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Kraft Pulp Mill Effluent Components Cause Liver Dysfunction in Trout

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Wide areas of water in Scandinavia and North America are polluted by both bleached and unbleached kraft pulp mill effluent (KME). Many kinds of sublethal physiological effects on fish have been described, including alterations in liver glycogen and blood glucose concentrations (McLEAY 1973, McLEAY & BROWN 1979), decline in numbers of circulating leucocytes (McLEAY 1975) and increase in ventilatory water volume and oxygen uptake (DAVIS 1973). All these acute effects may be grouped under the concept of "general stress syndrome" (WALDEN & HOWARD 1977) and they do not describe specific toxic actions of KME or its components in fish.

UDP-glucuronyltransferase (UDP-GT, EC 2.4.1.17) in the vertebrate liver participates in many types of glucuronic acid conjugations, both of xenobiotics as well as of such endogenous compounds as bilirubin and steroid hormones (DUTTON 1966). We have previously described that the resin acids, which constitute the main part of acutely toxic compounds in KME (LEACH & THAKORE 1977), inhibit the trout liver UDP-GT *in vivo* down to 20% of the control activity (CASTREN & OIKARI 1979, OIKARI et al. 1982). It is not known, however, if this degree of inhibition could be compensated by some other metabolic alternatives. If not, many kinds of liver-origin disorders, e.g. jaundice (KRUZYNSKI 1979), would result.

Here we report evidence on impaired liver function in fish exposed to KME components. This study is part of our efforts to develop more specific bioassays for detecting and monitoring the sublethal impact on fish of the pulp and paper industry.

MATERIALS AND METHODS

Juvenile rainbow trout (*Salmo gairdneri*), weighing 24 ± 5 g ($\bar{x}$ ± SD) and with length of 13.9 ± 0.9 cm, were acclimatized to dechlorinated Helsinki City tap water for two weeks before this study conducted in March. A 12L : 12D (08:00-20:00) cycle was applied in the aquarium room, and the fish were daily fed *ad lib* with pelleted food (Ewos). Water temperature (12.5 with range of ± 0.3 °C) was the same as during the tests.

The toxic material studied was a sulphate soap preparation used for tall oil production (Ahlström Co., Varkaus, Finland). Its chemical composition simulates well the toxic contribution of KME (termed KME-Sa; HOLMBOM & LEHTINEN 1980). The tall oil content was 89.8% of dry soap, the rest being residual Na-salts

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and lignin material. Of the resin acids, which all contributed 25.0% of the total tall oil content, the different acids occurred as follows: 6.85% abietic, 3.91% palustric, 3.41% dehydroabietic, 3.32% neoabietic, 2.61% pimaric, 2.50% levopimaric, 1.62% isopimaric, 0.48% sandaracopimaric and 0.33% secodehydroabietic acid. The fatty acid content was 51.5% and the linoleic (22.7%), oleic (13.7%) and pinolenic (6.3%) acids constituted most of it (analysed according to HOLMBOM 1977).

The 96h LC50 of KME-Sa to yearling rainbow trout was 0.53 mg total resin acids/L (95% confidence limits 0.43 - 0.66 mg/L), corresponding 1.9 mg dry substance/L, which is very similar to previous determinations (LEACH & THAKORE 1977, HOLMBOM & LEHTINEN 1980). Exposures, made in 200 L glass aquaria, were of flow-through type (3.5 L water/g fish/d). The KME-Sa stock was dosed by peristaltic pump so that the first group of 9-11 fish was exposed at 0.3 x 96h LC50 for 3 days, the second one at 0.15 x 96h LC50 for 11 days, and the third - without poison - served as control. No mortality was observed. Test water pH was 7.3-7.4 and O_2 concentration was kept between 8-9 mg/L by aeration. The animals were not fed during the four days' period before sampling, and each fish was kept for the last 24h in a restraining cylinder.

Fish were stunned by a blow on the head, weighed, and the blood samples aspirated from the caudal vessels into heparinized syringes. The bile was collected by puncturing the gall bladder. The liver was then quickly removed, weighed, and divided for different analyses. In each case the same part was used for a given determination. Subsamples for glycogen and UDP-GT were immediately frozen and stored in liquid nitrogen. Plasma was separated without delay by Beckman Microfuge B, and a part from it precipitated in 0.6 M perchloric acid for glucose determination. The rest of the plasma and the bile was kept in the dark at 3-4 ^{o}C. All plasma and bile analyses were made within 3-4h of sampling.

Glucose, bilirubin and ammonia analyses were made using commercial test kits (Boehringer Mannheim, BRG, Cat.No:s 124028, 12 3919 and 125857). Glycogen concentration was measured, after treatment of tissue pieces as described in HARRIS et al. (1974), as glucose equivalents. UDP-GT was assayed from homogenate at 25 ^{o}C according to HÄNNINEN (1966) using p-nitrophenol as substrate. Liver water was measured gravimetrically by drying the tissue pieces at 105 ^{o}C for 24h. Liver somatic index (LSI) is the percentage of liver weight of the total body weight. The statistical comparison to the controls was made by Student's t test.

RESULTS AND DISCUSSION

The results in Table 1 show that fairly effective depletion (-67%) of liver glycogen reserves takes place in three days. This observation agrees with earlier data on salmonid fishes (DAVIS 1976, McLEAY & BROWN 1975). Because no difference was seen in plasma glucose after three days (Table 1), it is possible that the peak

Table 1. Effects of KME-Sa on Rainbow Trout

		Control fish	Exposed for 3 days at 0.3 x 96h LC50	Exposed for 11 days at 0.15 x 96h LC50
LSI		0.88 ± 0.06 (11)	0.92 ± 0.07 (11)	1.24 ± 0.21 (8)***
Liver water	%	74.3 ± 0.6 (11)	74.2 ± 0.6 (9)	77.4 ± 0.9 (9)***
Liver glycogen	%	2.06 ± 0.54 (11)	0.67 ± 0.31 (11)***	0.58 ± 0.34 (9)***
Plasma glucose	g/L	0.91 ± 0.15 (10)	0.86 ± 0.33 (7)	0.58 ± 0.12 (5)***
Plasma ammonia	mM/L	0.71 ± 0.18 (9)	0.68 ± 0.12 (7)	2.08 ± 0.86 (7)***

TABLE 2.		Control fish	Exposed for 3 days at 0.3 x 96h LC50	Exposed for 11 days at 0.15 x 96h LC50
Liver UDP-GT	mU/g prot	223 ± 18 (9)	160 ± 19 (10)***	134 ± 13 (8)***
Plasma total bilirubin	mg/L	2.1 ± 0.5 (11)	1.6 ± 0.4 (7)	5.0 ± 0.7 (5)***
Bile direct bilirubin	mg/L	294 ± 88 (4)	167 ± 10 (3)*	190 ± 28 (3)
Bile bilirubin: direct/total	§)	0.99	0.83	0.88

Mean ± SD (n), * $P < 0.05$, *** $P < 0.001$. §) Mean ratio.

values were already passed at that moment, as in coho salmon (McLEAY 1977). After 11 days in 0.15 x 96h LC50 of KME-Sa the consequences in liver metabolism were more diverse. In spite of the lack of glycogen, the LSI was significantly increased. Since the length/weight ratio of trout remained unchanged, the main reason for this was the increased liver water concentration. Additionally, these fish displayed moderate hypoglycemia with accompanied increase in plasma ammonia concentration (Table 1). Less pronounced hypoglycemia was also observed in coho salmon after 25 d exposure to 30% (v/v) KME (McLEAY 1973). Elevation of plasma ammonia due to KME has not been described before, and may reflect the withdrawal of tissue proteins as a new energy source (PHILLIPS 1969). A probable source of blood ammonia is the lateral muscle which showed a significant decrease, by 13.5%, of its protein concentration after 30 d exposure to sublethal KME-Sa (OIKARI et al. unpublished). On the other hand, the actual site of ammonia production may be the liver (FORSTER & GOLDSTEIN 1969). As a possible consequence of higher blood ammonia concentration, the increase of Na^+ uptake from the environment should be mentioned (MAETZ & GARCIA-ROMEU 1964).

Inhibition of liver UDP-GT by KME-Sa was significant ($P < 0.001$) after only three days but even more pronounced (-40%) after 11 d at the lower exposure concentration (Table 2). The result is the same as that produced by pure resin acids (OIKARI et al. 1982). Simultaneously with the enzyme inhibition, decreases in concentration and in proportion of "direct" (presumably glucuronic acid conjugated) bilirubin seem to develop. However, for the increase of plasma bilirubin level a longer exposure was necessary. In the course of jaundice development the decreased capacity for conjugations must have been of primary importance (SCHMID & LESTER 1966). It remains to be seen if the transcellular transport of bilirubin glucuronides is also inhibited. Since all the plasma samples looked normal, some drastic increase in destruction of red blood cells is not an apparent explanation for the abnormal metabolic state.

In conclusion, our results indicate that the kraft mill effluent components (presumably resin acids) give rise to impairment of liver function in rainbow trout. At a late stage of intoxication jaundice is developed. It is preceded, however, by an inhibition of UDP-glucuronyltransferase activity and substantial depletion of liver glycogen.

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