Mixed Function Monooxygenase of Fish as an Indicator of Pollution of Aquatic Environment by Industrial Effluent

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The aquatic environment is subject to a heavy and diverse pollutant loading, particularly in industrial districts. The response to this loading by aquatic animals is largely unknown. An enzyme system that in mammals responds readily to the presence of foreign chemicals is the mixed function monooxygenase (MFO) system, the very enzymes involved in the elimination of foreign compounds. The MFO enzymes are induced by a large number of xenobiotics thus increasing the rate of their elimination (REMMER, 1972). On the other hand certain hepatotoxic compounds and 'dirty' environment have the opposite effect according to SASAME et al. (1968) and VESELL et al. (1973). It has been reported by DEWAIDE (1971) and PAYNE and PENROSE (1975) that fish in polluted water have a higher *in vitro* capacity to metabolize foreign compounds than those in clean water. In this study pike (*Esox lucius L.*) from heavily polluted Lake Vatianjärvi in the central Finland were studied and a largely opposite result is obtained.

Materials and methods

The pike (*Esox lucius L.*) used in this study were caught with bag-net or weir in May 1975. The following two groups were studied: *Experimental group* consisted of pike caught from Lake Vatianjärvi. This lake is heavily polluted by mixed effluents from pulp and chemical factories (sulphate and sulphite processes, bleaching etc.) and human communities in the town of Äänekoski (about 20 km upstream from the Lake Vatianjärvi; population of about 20,000). The fish were transferred into 1 sq. m (0.4 cub. m) aquaria of Vatia Fish Research Station (VFRS). The water supply (about 8 l/min/aquarium) was straight from the polluted lake. Water temperature was 7.5± 0.5 °C (the same as in the lake) and the O_2 concentration varied between 75 and 85 % of saturation. For further details on water quality see OIKARI and SOIVIO (1976).
Control group consited of pike caught from Vanhaselkä in the Lake Päijänne, which is considered relatively unpolluted according to HATTULA (1973). The fish were transported to Laukaa Fish Culture Research Station (LFCRS) (about 70 km) in oxygenated water tanks. The fish were allowed to recover for one week at 7.6±0.2°C in 4 sq. m fibre glass tanks which had their water supplied at about 50 l/min from the unpolluted Lake Perunkajärvi. The O_2 content of the water varied between

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75 and 85 % of saturation (OIKARI and SOIVIO, 1976).

Pikes of both groups (weighing from 500 to 2500 g and of both sexes) were handled and sampled identically as described elsewhere by OIKARI and SOIVIO (1976). The fish were stunned by a blow on the head, the livers were excised, subsequently to blood sampling, without a delay, wrapped in aluminium foil and immersed in liquid nitrogen for storage and transport.

The livers were thawed and homogenized by using a Potter-Elvehjem homogenizer to give a homogenate in 0.1 M Na/K phosphate buffer (pH 7.4) containing 200 mg liver per ml of homogenate. Mitochondria, nuclei and cell debris were sedimented by centrifugation at 10,000 x g for 20 min. Microsomes were obtained from the 10,000 x g supernatant by re-centrifuging it at 100,000 x g for 60 min.

Assays for cytochrome P-450 and NADPH-cytochrome c reductase were carried out according to the methods of GREIM et al. (1970) and MASTERS et al. (1967), respectively. The 3,4-benzpyrene hydroxylase activity was measured by the method of NEBERT and GELBOIN (1968), aminopyrine N-demethylase was measured by the method of NASH (1953), aniline hydroxylase was measured by the method of KATO and GILLETTE (1965) and lipoperoxidation was determined according to GHOSHAL and RECKNAGEL (1965).

Cytochrome P-450 and NADPH-cytochrome c reductase determinations were carried out at room temperature. The incubation temperature for the other assays was 30 °C which was established to be near the optimal temperature for pike as well as for several other species of fish.

Results and discussion

The results are compiled in the table 1. The overall trend, contrary to what was found by DEWAIDE (1971) and PAYNE and PENROSE (1975), was that the fish from the polluted water showed a reduced in vitro MFO capacity. The 3,4-benzpyrene hydroxylase activity was low in all fish in comparison to results obtained with trout or rat (AHOKAS et al., 1975), but in the fish of the polluted Lake Vatianjärvi the activity was only 1/6 of the activity in the control pike. Also aminopyrine N-demethylase activity in the fish from the polluted water was only about 70 % of that of the control fish. The differences in the aniline hydroxylase activity were not statistically significant. The above could be an indication that the pollution in the Lake Vatianjärvi is of a nature causing hepatotoxic effects leading to reduced MFO capacity.

More detailed investgation of the subcellular fractions indicate that there is a decreased amount of cytochrome P-450 in the hepatic microsomes of pike from the Lake Vatianjärvi. According to numerous models e.g. VAINIO (1973) cytochrome P-450, the terminal component of the MFO requires NADPH dependent reducing component in a close structural proximity

Table 1.

	Experimental group (Vatianjärvi)				Control group (Lake Päijänne)		
	Mean	SD	(n)	p<	Mean	SD	(n)
Cytochrome P-450, microsomal nmol/g liver	2.0	0.6	(7)	0.05	2.9	0.5	(6)
NADPH-cyt. c reductase nmol cyt. c reduced/ g liver/min							
microsomal	96	26	(7)	N.S.	119	25	(6)
'soluble'	598	211	(7)	0.001	284	25	(6)
3,4-Benzpyrene hydroxylase pmole/g liver/min	3	2	(7)	0.01	18	12	(6)
Aminopyrine N-demethylase nmol/g liver/min	4	1.7	(7)	0.05	5.1	5	(6)
Aniline hydroxylase nmol/g liver/min	1.1	1.7	(7)	N.S.	0.4	0.2	(6)
Lipoperoxidation AU/g liver/min	0.003	0.003	(4)	N.S.	0.004	0.001	(4)
Protein mg/g liver							
microsomal	26.9	5.4	(7)	N.S.	23.8	3.4	(7)
'soluble'	26.5	7.0	(7)	N.S.	23.5	5.0	(7)

AU = Absorbance units

in order to function normally. This cytochrome P-450 reductase, measured as NADPH-cytochrome c reductase, cannot be removed from the endoplasmic reticulum without having an impairing effect on the activity of cytochrome P-450 and cytochrome P-450 dependent xenobiotic oxidations. It can be noted that in the case of the Lake Vatianjärvi pikes a large amount of NADPH-cytochrome c reductase has been released into the 100,000 x g soluble fraction. The measurable total NADPH-cytochrome c reductase activity is higher in the experimental group. Calculating these values per mg of recovered protein does not alter the results as there is no marked variation in the protein contents. This finding is similar to what has been found with the effects of carbon tetrachloride in mammals where NADH-cytochrome c reductase was released from the endoplasmic reticulum to the extent that it was detectable in blood (YAMAMOTO et al., 1973). Lipoperoxidation has been attributed to cause solubilization of NADPH-cytochrome c reductase from the endoplasmic reticulum in a similar way (HÖGBERG et al., 1973). The fact that no appreciable amount of cytochrome P-450 is found in the 100,000 x g soluble fraction of either the control or Lake Vatianjärvi fish, disputes the thought that soluble fraction of the experimental fish liver was contaminated by less easily sedimentable microsomes and supports the thought that the pollution of Lake Vatianjärvi have similar harmful effects as some hepatotoxic chemicals like carbon tetrachloride. The release of NADPH-cytochrome c reductase from the endoplasmic reticulum may play a role in the lowered monooxygenase activity of the fish from the water polluted by effluent from wood processing industry. If found consistent, the above finding could be used as an indicator of the severity of certain kind of pollution and provide a clear distinction between the pollution caused by petroleum products which have been found to cause induction of MFO (PAYNE and PENROSE, 1975). However, it must be brought to light that preliminary exposure studies in which two other species of fish (Salmo trutta lacustris and Salmo gairdneri) were exposed to the Lake Vatianjärvi water for one and four week periods, did not develop changes such as those seen in pike chronically exposed to the contaminated waters.

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