

Hepatic cytochrome P450IA of feral perch (*Perca fluviatilis*) affected by unbleached pulp mill effluents

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Polysubstrate monooxygenase (PSMO) enzymes act in xenobiotic metabolism by decreasing lipid solubility of organic contaminants, thereby facilitating excretion of the pollutants. PMSO enzyme activities measured from feral and caged fish have been used as bioindicators in monitoring the effect of bleached pulp mill effluents containing e.g. numerous chlorinated compounds known as potentially strong inducers^{1,2}.

Previous studies have shown that also unbleached pulp mill effluents have a slight effect on PMSO enzyme activities measured from fish liver³. This has been demonstrated by 7-ethoxyresorufin O-deethylase (EROD) activity, which is catalyzed by cytochrome P450IA proteins in fish. The aim of the present study was to determine if this elevation of EROD activity is based on the increase of the amount of cytochrome P450IA.

The mill releasing studied effluents is situated in Eastern Finland. It produces unbleached pulp and cellular board by applying neutral ammoniumsulphite process. The raw material used is mainly birch. The effluents undergo mechanical and biological treatment (anaerobic process). The purified effluent is drained into Lake Kallavesi about 100 meters from the mill shore and at that point passively diluted into the lake water to about 1 % concentration. The mill studied does not use chlorinated bleaching compounds.

Perch were caught at four seasons during the year 1990. Fish were caught from the polluted area downstream the sewer outlet and from the control site upstream the mill. Fish were killed, and the liver was removed and stored in liquid nitrogen. The isolated fractions of liver microsomes were prepared for enzyme analyses and immunoplotting.

The deethylation of 7-ethoxyresorufin was recorded by Perkin-Elmer spectrofluorometer in a kinetic reaction with resorufin as reference⁴.

Part of the winter and summer material were chosen for immunoblotting and P450IA protein detection. These samples were prepared by boiling with standard treatment buffer and send to USA.

Measurement of the perch cytochrome P450IA1 equivalents was performed as described previously³. 45 µg microsomal protein out of each sample and several standards from 0.125 to 1.5 pmol of scup, a marine teleost, cytochrome P450IA1 (scup P450E) were subjected to separation with SDS polyacrylamide gel electrophoresis in an 8 - 15 % acrylamide gradient gel. The proteins were electrophoretically transferred onto nitrocellulose and incubated with monoclonal antibody (Mab 1-12-3) to scup P450E and then with secondary antibody (goat

anti-mouse IgG linked to alkaline phosphatase (Bio-Rad)). The nitrocellulose was exposed to color developer and qualified by video imaging densitometry (Master Scan, Scanalytics/CSPI, Billerica, MA).

Biotreated unbleached effluents increased hepatic EROD activities in feral perch in winter, spring and summer, compared to control activities. The activities were 1.3 to 1.5 fold, 1.2 to 1.3 fold and 1.9 to 2.9 fold, compared to controls, in perch caught in winter, spring and summer, respectively. In autumn, unfortunately, no control fishes were caught though the activities at this season, too, were closely comparable to earlier ones.

Relative P450IA protein content of the perch samples gathered from the polluted area was higher both in winter and summer samples, compared to controls. The contents were 2.7 to 3.2 fold, compared to controls, in winter and 3.3 to 9.5 fold during the summer sampling.

The biotreated unbleached effluent received from the pulp and cellular board mill contain compounds which affect especially EROD activities. The induction of this monooxygenase enzyme is not high but clear. The most enhanced EROD activities were seen in summer.

The studies both with EROD activities and P450IA content expressed similar elevated trends at the polluted site, compared to controls. However, the cytochrome P450IA in exposed perch showed much stronger effects indicating the trend seen in catalytic activities to be real. Based on these findings it can be claimed that unbleached pulp mill effluents contain inducers acting at least like PAH-type inducers, the source and structure of which remain to be studied.

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