Bioaccumulation in Fish of Chlorinated Phenols from Kraft Pulp Mill Bleachery Effluents

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During the last ten years the gross water pollution caused by oxygen consuming organic substances in the effluents from the pulp and paper industry has been considerably reduced. The greatest remaining pollution problem in the pulp industry is connected with the bleaching process. In this process the pulp is treated first with chlorine or a mixture of chlorine and chlorine dioxide in several steps and thereafter with strong alkali in order to extract the colored breakdown products of lignin from the pulp. The effluents from this multi-step bleaching procedure partly possess a strong brownish colour and generally constitute about half of the total effluent volume from a kraft pulp mill. This means that only in Sweden the kraft pulp bleacheries discharge about 300 million m^3 of waste water per annum.

To get a detailed description of the chemical composition of the very complex mixture of different organic compounds discharged from the cellulose pulp mills is a formidable task, still to a great extent refractory to fulfilment. An interesting approach to this problem has recently been used by the Canadian workers LEACH and THAKORE (1973, 1975), who concentrated their identification work on those compounds that could be shown to cause clear-cut biological effects on aquatic organisms.

In their studies on effluents from the first extraction step in kraft pulp bleacheries, LEACH and THAKORE (1975) were able to show that almost the total acute toxic effect to rainbow trout was exerted by a limited number of resin acid derivatives and chlorinated lignin breakdown products. The compounds responsible for 90 percent of the acute toxic action to fish were mono- and dichlorodehydroabietic acid, tri- and tetrachloroguaiacol and epoxystearic acid. This result relates of course only

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to that particular raw material used by the mills in western Canada that were included in the investigation and cannot be directly generalized to European conditions.

No studies have so far - to our knowledge - been reported on the accumulation or long term effects of these compounds on fish or other aquatic animals.

The first characterization of the acid-phenolic fraction of European, pine-wood pulp bleachery effluents, with respect to low-molecular chlorinated compounds was carried out by LINDSTRÖM and NORDIN (1976). They identified trichlorophenol, isomeric trichlorocatechols and tetrachlorocatechol in the effluents from the first chlorination step and trichlorophenol, isomeric trichloroguaiacols and tetrachloroguaiacol in the effluents from the first alkaline extraction step. However, the neutral fraction of these effluents still remains to be characterized for low-molecular chlorinated compounds.

The present work is an attempt to find out if the low-molecular chlorinated compounds, identified to be present in low concentration in the effluents from Swedish kraft pulp bleacheries and some of which have been shown to be acute-toxic to fish, furthermore show tendencies to accumulate in fish. If such a bioaccumulation is demonstrated to occur, this would be a strong rationale for further studies on possible long term or chronic effects of this group of compounds.

MATERIAL AND METHODS

Accumulation experiments

Rainbow trout yearlings (Salmo gairdineri) were exposed to effluents from the chlorination (C-step) and extraction steps (E-step), respectively, in a dilution of about 40 times in Baltic Sea water (salinity 7 ppt) in flow-through whole glass aquaria. Fresh effluent samples were taken every week at the mills, producing full bleach pine kraft pulp. The samples were stored in completely filled and closed polyeten containers at 3°C before use. Two different E-step effluents were tested; one after a C-step using 100 per cent chlorine and the other after a C-step where 5 per cent of the chlorine was replaced by chlorine dioxide (C_D-step). In both cases, the chlorine addition in the C-step was 70-90 kg/ton pulp and the addition of caustic in the E-step about 60 kg/ton pulp.

The effluent volumes were: from the C-step ~40 m^3/ton pulp and from the E-step ~20 m^3/ton pulp.

All exposures were carried out at a temperature of 8-10°C. The pH of the solutions was 7 ± 0.5. Water flow was adjusted to about 10 l/h and the fish density was about 10 g/l. In one of the experiments, exposure was discontinued after 7 weeks and the fish were allowed to excrete the accumulated compounds during 8 further weeks in nonpolluted sea water. Liver samples were composite samples from 4 to 6 individuals, whereas muscle samples were from individual fish.

Analytical

Sample preparation

Preparation of fish tissues and isolation of the chlorophenols in a benzene extract was based on the procedure by RENBERG (1974). The benzene extract was then evaporated to about 0.5 ml, derivatized with diazoethane LINDSTRÖM and NORDIN (1976) and finally purified on a column (2 cm x 0.5 cm i.d.) packed with silicic acid (Mallinchrodt, 100 mesh analytical grade) which was prewashed with benzene. The eluate (about 0.5 ml) was taken to gas chromatographic analysis.

Gas chromatography

A Perkin-Elmer gas chromatograph model 900 equipped with an electron capture detector (ECD) was used. The chromatograph was reconstructed to suit glass open-tubular columns with a Grob type injector and a purge gas system. The column used was a SE-30 glass open-tubular column (25 m x 0.25 mm i.d.) kindly given to us by Johan Roraade at the Swedish Tobacco Company. The column temperature was 170°C, injector temperature 250°C and manifold and ECD temperatures 200°C throughout the experiments. Carrier gas was argon-methane 95:5 with a column flow of 0.35 ml/minute. Split ratio was 1:20. The purge gas flow was 90 ml/minute.

GC-MID

A Finnigan gas chromatograph (9500)- mass spectrometer (3200 F) with an on-line computer (6000) was used. To obtain interpretable mass spectra, the small sample size is a limitation. However, the sensitivity can be increased by monitoring on specific ions (multiple ion detection, MID). Gas chromatographic data were as described above except for carrier gas flow (0.60 ml/minute, He).

The platinum capillary interface and ion source temperatures were 250°C respectively.

The instrument was calibrated before each run to 0.1 a.m.u. Detection of ions was performed at m/e 195.9, 197.9, 251.9 and 253.9 in the first cluster, 210.9, 212.9, 225.9 and 227.9 in the second, 244.9, 246.9, 259.9 and 261.9 in the third. Maximum run time for each cluster was 6, 3.5 and 2.5 minutes, respectively. So that the total run was 12 minutes. The first cluster had ions of ethylated 2.4.6-trichlorophenol and 2.6-dibromophenol, the second 4.5.6-trichloroguaiacol and the third tetrachloroguaiacol. Each ion was monitored for 64 msec every 200 msec.

Ion source controls were set as follows:

Emission current: 0.5 mA
Electron energy: 70 eV
Ion energy: ~10 V
Electron multiplier: 2.5 kV
Preamplifier sensitivity: 10^{-8} Amps/V

Reference compounds

2.4.6-trichlorophenol (Fluka AG, pract. grade). 4.5.6-trichloroguaiacol (m.p. 112.6° - 113.3°C) and tetrachloroguaiacol (m.p. 122.3°C - 122.6°C) were synthetized by Lars Strömberg *et al.* at the Swedish Forest Products Research Laboratory and kindly given to us.

Determination

Internal standard (10 µl of 260 µg 2.6-dibromophenol per ml ethanol) was added to the benzene extract before the ion exchange procedure RENBERG (1974). Quantitative analysis was performed on the gas chromatograph (ECD) as previously described LINDSTRÖM and NORDIN (1976). All samples were also quantified by means of MID as a double check. The various procedures agreed fairly well.

The precision of the GC analysis on replicate injections was around ± 1.5 % (calculated on 4.5.6-trichloroguaiacol). The overall accuracy had a coefficient of variation of about 12 % based on a triplicate analysis of various fat solutions (25 mg fat/ml benzene) spiked with the reference substances (about 1 µg/ml). All peaks quantified were within the linearity of the detector.

RESULTS AND DISCUSSION

As can be seen from table 1, a several week long exposure of rainbow trout for caustic extraction effluents diluted in Baltic Sea water to 2.5 % resulted in a significant uptake of at least three different chlorinated phenols into the fish body. Both the E-step effluent after a pure C-step and that after a C_D-step supported the uptake of the three chlorinated phenols. However, the first mentioned E-step effluent resulted in the highest equilibrium concentrations in the fish liver. A somewhat lower uptake of chlorinated phenols followed the exposure to chlorination effluents of similar concentration. In particular, tetrachloroguaiacol was taken up only to a small extent after exposure to C-step effluents. This difference might be due to the fact that the C-step effluents contain only low concentrations of chlorinated guaiacols, but instead relatively high amounts of the corresponding catechols (LINDSTRÖM and NORDIN, 1976). These latter compounds have a more polar character than the guaiacols and may be more readily cleared from the fish body.

In table 1 it is also indicated that the uptake in the liver was more efficient than that in the muscle. This was particularly evident for trichloroguaiacol.

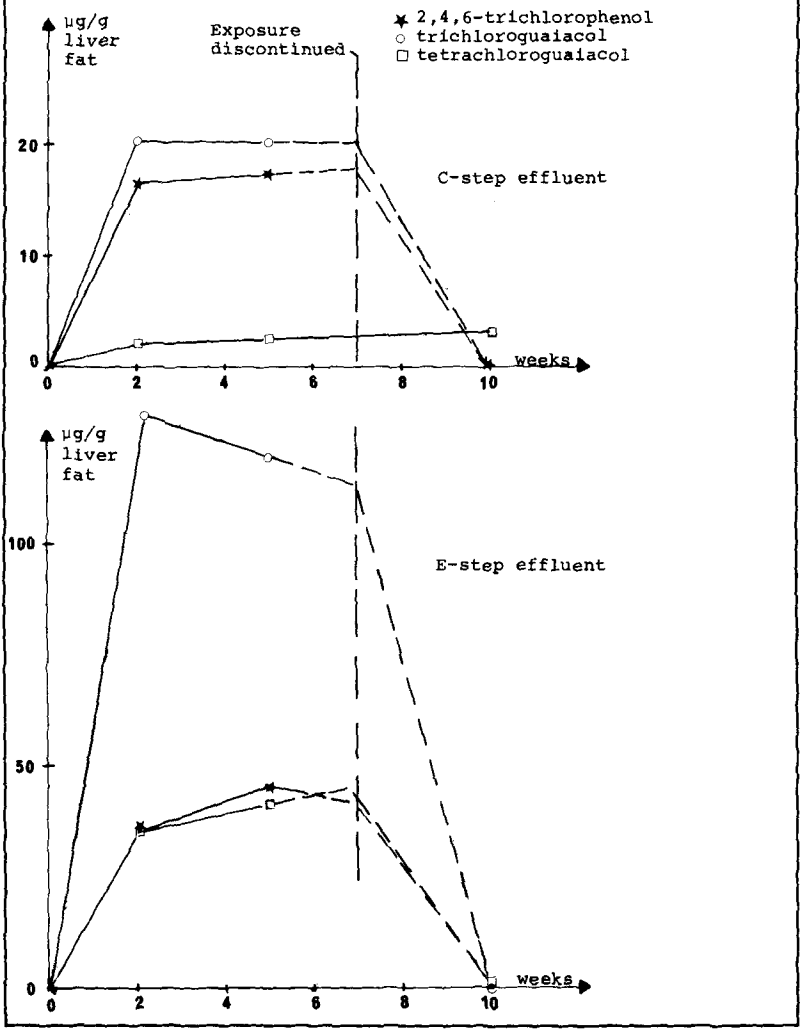
Figure 1 summarizes some preliminary data showing the rate of uptake of chlorinated phenols into fish liver during exposure to bleachery effluents. It also gives a preliminary idea of the rate of clearance after discontinuation of exposure. A steady state, where uptake is balanced by excretion and/or metabolism, seems to be achieved already after two weeks, at least for trichloroguaiacol. The here obtained rate of clearance for 2.4.6-trichlorophenol and trichloroguaiacol gives a tentative biological half life in the liver of less than 10 days. For tetrachloroguaiacol the picture is still somewhat obscure.

TABLE 1

Chlorinated phenols in body fat of rainbow trout after experimental exposure to sulphate pulp bleachery effluents diluted 40 times in Baltic Sea brackish water. Liver samples are composite samples of 4 to 6 individuals.

Effluent	Exposure time (weeks)	Fish mean weight (g)	Tissue	Fat content (%)	Concentration (µg/g fat) of: 2.4.6-trichlorophenol	Trichloroguaiacol	Tetrachloroguaiacol
C-step	2	150	Liver	2.6	16	19	3.0
"	5	150	Liver	2.3	17	20	2.5
E-step	2	120	Liver	2.9	35	130	35
"	5	130	Liver	2.5	45	120	42
Control	-	130	Liver	2.3	<0.1	<0.1	<0.1
E-step	6	63	Liver	2.5	2.9	3.8	23
after C_D-step	6	62	Liver	2.9	1.5	6.7	19
"	11	59	Liver	2.8	2.8	25	10
"	11	38	Liver	3.4	2.1	37	11
"	11	86	Muscle	1.5	trace	trace	1.3
"	11	50	Muscle	2.6	1.0	trace	5.9
"	11	34	Muscle	1.6	0.8	trace	3.6
Control	-	60	Liver	2.8	<0.1	<0.1	<0.1
"	-	70	Muscle	2.5	<0.1	<0.1	<0.1
ECD Response factor (calculated on weight of pure phenol)					0.64	0.75	0.25

Figure 1	Uptake and clearance of chlorinated phenols in rainbow trout liver fat after exposure to bleachery effluents diluted 40 times.	



The fishes exposed to C-step effluents did not begin to clear this latter compound from their liver after discontinuation of the exposure. This might be due to the fact that tetrachloroguaiacol showed a stronger tendency to be accumulated in the muscle than the other two compounds. During the clearance period, tetrachloroguaiacol residing in the muscle might be carried to the liver to be metabolized, which may result in a temporary storage of this compound in the liver.

Furthermore, tetrachlorocatechol, which is a possible metabolite of the corresponding guaiacol, was detected at a concentration of 3.9 µg/g liver fat about 8 weeks after discontinuation of exposure to E-step effluent.

These preliminary data indicate that the three chlorinated phenols here studied are rapidly taken up from water to fish, resulting in a steady state concentration in the liver fat already after a couple of weeks. They also indicate that clearance from the fish tissues is relatively rapid after discontinuation of exposure and that at least tetrachloroguaiacol may be metabolized by the fish before excretion.

For the similar compound, pentachlorophenol, the biological half life in the Guppy fish (Lebistes reticulatus) has been estimated at about 30 days (AHLING and JERNELÖV, 1969).

The necessary connection between laboratory experiments and field conditions is provided by the data in table 2. Thus, it is obvious that the same compounds that were found to be accumulated in rainbow trout during experimental exposures in the laboratory, were also identified in two species of fish caught in the vicinity of a mill producing full bleach kraft pulp. It can be seen that the relation between the three compounds identified in the fish livers varies from one individual to another, indicating that the turnover of the compounds probably is different.

Comparisons between the gas chromatograms from perch liver, rainbow trout liver and raw caustic extraction effluent (see Figure 2) show that most of the peaks are recurrent in all three chromatograms.

The more lipophilic chloroguaiacols are predominating, whereas most of the remaining compounds, particularly the chlorocatechols, occur to a less extent. It is obvious that accumulation of chloroguaiacols is most efficient

TABLE 2

Chlorinated phenols in liver fat of fish caught in the vicinity of a pulp mill producing full bleach sulphate pulp.

Species	Weight of fish (g)	Fat content (%)	Concentration (µg/g fat) of: 2.4.6-trichlorophenol	trichloroguaiacol	tetrachloroguaiacol
Perch (*Perca fluviatilis*)	200	2.3	2.7	11.5	8.2
Northern pike:					
(*Esox lucius*)	370	10.1	0.4	2.0	0.5
" "	600	5.5	0.5	1.5	4.4

although the chlorocatechols are predominating in the C-step effluent (LINDSTRÖM and NORDIN, 1976). As in extract from the E-step effluent the two isomeric trichloroguaiacols (peaks 10, 11) appear in the chromatogram as does the third isomer (peak 7). Although several compounds occurring in the E-step effluent were also found in the fat extract of fish liver, only three have been quantified in this work. This is due to the fact that these substances predominate in the extract. Furthermore, they were available at the laboratory so that response factors could be calculated on both MID and ECD. Compounds 10 and 11 (isomeric trichloroguaiacols) were assumed to have equal response factors and thus calculated as one peak. The isomer 4.5.6-trichloroguaiacol is the most abundant of the two compounds.

By optimizing the spectrometer it was possible to obtain mass spectra from the perch liver extract too, despite the fact that the amount detected was in the range of only 400 pg. The spectra were not perfect but the ions in the clusters obtained from the isoptops ^{35}Cl and ^{37}Cl were distinct and there was no doubt about the origin of the substances interpreted. The ratios of peak intensities were checked by means of MID.

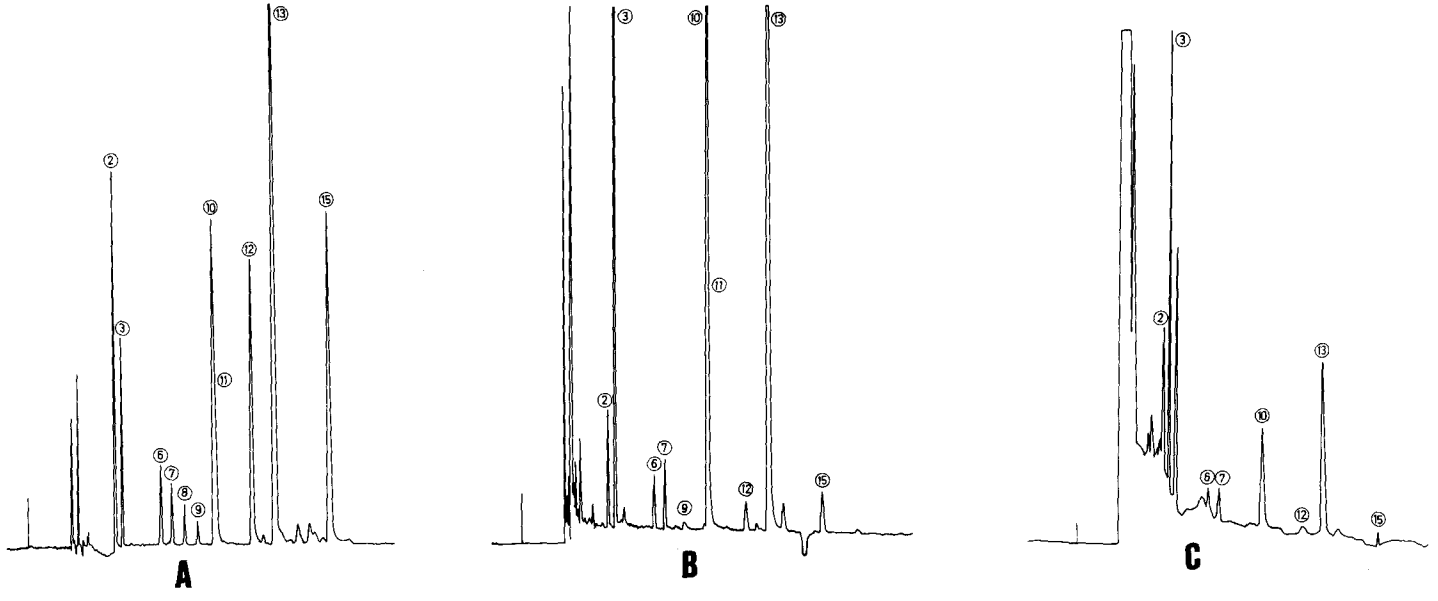


Figure 2. Gas chromatograms of ethylated chlorofenols.

2. 2.4.6-trichlorophenol 3. 2.6-dibromophenol (internal std.) 6. Dichloroguaiacol 7. Trichloroguaiacol 8. Dichlorocatechol 9. Trichlorocatechol 10. isomer to 7 11. isomer to 7 12. isomer to 9 13. Tetrachloroguaiacol 15. Tetrachlorocatechol. Column: 25 m x 0.25 mm i.d. SE 30 (glass). Detector: EC. Recorder chart speed 1 cm/minute.

A : Sample from a bleachery effluent (E-stage).
B : Liver extract from rainbow trouts exposed to effluent from the E-step.
C : Liver extract from a perch caught in the vicinity of a kraft pulp bleachery.

We would like to stress that the data presented in this report only demonstrate that a few chlorinated phenols occurring in sulphate pulp mill bleachery effluents, are bioaccumulated in fish. This does not tell us anything about their possible detrimental effects on fish or other parts of the aquatic ecosystem. Studies are now under way in our laboratories to check possible chronic effects on the fish of these compounds after accumulation to high levels. After continuous exposure for 2 weeks to caustic extraction effluents, diluted about 40 times, the maximum concentration of trichloroguaiacol detected in the liver of rainbow trout was about 3 µg/g fresh weight (130 µg/g fat).

For the sake of comparison, the maximum concentration of tetrachloroguaiacol obtained in fish muscle in these experiments (~0.15 µg/g wet weight) may be related to data on other chlorinated compounds reported in the literature. PCB concentrations in fresh water fish have been found in the range of 10-20 µg/g (in Japan) and between 2 and 400 µg/g in the USA, both calculated on wet weight basis (WHO, 1975). In marine fish from polluted areas MARTINSEN et al. (1976) have detected up to 1.8 µg/g hexachlorobenzene and 4.7 µg/g octachlorostyrene, respectively, calculated on wet weight basis. WESTÖÖ (1974), finally, found up to 3.2 µg/g of dieldrin in fish from a polluted river.

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