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# Metabolites of Xenobiotics in the Bile of Fish in Waterways Polluted by Pulpmill Effluents

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About ten years ago Lech and coworkers (Lech et al. 1973; Statham et al. 1976) proposed that metabolites of xenobiotics in trout bile could be used as a qualitative monitoring aid for certain types of aquatic contaminants. The fate of the parent compounds in trout were traced using radioactive isotopes, and high activity ratios between the bile and the water were detected e.g. 1065 for 3-trifluoromethyl-4-nitrophenol and 5360 for pentachlorophenol (Statham et al. 1976).

Waste waters from kraft pulp and paper mills contain a myriad of small molecular organic substances toxic to the aquatic biota such as fishes (Leach and Thakore 1977). Among these xenobiotics are chlorophenolic (CP) substances, like phenols and guaiacols, as well as unchlorinated and chlorinated resin acids (RA, Lindström and Nordin 1976; Holmbom 1980; Voss et al. 1980). We recently observed, that CP and RA absorbed into rainbow trout are effectively metabolized into glucuronide and/or sulphate conjugates in their livers (Oikari et al. 1984; Oikari and Ånäs 1985). Interestingly, a good positive correlation was noted between the mean percentage dilution of bleached kraft mill effluent (BKME) and the concentration of CP and RA conjugates in trout bile (Oikari and Niittylä 1985). Thus, the concentration-effect relationship apparently was quantitative in its nature. This study was initiated to see, whether or not the same was true also in natural fish populations living in waters into which BKME flowed continuously.

## MATERIALS AND METHODS

The fish were collected in early June (1983) by wire traps and seine nets at different locations upstream and downstream from a mill (Figure 1; Southern Lake Saimaa, Finland) annually producing ca. 275 000 t bleached kraft pulp and ca. 160 000 t LWC printing paper. The chlorine bleaching sequence in the mill is conventional (D/E-E-D-E-D; D = chlorination, E = extraction), and both soft (1/3) and hard (2/3) woods are used as raw materials. The waste waters, totalling ca. 225 000 $m^3$ /day, were first clarified by the removal of easily settleable suspended solids and

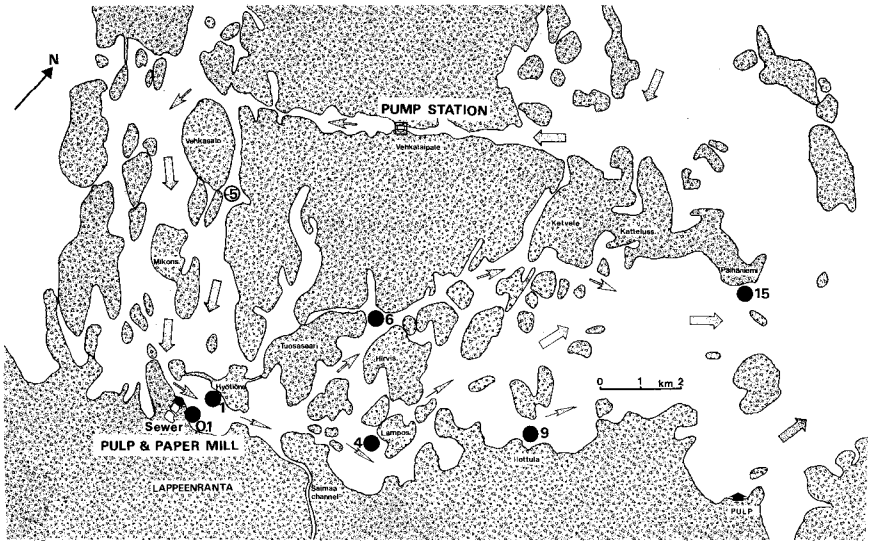


Figure 1. The study area at the Southern Lake Saimaa, SE Finland. Sampling areas downstream to the common sewer (black arrow) of a pulp and paper mill are indicated with black circles, the adjacent numbers denoting their distance in kilometers. The upstream control area (- 5 km) is indicated by an open circle. The approximate directions of lake water current are shown by grey arrows; the pumping station displacing ca. 40 $m^3$ /sec clean Lake Saimaa water maintains the circulation as shown.

then directed to an aerobic biological treatment system with an approximate retention time of 2-3 days. The quality of treated effluents, and distribution of certain chlorophenolics and resin acids in the lake receiving BKME have been described before (Oikari et al. 1985).

The water temperature was 16-19 $^{o}$C. The total length of roach (Rutilus rutilus L.) studied varied from 13 to 21 cm and that of perch (Perca fluviatilis L.) from 11 to 21 cm. Fish were sampled, both sexes in random, within 1.5 h of captured. Until that they were kept in the same water they were caught in. The nutritional status of animals was not controlled. Sampling of the bile was done by opening up the fish and aspirating the fluid inside the gall bladder with a fine needle (26 G) into a disposable syringe. The bile of several animals were pooled (0.4-1 mL). The samples were instantly frozen in dry ice and kept frozen (-20 $^{o}$C or less) in darkness until analysed.

Free and combined CP and RA were analysed by GC as described before (Oikari et al. 1984; Oikari and Ånäs 1985). The free substances were extracted first with hexane-acetone (3:1) from an acidified

(pH 3.5) sample. The rest was then subjected to alkaline hydrolysis (0.5 M KOH in ethanol, 70 °C, 3 h) and CP and RA freed were extracted again with hexane-acetone. The extracts were dryed under a nitrogen stream, and the substances silylated with BSTFA (= bis(trimethylsilyl)-trifluoroacetamide, Merck; 1 h, 60 °C). Concentrations were measured on a Varian 6000 or Hewlett Packard 5890A with a 25 m/0.25 mm i.d. fused silica capillary column coated with SE-30 (oven temperature 80 —> 270 °C, 4 °C / min). Signals from ECD and FID were simultaneously integrated, and identified CP with 3-5 chlorine substituents were monitored (cf. Holmbom 1980).

## RESULTS AND DISCUSSION

The concentrations of free CP and RA in the bile of fish living 0.1 - 6 km downstream from the effluent pipe were fairly low, ranging on an average from about 1 to 8 ug/mL for CP and from 1 to 9 ug/mL for RA (Table 1). The material obtained was insufficient for concluding whether there are any differences between the species in this respect. Of the free CP, tetrachloroguaiacol and pentachlorophenol were the most abundant substances, whereas free RA was completely made up of dehydroabietic, abietic and isopimaric acids. In all, the free CP in particular reflected fairly well increasing distance from the BKME source and, furthermore, this corresponded with previous analyses of these xenobiotics in the water (Oikari et al. 1985).

The average total concentrations of conjugated CP and RA in the fish bile were up to 100-200 times higher than for the free substances. Figure 2 shows, that the concentration of conjugated CP in the roach bile decreased continually with increasing distance from the pulpmill. At 15 km from the effluent pipe, i.e. in the area also important for the local fisheries (vendace, bream, perch etc.), the levels of conjugated xenobiotics still were significantly ($P < 0.001$) higher than in the premill reference area. Compared to CP, conjugated RA decreased at first faster but they were still present 9-15 km from the BKME pipe (Fig. 2). Thus the roach, possibly being a fairly local species, nicely reflected the average concentration of these xenobiotics in the animal's ususal cruising area. The span that the roach bile analyses actually are averaging, however, need to be investigated in more detail. Anyway, it is conceivable that roach may be a suitable species for quantitatively monitoring such low-level chemical loads which originate in pulp mills and are directed towards fish populations in local waterways.

Unlike roach, the results obtained from perch did not reveal such a consistent relationship between the distance of BKME source and the bile concentrations of CP conjugates (Fig. 3). In particular, the levels closest to the mill were, despite obviously higher concentrations in water (cf. Oikari et al. 1985), lower than

Table 1. Concentrations ( ug/mL ) of free chlorophenolics and resin acids in the bile of roach and perch caught at various distances from the sewer of bleached pulp mill effluents.

| Distance | *Rutilus rutilus* | | *Perca fluviatilis* | |
|---|---|---|---|---|
| km | Phenolics | Resin acids | Phenolics | Resin acids |
| 0.1 | NA | NA | 6.3 ± 3.1 (5) | 8.6 ± 4.2 (5) [1] |
| 1 | 7.5 ± 3.7 (2) | 3.2 ± 1.7 (2) | 5.8 ± 1.4 (3) | 8.1 ± 8.3 (3) [2] |
| 4 | 3.5 (1) | NA | 3.4 ± 1.9 (3) | 1.0 (3) [1] |
| 6 | 3.4 (1) | 1.9 (1) | 2.0 ± 1.0 (3) | 1.1 (2) [1] |
| 9 | Traces | 3.6 (1) | NA | NA |
| 15 | NA | NA | 1.1 (2) [1] | 0.4 (2) [1] |

Mean ± SD (N = number of sample pools, each consisting of 2-5 fish)
NA = not analysed
1) Being non-detectable (< 0.02 ug/mL) in one sample
2) Being non-detectable in two samples

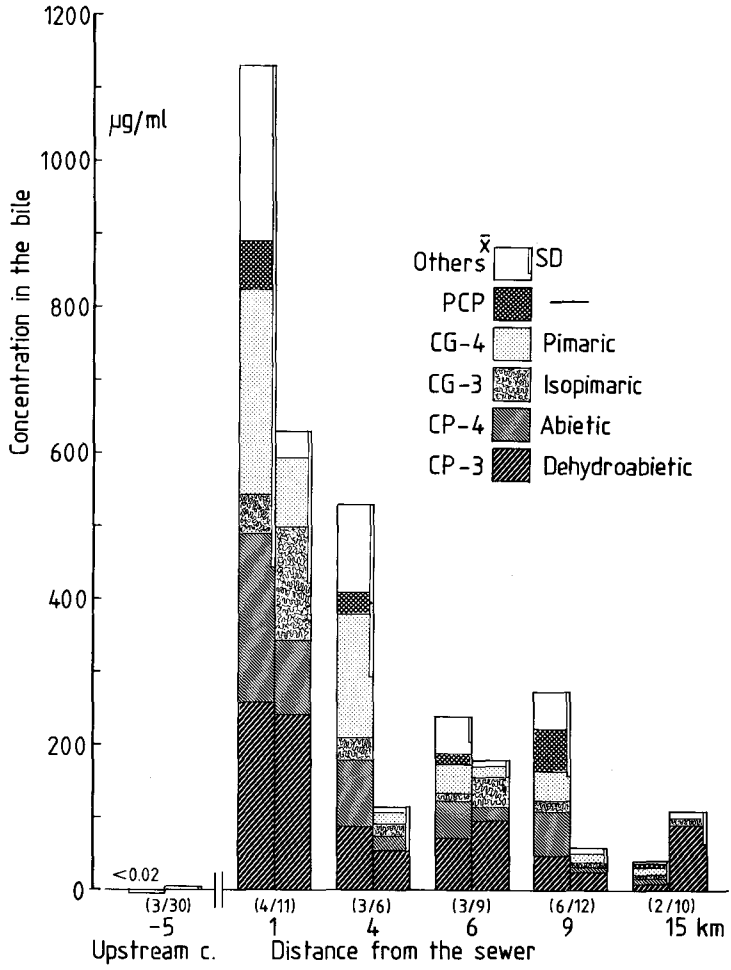


Figure 2. Concentrations of conjugated chlorophenolics (left column) and resin acids (right column) in the bile of roach, Rutilus rutilus (L.), caught downstream from an integrated pulp and paper mill at southern Lake Saimaa. For the location of sampling areas, see Fig. 1. Below each pair of columns, (number of sample pools analysed / number of animals sampled) are given.
Abbreviations: CP-3 = 2,4,6-trichlorophenol, CP-4 = 2,3,4,6-tetrachlorophenol, CG-3 = 4,5,6-trichloroguaiacol, CG-4 = 3,4,5,6-tetrachloroguaiacol and PCP = pentachlorophenol; SD = standard deviation.

at the more distant sampling localities. There are, at least, two different explanations for this. Firstly, perch may make periodical trips away from the vicinity of the final catching site and some specimens may have entered the area from relatively clean upstream habitats just before they were caught. Thus those animals which

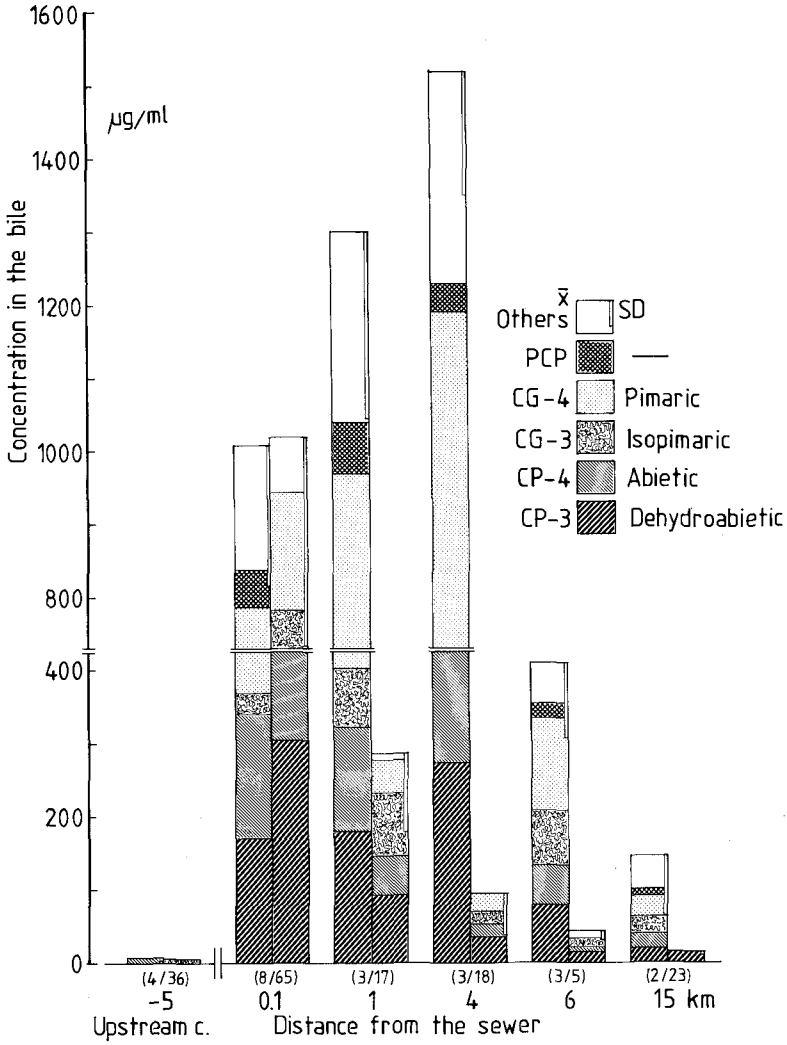


Figure 3. Concentrations of conjugated chlorophenolics (left column) and resin acids (right column) in the bile of perch, Perca fluviatilis (L.), caught downstream from an integrated pulp and paper mill at southern Lake Saimaa. For the location of sampling areas and for the further details, see Figs 1 & 2.

may have been in the polluted waters only for a short period would have lower concentrations of the conjugates in the bile. Secondly, environmental conditions, including high levels of toxicants themselves, may interfere with the metabolic processes in the liver of fish living close to the mill sewer. For instance, water oxygen concentration in the first three kilometers from the mill was generally found to be 5 mg/L or less (in June; cf. Oikari et al. 1985 ) and this may well interfere with detoxication reactions through cellular hypoxia. At more distant areas, e.g. 6-15 km from

the sewer, water oxygen concentration is sufficien for maximum production of biliary conjugates in perch.

On the other hand, the behaviour of RA conjugations in perch was ideal i.e. their level steadily decreased with increasing distance from the pulpmill effluent pipe (Fig. 3, right columns). In my opinion this observation is as such in contradiction with both viewpoints given for the simultaneous inconsistency of CP conjugations. All in all, however, it has been hypothesised that there are different or partially separated cellular mechanisms in biotransformation or transport, or in both, for the two classes of xenobiotics (cf. Klaassen and Watkins 1984).

To our knowlwdge this is the first time that the basic observations made using radiolabelled model compounds (Lech et al. 1973) have been shown to be true also in natural fish populations living downstream from a pulp and paper mill. Furthermore, bile analyses from roach revealed a good quantitative distance-response relationship with respect to chlorophenolics and resin acids. To clear up the primary action of characteristic BKME on fish health and on fish populations, research on the metabolic connections between the excretion of BKME xenobiotics and endogenous physiological substances (such as steroid hormones, bile acids and bilirubin) may be very fruitful.

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