2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN EQUIVALENTS IN EXTRACTS OF BALTIC WHITE-TAILED SEA EAGLES

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1. Abstract

Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as TCDD equivalents (TEQ) were measured in extracts of Baltic white-tailed sea-eagle tissues and eggs. The concentrations of TEQs determined by the H4IIE rat hepatoma cell line bioassay (TEQ_{Bio}) were compared with toxic equivalents derived from instrumental chemical analyses (TEQ_{Chem}) in total extracts and fractions containing PCDD/PCDFs or coplanar PCBs. TEQs obtained from bioassay and chemical analyses were similar. For example, TEQ_{Bio} of the PCDD/PCDF fraction of the most contaminated eagle was 240 pg/g, fresh weight (fw) and TEQ_{Chem} of the same fraction was 270 pg/g fw.

2. Introduction

Polychlorinated-dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are toxic polychlorinated diaromatic hydrocarbons (PCDHs) which occur widely in the environment and can elicit toxic effects in species at the top of the food chain[1]. These compounds have been observed to be concentrated in predatory species like white-tailed sea eagles and therefore, adverse health effects are more likely to be detected in these species[2]. PCDHs originate from numerous sources including combustion processes, chlorine bleaching and production of other chemicals[3].

TCDD is the most toxic congener of PCDDs and PCDFs and causes a number of toxic effects, including immunological and reproductive effects[4]. The toxic responses of dioxin-like PCDHs are mediated through the aryl hydrocarbon receptor (Ah-r) and have been reported to correlate with their potency to induce microsomal mixed-function oxidases[4-6]. The effects of complex mixtures

of PCDHs can be evaluated by determining the total 2,3,7,8-dioxin like activity in extracts of tissues. This can be accomplished by the use of instrumental chemical analyses or bioassays[3]. Induction of hepatic microsomal ethoxyresorufin-o-deethylase (EROD), for example, can be used as an indicator for dioxin-like compounds[7]. The rat H4IIE hepatoma cell line have been used to determine TCDD equivalency factors (TEFs) of chlorinated compounds relative to 2,3,7,8-TCDD and to measure biological activity of environmental extracts[8-10].

The use of bioassays have been developed to measure total concentrations of TCDD equivalents (TEQ_{Bio}) in extracts directly to replace some chemical analyses which often are time consuming and expensive. Furthermore, all compounds responsible for TEQ can not be analyzed chemically. Bioassays measure the responses of all of the compounds in the mixture and account for interactions among compounds[1]. These interactions are primarily additive, but could also be infraadditive. Concentrations measured in instrumental chemical analyses can be converted to TEQs by multipling the concentration with a TEF measured in bioassays. The total activity of TEQ_{Chem} is determined by summing these products in an additive model.

This study was a part of the study on the concentrations and TEQs of chlorinated compounds in Baltic white-tailed sea eagle[11]. The purpose of the study was to measure concentrations of PCDDs, PCDFs and PCBs by chemical analyses in Finnish white-tailed sea eagles and to compare TEQs derived from chemical analyses with TEQs obtained from bioassays. This was done to determine if all of the TEQ_{Chem} could be accounted for by PCDDs, PCDFs and non-ortho-substituted PCBs.

2. Materials and methods

White-tailed sea eagle (*Halieetus Albicilla* L.) tissue and egg extracts analyzed in this study were collected from the Baltic Sea environment (see details of sample data in ref. [11]). Samples presented in this paper were from two breast muscle and one egg. Breast muscle samples were from female white-tailed sea eagles found in 1981 (Central Finland) and 1991 (Åland Islands). The egg was found addled in the Archipelago of Turku in 1991.

The extraction of samples was performed as described earlier[11,12]. Briefly, samples (5-10 g) were homogenized with four fould amount of sodium sulphate and were extracted in a Soxhlet-apparatus for 6 h with a solvent mixture of petroleum ether:acetone:hexane:diethyl ether (PAHE) (18:11:5:2; v/v/v/v). Separate extracts were prepared for H4IIE bioassay and for chemical measurements. Internal standards were added to extracts to be used for instrumental analyses.

After extraction, the lipid content was measured and each extract was divided into two aliquants. Details of the analytical scheme for the isolation, purification and chemical analyses of PCDDs, PCDFs, PCBs, PCDEs and other chlorinated compounds are presented elsewhere[11,12]. Removal of interfering compounds and most of the lipid was accomplished with concentrated sulphuric acid. For H4IIE bioassay, sulphuric layer of these samples was washed with hexane which was combined with the extract to minimize losses during this purification step.

For chemical analyses non-ortho-substituted (coplanar) PCBs were isolated from mono- and di-ortho substituted congeners using column chromatography on both activated carbon and basic alumina microcolumns[12]. PCDDs and PCDFs were analyzed from the other part of the extract. These compounds were first isolated from PCDEs and PCBs by adsorption column chromatography on florisil and were then purified on activated carbon[12].

Major PCB congeners and chlorinated hydrocarbons were analyzed after sulphuric acid cleanup by high resolution gas chromatography combined with electron capture detection (HRGC-ECD). Non-ortho substituted PCBs (77, 126 and 169) were analyzed by high resolution gas chromatography/low resolution mass spectrometry (HRGC/LRMS) using a Hewlett-Packard 5970 mass selective detector system. Analyses of toxic PCDDs and PCDFs were performed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) with a VG AutoSpec instrument. A resolution of 10,000 in selected ion monitoring (SIM) mode was utilized.

The induction of EROD activity in H4IIE cells of the total extracts was determined after sulphuric acid purification. For the H4IIE bioassay of fractions, PCDD/PCDFs were first isolated from PCBs and PCDEs on florisil column and were then purified further on carbon column similarly to chemical analyses presented above. The PCB/PCDE fraction from florisil contained all PCBs including coplanar PCBs. This fraction was analyzed in the H4IIE bioassay both before and after fractionation on carbon, which separated coplanar PCB congeners from non-planar PCBs.

The H4IIE bioassay was conducted as described previously[11,13]. Briefly, the H4IIE cells were grown in Dulbecco's Modified Eagle's Medium (D-MEM) supplemented with fetal bovine serum (FBS) and antibiotic-antimycotic mixture until cells reached 85% confluency. For the assay, the culture was trypsinized and seeded in culture 96-multiwell plates. After cells had grown in the plates for 24 hr, dose solutions (5µl) in iso-octane were added to each well. The cells were then incubated for 72 hr. The EROD activity and protein were measured fluorimetrically using a Cytofluor 2300 fluorescence detector (Millipore)[13]. A TCDD standard curve was developed with every set of samples and used to calculate the relative potency of samples.

4. Results

Concentrations of TEQ_{Bio} obtained from the H4IIE rat hepatoma cell bioassay and those of TEQ_{Chem} are presented in Figures 1 and 2. Concentrations of PCDHs were used to calculate the TEQ_{Chem} in each fraction. Concentrations of PCBs have been presented in Table 1.

TEQ_{Chem} was calculated by use of an additive model in which the concentrations of individual congeners were multiplied by the H4IIE bioassay-based TCDD equivalency factors (TEF)[14] and were summed to give a total concentration of TEQs. The concentrations of individual Ah-r-active congeners were determined by isotope dilution so they were corrected for recovery. Concentrations of TEQ_{Bio} were not corrected for recovery, since recovery experiments with standards on fractionation columns showed that recoveries of Ah-r active compounds were sufficiently high on different fractionation column not to cause too high errors in comparison.

The effect of interactions to the TEQs was omitted by separation of the Ahr-active compounds from the inactive compounds by column chromatography thus correction due to interactivity between compounds was unnecessary to compare TEQ_{Bio} to TEQ_{Chem}.

The lesser concentrations of TEQ_{Bio} contributed by the PCB and PCDD/PCDF fractions compared to that of the total extracts (Figure 1 and 2) indicates that there are compounds other than PCDDs, PCDFs and PCBs that contribute to TEQ_{Bio}. TEQ_{Chem} contributed by PCDDs and PCDFs in extracts were similar to those of TEQ_{Bio} in the PCDD/PCDF fraction (Figure 2). Coplanar PCBs contributed in most of the the toxic load in eagles (Figure 2).

Table 1. Concentrations of PCDD, PCDF (pg/g fw) and PCB congeners (ng/g fw) in Baltic white-tailed sea eagle muscle (EM) and egg (EE).

	EM1	EM3	EE4
2378-TCDD	91	14	27
12378-PeCDD	176	30	74
123678-HxCDD	87	11	32
2378-TCDF	<5	6	19
23478-PeCDF	346	73	25
PCB 156	1030	127	143
PCB 77	3.8	0.70	1.0
PCB 126	32	4.5	5.9
PCB 169	6.4	0.77	1.3

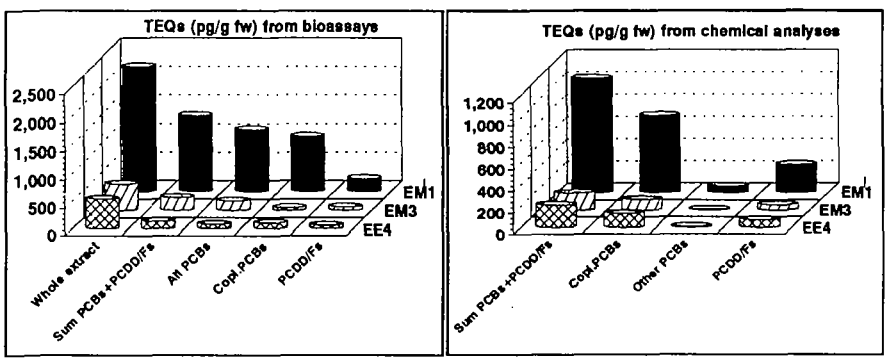

Figure 1. A) TEQ_{Bio} of whole extract and fractions of eagle extracts. B) TEQ_{Chem} of PCBs and PCDD/PCDFs of eagle extracts.

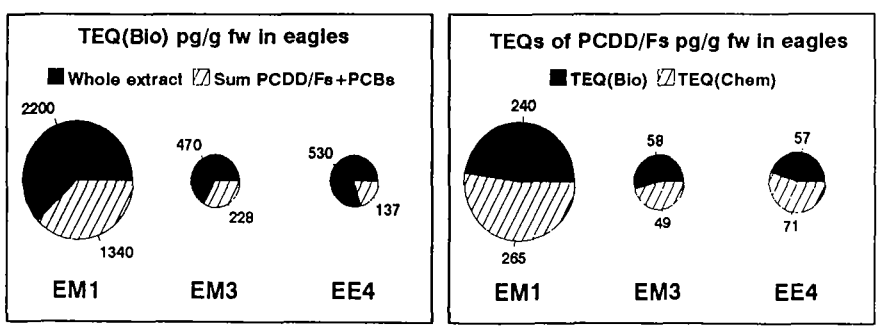

Figure 2. A) TEQ_{Bio} of whole extract compared with TEQ_{Bio} of PCDD/PCDF+PCBs of eagles. B) Comparison of TEQ_{Bio} and TEQ_{Chem} of the PCDD/PCDF fraction of eagle extracts.

The relative hazard of current concentrations of TEQ_{Bio} to eagles as determined by calculating hazard quotients (HQ) by dividing the concentrations of TEQ_{Bio} in eggs by an estimated no observable adverse effect concentration (NOAEC) was greater than 1.0 in egg EE4. This indicates that current concentrations of TEQ_{Bio} in eggs are likely causing adverse effects in the Baltic populations of white-tailed sea eagles.

5. Conclusions

This study indicated that the H4IIE rat hepatoma cell line is useful for monitoring the presence and biological activity of TCDD-like compounds in white-tailed sea eagles. Samples having greater concentrations of PCDHs were also more active as inducers of EROD activity in H4IIE cells. This bioassay gives the level of toxic compounds such as coplanar PCBs and dioxins without the need for expensive instrumental quantification of individual PCDH congeners as well as accounting for interactions among these congeners.

The two methods used to estimate the concentrations of TEQs resulted in similar values. This on hand refers to that that the extraction efficiencies were similar for both methods. By summing the results of assays with different fractions, there was no need to correct for interactions among congeners when determining the concentrations of TEQ_{Bio}. On the other hand, similar results mean that the PCDDs, PCDFs and coplanar PCBs account for most of the TEQ_{Bio} measured in the corresponding extract fractions.

Concentrations of TEQ_{Bio} in whole extracts were greater than the sum of TEQ_{Bio} concentrations contributed by the separate PCDD/PCDFs and PCB fractions. This indicates that other compounds also contribute to the TEQ_{Bio}. Currently, the cause of the unexplained TEQ_{Bio} is unknown. However, there are a large number of compounds that based on their structure could bind to the Ah-r and induce EROD activity.

6. References

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