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Induction of CYP1A and Porphyrin Accumulation in Fish Hepatoma Cells (PLHC-1) Exposed to Sediment or Water from a PCB-Contaminated Lake (Lake Kernaala, Finland)

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ABSTRACT

Surficial sediments and lake waters from a polychlorinated biphenyl (PCB) polluted lake, Lake Kernaala, were monitored with a fish hepatoma cell line (PLHC-1). The cells were exposed to the lipid soluble fraction of the samples in 48-multiwell plates. 7-Ethoxyresorufin O-deethylase (EROD) activity, total protein content and porphyrin content were detected with a plate reader. Lake Kernaala sediment extracts induced CYP1A and porphyrins in the cells more than the reference sediment extracts from Lake Alasjärvi. ED_{50}s for EROD activity were achieved at lower sediment extract doses than ED_{50}s for porphyrin content. The pollutants were in a concentrated form in the sediment extracts. Thus, these samples more likely expressed the potential rather than the actual hazard of the chemicals. Lake Kernaala water extracts did not increase EROD activity in the cells at doses that corresponded to the actual concentration in the lake. However, the water extracts elevated EROD activity when concentrated and in this way expressed CYP1A induction. Based on our findings in sediment and water exposures, PLHC-1 cells are suggested for use in monitoring and comparing different water areas. © *1998 Elsevier Science Ltd. All rights reserved*

A Finnish lake, Lake Kernaala, is known to be polluted by polychlorinated biphenyls (PCBs). In a paper mill, located upstream from the lake, PCB chemicals were used for quality control (about 10001 during 1956–1983) and finally discharged into Lake Kernaala. Nowadays, the use of PCBs at the mill has ended; however, there are still reserves of these persistent halogenated compounds in the lake sediment. High levels of accumulated PCBs in the tissues of fish have also been measured (Koistinen *et al.*, 1989).

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Some polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons (HAHs) are documented to cause *Ah* receptor mediated induction of cytochrome P4501A (CYP1A) in a fish hepatoma cell line, PLHC-1 (Hahn *et al.*, 1993, 1996; Brüschweiler *et al.*, 1996; Celander *et al.*, 1997). High concentrations of the compounds can also inhibit CYP1A. This produces biphasic CYP1A induction curves.

Further, treatment with planar HAHs can lead to accumulation of porphyrins in PLHC-1 cells (Hahn and Chandran, 1996). HAHs cause a disruption in heme biosynthesis by stimulating the oxidation of heme precursors to porphyrins. The ability of PCB congeners to cause porphyria was correlated with their ability to induce 7-ethoxyresorufin *O*-deethylase (EROD) activity and CYP1A protein in the PLHC-1 cells, suggesting direct or indirect regulation of porphyrin accumulation via the *Ah* receptor and/or the induced CYP1A (Hahn and Chandran, 1996). Further, EC_{50} values for porphyrin accumulation were similar to, or slightly higher than, the concentrations at which peak EROD activities have been obtained. This suggests a relationship between the decline in EROD activity and enhanced porphyrin accumulation (Hahn and Chandran, 1996).

We studied whether CYP1A induction and porphyrin accumulation in the PLHC-1 cells can be used to detect the pollution in Lake Kernaala. Our aim was to survey the lake for contaminants. Therefore, the top layer of the sediments and surface waters were selected for test material.

In Lake Kernaala, sediments (with a grab sampler) and waters were collected from the southern (nearest to the mill), middle and northern part of the lake. In addition, sediments were collected from Lake Alasjärvi, a reference lake located upstream from the mill. The sediments were first dried at 65°C to constant weight and then, to obtain a lipid soluble fraction, extracted with dichloromethane in a Soxhlet apparatus. The waters were extracted separately with dichloromethane and diethylether by shaking in a separatory funnel.

Our bioassay material, PLHC-1 cells (derived from a hepatocellular carcinoma of a topminnow, *Poeciliopsis lucida*), were grown as monolayers in 75-cm^2 flasks at 30°C in Eagle's Minimal Essential Medium (containing Earle's salts, non-essential amino acids, L-glutamine and 10% of calf serum). The cells were subcultured as previously described (Hahn *et al.*, 1993). The PLHC-1 cells were exposed to the extracts dissolved in DMSO in 48-multiwell plates for 24 h. After this, 7-ethoxyresorufin *O*-deethylase (EROD) activity, total protein content and porphyrin content in the cells were measured with the Cytofluor 2300 fluorescent plate reader (Millipore) (for details see Kennedy *et al.*, 1995; Hahn and Chandran, 1996; Hahn *et al.*, 1996). In the first exposure with the sediments, EROD activity and total protein content were measured in two parallel samples. In the second experiment, all three parameters were measured in the same sample. In the water exposure, only EROD activity and total protein content were measured. After analyses, fluorescence data were imported into SigmaPlot (Jandel Scientific) for analysis and curve fitting. To obtain ED_{50} values for sediment extracts and EC_{50} for positive control, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), EROD and porphyrin data were fitted to a modified Gaussian function (for biphasic relationships) or to a logistic function (for sigmoid relationships) (Kennedy *et al.*, 1993).

The sediment extracts induced both EROD activity and porphyrin content in the PLHC-1 cells. The peak of EROD activities ranged from 50 to 86 pmol $min^{-1}mg^{-1}$ in the first sediment exposure (Fig. 1(A); only one sample from each site presented) and from 50 to 69 pmol $min^{-1}mg^{-1}$ in the second (Fig. 1(B)). Peak values were achieved at higher sediment extract doses at an upstream reference lake than in Lake Kernaala. The peak of porphyrin

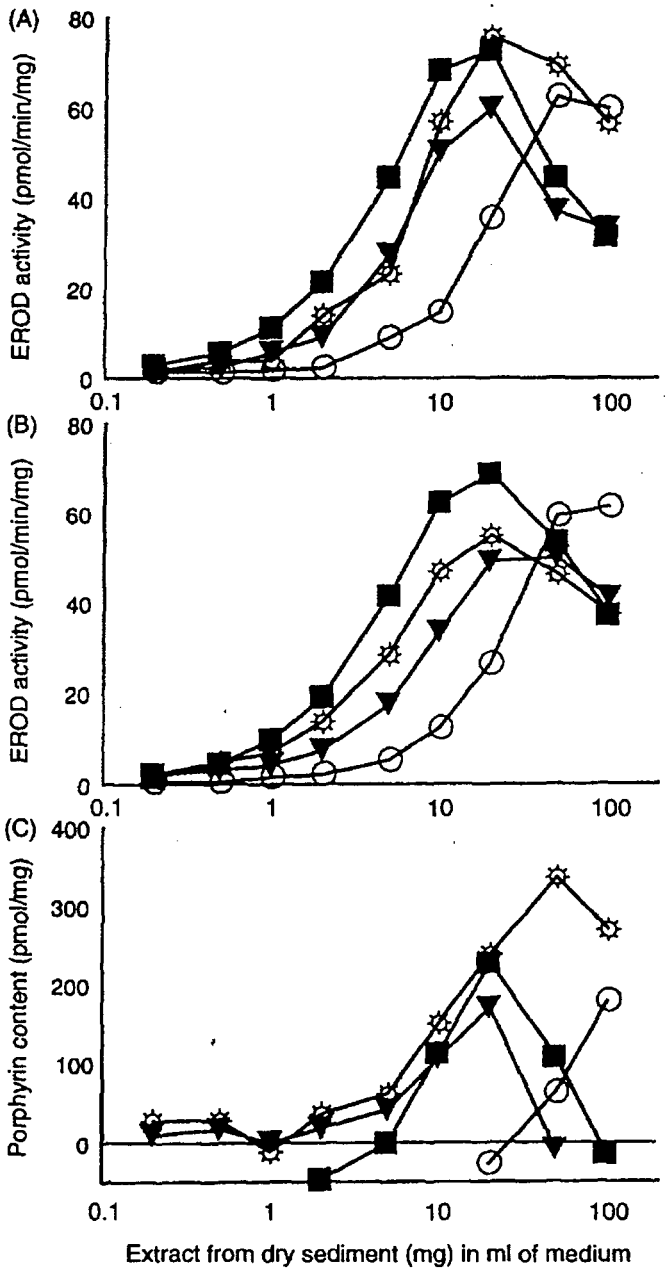

Fig. 1. EROD activity (pmol min^{-1} mg prot.$^{-1}$) in PLHC-1 cells after the (A) first and (B) second sediment exposure, as well as (C) porphyrin content (pmol mg prot.$^{-1}$) in PLHC-1 cells after the second sediment exposure. All the experiments illustrated here are made from the same samples. The surficial sediments were collected from Lake Alasjärvi (○) and from Lake Kernaala (south ■; middle ☼; north ▼). The mean response of three wells.

contents ranged from 171 to 336 pmol mg^{-1} (Fig. 1(C)). These values were reached at 20 or 50 mg ml^{-1} dose in Lake Kernaala and at 100 mg ml^{-1} dose in Lake Alasjärvi.

Thus, all the sediment extracts seemed to contain compounds which were able to induce CYP1A in the cells. Without chemical analyses we do not know whether these compounds were PCBs or other lipid soluble compounds which had been dissolved by

dichloromethane. Most probably there were a lot of PAHs involved. This could explain why the sediments collected from a proposed reference site, which was supposed to be free of PCBs, also increased EROD activity. The presence of some unknown natural compounds is also possible.

An increase of porphyrin content in the PLHC-1 cells is previously observed in studies with model HAHs (Hahn and Chandran, 1996). The porphyrin accumulation caused by our Lake Kernaala sediment extracts suggests that the samples contain planar HAHs. However, whether PAH can also cause porphyrin accumulation in PLHC-1 cells is not known. If porphyrin accumulation can be enhanced by PAHs, it is possible that these compounds, together with planar HAHs, were affecting the cells in our samples.

In our study, porphyrin content in the PLHC-1 cells was decreased at higher doses of sediment extract after reaching a peak at lower doses, suggesting biphasic induction curves as with CYP1A. A diminished porphyrin content at higher concentrations has earlier been detected with some samples in a study in which chicken embryo hepatocyte cultures were treated with individual HAHs as well as with a commercial mixture of PCBs (Aroclor 1254) (Lorenzen *et al.*, 1997). Cytotoxic effects could explain the biphasic nature of the EROD and porphyrin curves at high sediment extract doses. In our study, cytotoxicity in the cells treated with the sediment extracts was not measured with a separate assay. However, total protein content in the cells, which appears to be a rough measure for cytotoxicity, was not diminished more than 24% at any doses tested and not more than 22% at any of the highest dose.

The ED_{50} values of sediment extracts suggested a difference in the induction potency between exposed sites and reference site. Thus, sediments from Lake Kernaala were more potent than sediments from the reference Lake Alasjärvi as inducers of CYP1A activity and porphyrin accumulation. In the first sediment exposure, ED_{50} values for EROD activity in the cells ranged from 3.3 to 7.0 mg ml^{-1} in Lake Kernaala and were 15 and 18 mg ml^{-1} in Lake Alasjärvi (not illustrated). In the second exposure the ED_{50}s were similar: 3.8–7.8 mg ml^{-1} in Lake Kernaala and 23 mg ml^{-1} in Lake Alasjärvi. ED_{50}s for porphyrin content were 7.9, 12.3 and 8.6 mg ml^{-1} in the south, middle and north of Lake Kernaala, respectively (not illustrated). The ED_{50} for porphyrin content was 53 mg ml^{-1} at the reference lake. TCDD, a positive control, gave 0.263 nM and 0.598 nM as for EC_{50} for EROD activity and for porphyrin content, respectively. As for comparison for contaminated sediments, in earlier studies in River Narva, Estonia, a polluted sediment (744 ng total PAH/g dry sediment) showed ED_{50} value of 2.6 mg ml^{-1} for EROD activity in the PLHC-1 cells (Huuskonen *et al.*, 1998). In the same study, an accidentally and highly polluted sediment (278 400 ng total PAH/g dry sediment) gave 0.062 and 0.063 mg ml^{-1} as for ED_{50} values.

The Lake Kernaala water extracts induced EROD activity in the cells at high doses but not at a dose that corresponded to the actual concentration in the lake (Fig. 2). Water extracts from the northern part of the lake were less potent to induce EROD activity than the water extracts from the southern or middle part. We could not see clear differences between the effects of dichloromethane and diethylether extracted waters (see Fig. 2 for dichloromethane extracted waters). The potency of the sediment extracts to induce EROD activity was higher than the potency of the water extracts (not shown). This is consistent with the knowledge that sediments contain much higher concentrations of contaminants than overlying waters (Schindler *et al.*, 1995). However, since our studies examined

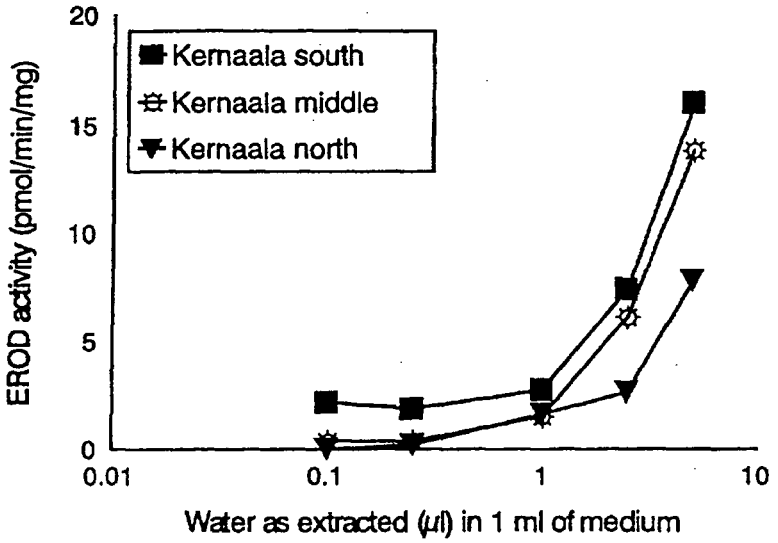

Fig. 2. EROD activity (pmol min^{-1} mg $prot.^{-1}$) in PLHC-1 cells treated with dichloromethane-extracts of waters from Lake Kernaala. The dose of water extract corresponding the actual concentration in the lake is calculated to be 1 $\mu l\,ml^{-1}$. For further information see Fig. 1.

organic extracts, differences between sediment and water in bioavailability of contaminants were not addressed.

There is no previous data whether Lake Kernaala waters are able to induce CYP1A in fish. Therefore, we were not able to make comparisons with our cell results and fish studies. That kind of comparison would help to evaluate the use of the PLHC-1 cells in biomonitoring.

In conclusion, PLHC-1 bioassays revealed that sediment and water extracts from Lake Kernaala contained PAHs, HAHs and/or other compounds with the capacity to induce CYP1A and porphyrin accumulation. Because of their ability to detect such contaminants, PLHC-1 cells could be used in monitoring and comparing other water areas as well.

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