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LAKE KERNAALA SEDIMENTS AND WATERS CONTAIN INDUCERS OF CYP1A AND PORPHYRINS

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Introduction

A Finnish lake, Lake Kernaala, has a history of polychlorinated biphenyl (PCB) pollution caused by an upstream pulp and paper mill since 1950's. During 1956-84 the mill used altogether about 1,000 litres of PCB chemicals for quality controlling. PCBs were finally discharged into L. Kernaala as part of the sewage water from the mill. Nowadays the use of PCBs at the mill has ended; however, because of the persistent nature of these compounds, there are still reserves of halogenated compounds in the lake sediment. High levels of accumulated PCBs in the tissues of fish have also been measured (1).

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 (2, 3, 4). In these studies CYP1A induction was measured as both CYP1A protein amount and catalytic enzyme activity (7-ethoxyresorufin *O*-deethylase activity, EROD). High concentrations of the compounds can also inhibit CYP1A. This produces biphasic CYP1A induction curves.

It has also been observed that treatment with planar HAHs can lead to accumulation of porphyrins in PLHC-1 cells (5). HAHs cause a disruption in heme biosynthesis by oxidizing heme precursors to porphyrins. The ability of PCB congeners to cause porphyria was correlated with their ability to induce 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 (5). Further, EC50 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 (5).

We studied whether CYP1A induction and porphyrin accumulation in the PLHC-1 cells can be used to detect the pollution in L. Kernaala. Our aim was to get an overview about the present situation in the lake. Therefore, the top layer of the sediments and lake waters were selected for test material.

Materials and methods

Representative samples from the upper layer (5 cm) of the sediment were collected from three sites in L. Kernaala and from one site in Lake Alasjärvi, an upstream reference lake. In L. Kernaala, sediments were collected from the southern (nearest to the mill), middle and the northern part of the lake. The sediments were first dried at 65°C up to constant weight and then extracted with dichloromethane in a Soxhlet apparatus. Surface water samples were collected from the study sites in L. Kernaala. The waters were extracted separately with dichloromethane and diethylether by shaking in a separatory funnel.

The PLHC-1 cells were exposed to the extracts in 48-well plates at 30°C for one day. After this, 7-ethoxyresorufin *O*-deethylase (EROD) activity, total protein content and porphyrin contents in the cells were measured with the Cytofluor 2300 fluorescent plate reader (Millipore) (for details see 3, 5, 6). All

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these parameters were studied in the sediment exposures. In the water exposure, only EROD activity and total protein content were detected. After analyses, fluorescence data was imported into SigmaPlot (Jandel Scientific) for analysis and curve fitting.

Results and discussion

All the sediments induced EROD activity in the PLHC-1 cells (Fig. 1). This indicates that there were CYP1A inducing compounds at all study sites. 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.

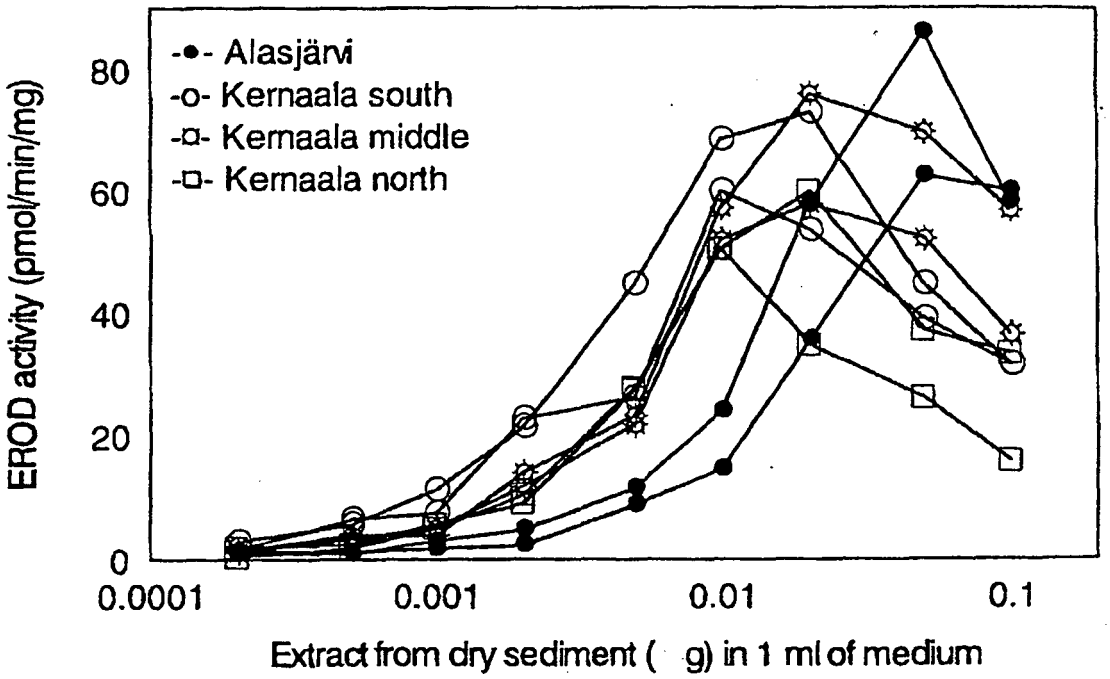

Fig. 1. EROD activity (pmol/min/mg prot.) in PLHC-1 cells after exposure to sediments extracted with dichloromethane. The surficial sediments were collected from L. Alasjärvi and from L. Kernaala (south, middle, north).

The peak of EROD activities ranged from 50 to 86 pmol/min/mg protein and was achieved at higher dose of sediment from the upstream reference site (extract from 50 mg dry sediment per 1 ml of medium) than from the L. Kernaala sediments (maximum at 10 or 20 mg/ml; Fig. 1). The highest potency to induce CYP1A was seen in the sediment samples collected from the site nearest to the mill (ED50 values for EROD activity 3.8 and 3.7 mg/ml in parallel samples). Also other sites showed relatively similar ED50 values (7.0 and 5.9 mg/ml in the middle and 5.0 and 3.4 mg/ml in the north). At the reference site the ED50 values (18 and 15 mg/ml) were reached at 2-5 times higher sediment doses than at the exposed sites. EC50 of a positive control, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), was reached at 0.26 nM concentration.

The ED50 values of sediments suggest a difference in the induction potency between exposed sites and reference site. This difference roughly characterizes the unpolluted and polluted areas in our study. A comparison of ED50 values between reference and polluted sediments could be used to categorize study sites in other waters, as well. As comparison for contaminated sediments, in earlier studies in River Narva, Estonia, a polluted sediment (744 ng total PAH/g dry sediment) showed ED50 value of 2.6 mg/ml for EROD activity in the PLHC-1 cells which were exposed to sediment extract for one day (7). In the same study, a highly polluted sediment (278,400 ng total PAH/g dry sediment; accidental release of asphalt as a cause of pollution) gave 0.062 and 0.063 mg/ml as for ED50 values for EROD activity.

Porphyrin concentrations in the cells were increased by treatment with the sediment extracts (not illustrated). ED50 values for porphyrin content in L. Kernaala were 8.9 mg/ml near the mill, 12 mg/ml in the middle of the lake and 10 mg/ml in the north. In L. Alasjärvi ED50 for porphyrin content was 52 mg/ml. EC50 of TCDD was reached at 0.60 nM concentration. All the ED50s for porphyrin were reached near the sediment dose where the peak of respective EROD activity was seen. This mode of action was similar to that seen with model compounds in earlier studies (5).

The L. Kernaala water extracts induced EROD activity in the cells at high doses (highest EROD activity around 15 pmol/min/mg prot.) but not at a dose that corresponded to the actual concentration in the lake (not illustrated). We could not see clear differences with the dichloromethane and diethylether extracted waters. The potency of the sediment extracts to induce EROD activity was higher than the potency of the water extracts. This is consistent with the knowledge that sediments contain much higher concentrations of contaminants than overlying waters (8).

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

In conclusion, PLHC-1 bioassays revealed that sediments and waters from L. Kernaala contained PAHs and/or HAHs which expressed capacity to induce CYP1A and porphyrins. With the property of identifying harmful chemicals, PLHC-1 cells could be used in monitoring and comparing other water areas, as well.

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