic aryl-hydrocarbon hydroxylase (Bucher et al. in preparation). Oikari & Kukkonen (1988) found a lower bioaccumulation of a phenol compound in pre-exposed roach than in control animals, and attributed this phenomenon to an induction of conjugation enzymes, which play an important role in detoxification.

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