# Monitoring exposure and effects of polyhalogenated aromatic hydrocarbons (PHAHs) in European otters (*Lutra lutra*)

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## 1. Abstract

PHAH levels in otter livers, fish and sediments have been determined using the *in vitro CALUX* (chemical activated luciferase gene expression) bioassay for Ah-receptor active compounds. For fish and otter liver extracts these levels, expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs), correlated well with chemically derived TEQs (GC-TEQs) based on non-, mono-, and di-ortho substituted PCBs. For sediments, however, the so-called *CALUX*-TEQ was much higher than the chemically derived TEQs. In livers from relatively fresh otters, retinol and retinylpalmitate levels were measured, and these vitamin A levels correlated negatively with hepatic GC-TEQ levels. In addition, a strong correlation was observed between PHAH levels in blood and liver on a lipid basis, either expressed as TEQs or individual PHAHs. From these observations it can be concluded that (*CALUX*-)TEQ levels in blood can be used as non-destructive biomarker for PHAH-exposure and accompanying hepatic vitamin A reduction.

## 2. Introduction

Otter (*lutra lutra*) populations in Europe have declined markedly over the last decades. Polyhalogenated hydrocarbon (PHAH) pollution is considered to be one of the major factors in this decline, in addition to physical threats, such as habitat destruction, traffic accidents and drowning in fishing nets. This assumption was based on toxicological studies with the mink (*Mustela vison*) which is often used as a model for the otter, and on associations between high PCB levels in otters and declining or endangered populations[1,2]. For practical and ethical reasons no toxicological experiments have been conducted with the otter itself. Because the Dutch government aims at the return of the otter in the Netherlands, insight in the environmentally safe levels of PHAHs permitting survival of viable otter populations is needed[3]. In addition non-destructive biomarkers are needed to monitor exposure and health status of otters, before and after re-introduction in their natural environment.

For hazard and risk assessment of mixtures of PHAHs the concentrations of individual PHAHs multiplied by their respective toxic equivalency factors (TEFs) are added up to give the total TCDD toxic

equivalency of the mixture[5]. However, given the complexity of mixtures of PHAHs in environmental matrices, even an extensive chemical analysis can only give an impression of the potency of a mixture[6]. In this study we used a rapid, sensitive *in vitro* assay for assessing the toxic potency of mixtures of PHAHs in livers of accidentally killed otters and in sediment and fish samples collected in the same area. The response in the *CALUX* assay[7,8], using recombinant rat (H4IIE) hepatoma cell lines, is compared with PCB-levels determined using gas chromatography combined with Electron Capture Detection (ECD) or Ion Trap Detection (ITD)[9]. Whenever possible, PHAH levels in the otter livers were compared with PHAH levels in blood samples of the same otters.

One of the mechanisms of toxic action of PHAHs is the reduction of the vitamin A storage in liver, which has been demonstrated in both experimental and field studies[10,11,12]. Vitamin A plays an important role in tissue development in foetuses, reproduction, and resistance against infectious diseases. In relatively fresh otter livers also vitamin A (retinol and retinylpalmitate) levels were determined, and compared with hepatic TEQ-levels. This paper presents the first results of a joint study of which the general outline is presented separately at Dioxin '96[4].

## 3. Methods

### *Sample collection and preparation*

Dead otters were collected from 1992 to 1993 in Denmark, and the health status was recorded[13]. Relatively fresh liver aliquots of 10-20 g from 12 otters were prepared separately for *CALUX* and GC-ITD or ECD measurement (see below). Samples of about 1 gr were prepared for hepatic retinoid analysis. Blood was collected for chemical and *CALUX* analysis. In five areas in Denmark fish and sediment samples, representative for the otter diet and habitat, were collected in 1995. From each fish species 25 individuals were homogenized using a blender. Fish samples were lyophilized, and sediment samples dried by 60°C.

### *CALUX-assay*

Rat H4IIE.pGudluc1.1 cells prepared as previously described[7,8] were exposed in 24-well cell culture plates and the assay performed as described elsewhere[6]. For calculation of *CALUX*-TEQs a standard curve of TCDD was fitted, and the *CALUX*-TEQ value for the unknown sample was interpolated on this curve.

### *Chemical analysis*

After Soxhlet extraction, lipids were gravimetrically determined in 10% of the liver, blood and fish extract. Another part of the extract (10%) was cleaned over 33% $H_2SO_4$ deactivated silica. The sediment extract was cleaned over a multi layer column filled with alumina oxide (deactivated with 5% $H_2O$). This extract was evaporated and partly used for the *CALUX*-assay. The rest was fractionated over a silica gel (5% $H_20$ deactivated) column and was further separated into three fractions containing non-, mono- and di-ortho substituted PCBs, using a PYE HPLC column[9]. The di- and mono-ortho fractions were measured with GC-ECD, the non-ortho fraction using GC-ITD. The so-called GC-TEQs were caluclated based on TEF-values as described before[5].

***Analysis of hepatic retinoids*** was performed according to Brouwer et al.[10] with aliquots of 50 µl liver homogenate on a reversed phase silica C18 column. Retinoids were detected at 326 nm.

**4. Results and discussion**

Good correlations were observed between the *CALUX*-TEQs and GC-TEQs for fish and otter liver samples (Figures 1a and 1b). The absolute TEQs were in the same order of magnitude. This was to be expected as TEQs in biota are mainly determinded by non- and mono-ortho PCBs[9,14]. However, for fish sample extracts the TEQ indicated by the *CALUX* -assay was slightly less than the chemically derived TEQs. This could be a consequence of the relatively high levels of di-ortho PCBs in fish compared with the otter[15]. Di-ortho PCBs have been demonstrated to have a slightly antagonistic effect on planar PHAHs in H4IIE cells[7]. The *CALUX*-TEQ and GC-TEQ for sediment extracts also correlated well (Figure 1c). The *CALUX*-TEQs, however, were much (more than a factor of 20) greater than the GC-TEQ. This is probably due to the presence chemically not quantified chemicals, such polyaromatic hydrocarbons (PAHs), which may also be good Ah-receptor agonists, and therefore are determined by the *CALUX* assay. As PAHs hardly bioaccumulate, such differences are not observed in the biotic samples. Relatively little sample preparation and clean-up is needed for the *CALUX* assay [6]. Therefore this assay is a good and fast alternative for more extensive chemical PHAH analysis.

The bioconcentration factors of the sum of 7 PCBs and TEQs from sediment to fish and to otter will be presented separately at the Dioxin '96 conference[4,16].

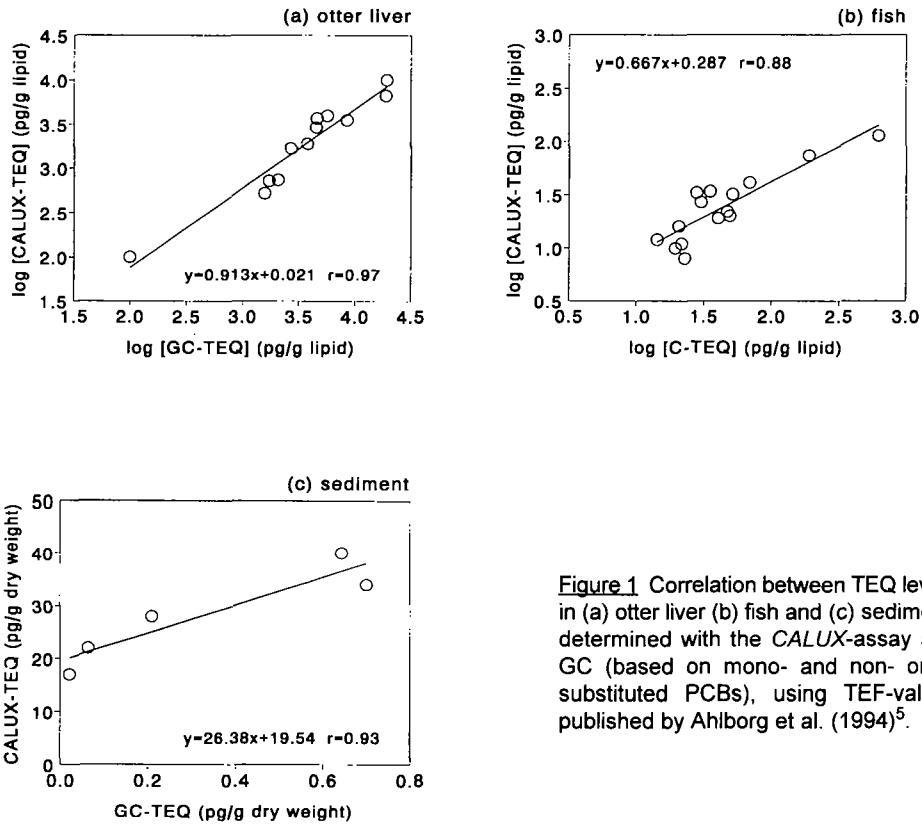


Figure 1 Correlation between TEQ levels in (a) otter liver (b) fish and (c) sediment, determined with the *CALUX*-assay and GC (based on mono- and non- ortho substituted PCBs), using TEF-values published by Ahlborg et al. (1994)[5].

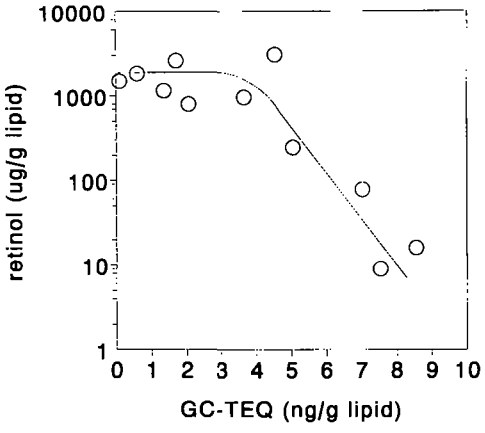


Figure 2 Correlation between TEQ and retinol levels in relatively fresh livers of otters. TEQ-calculations are based on chemically determined PCB-levels using TEF-values published by Ahlborg et al. (1994)[5].

A strong decline of hepatic retinol (Figure 2) and retinylpalmitate (data not shown) levels is observed with a relatively small increase in hepatic GC-TEQ levels. This correlation on TEQ-basis is much better than on the basis of the sum of the 7 standard PCBs. A comparable decrease in hepatic retinol levels was reported for flounder (*Platichthys flesus*) exposed to polluted harbour sediment, although hepatic retinylpalmitate levels did not decrease in these fishes[17]. More otter samples will be analysed to study these relationships further.

Good correlations were observed between total PCB-levels in liver and blood on a lipid basis (y=0.97x-0.043 ; r=0.89, data not shown). This implies that TEQ-levels in liver can be estimated based on TEQ-levels in blood, which can be collected in a non-destructive manner. There were too litle dead otters available with both a relatively fresh liver and enough blood to measure both hepatic vitamin A and TEQs in blood. However, the correlations observed so far between TEQs in liver and blood, and between TEQ in liver and hepatic vitamin A, suggests that at least a rough estimate of the vitamin A reduction in the liver could be obtained based on TEQ-levels in blood.

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