Use of *in vitro* bioassays in screening environmental samples for MC- and PB-type enzyme activity

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Introduction

Because of the many different known and possibly also unknown compounds expressing dioxin-like toxicity and the difficulties in analysing them chemically the H-4-II E enzyme induction bioassay has been developed and used for screening samples for dioxin-like enzyme induction activity (7-ethoxyresorufin O-deethylase; EROD). The assay has been used to measure dioxin-like activity of pure substances as well as of complex mixtures, such as environmental samples[1,2,3].
As some non-dioxin-like PCB congeners, which cause phenobarbital-like (PB-like) enzyme induction, have been shown to contribute to the toxicity of PCBs (e.g. in the case of tumour promotion) a bioassay for screening samples for PB-type enzyme induction has been developed. In this assay the induction of 7-penthoxyresorufin O-depenthylase (PROD) is measured in C2Rev7 cells.

Materials and Methods

The PCB congeners were kind gifts from Dr Åke Bergman at the Department of Environmental Chemistry, Wallenberg Laboratory, University of Stockholm. The sediment samples were extracted according to Jensen *et al.*[4]. Sludge samples were Soxhlet-extracted with toluene. The bioassay was run on the hexane or toluene extract, which contained all lipophilic polyhalogenated hydrocarbons as well as other lipophilic substances. The sediment and sludge samples have been chemically analysed for PCDDs and PCDFs, and in some cases also for non-*ortho* PCBs and PCNs[5,6,7,8].
EROD-induction in H-4-II E cells was measured using the method described by Sawyer & Safe[9] and Pohl & Fouts[10]. PROD-induction in C2Rev7 cells was measured using a modification of the method by Dr Friedrich Wiebel, Institut für Toxikologie und Biochemie, Neuherburg, Germany. TCDD and phenobarbital were used as standards in the EROD and PROD bioassays, respectively.

Results and Discussion

In the EROD bioassay the results of the sediment and sludge exposures were expressed as TCDD-equivalents (TEQs). These results were compared to the TEQs calculated from the chemical analyses of PCDDs and PCDFs using the Nordic TCDD-equivalency factors[11]. The bioassay TEQs were higher than the chemical TEQs for all samples (1.3-11.7 times the chemical TEQs). However, when the contribution to total TEQ from non-*ortho* PCB and PCN congeners were taken into account the bioassay and chemical TEQs were relatively similar. The PROD bioassay has been developed to be able to measure also PB-type enzyme inducing compounds (primarily PCB congeners) in environmental samples. The maximal PROD-induction in the C2Rev7 cells is lower than the maximal EROD-induction in H-4-II E cells (14 and 40 times that of control cells, respectively for phenobarbital and TCDD). Individual PCB congeners have even lower maximal inductions (e.g. 5 times for 2,3',4,4',5-PeCB). In addition, the ED_{50} for 2,3',4,4',5-PeCB (PROD-induction) is approximately 0.3 μmol compared to 0.1 pmol for TCDD (EROD-induction). However, the concentrations of the PROD-inducing PCB congeners are relatively high in environmental samples. For example, the concentration ratios between the PROD-inducing 2,2',4,4',5,5'-HxCB and TCDD are 8 000 - 15 000 000 in different wild-life species[12]. We expect the PROD-bioassay to become a useful complement to the EROD-bioassay in the assessment of toxic potency of pollutants in environmental samples.

References

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