ASSESSMENT OF DIOXIN-LIKE TOXICITIES OF WATER-EXPOSED SPMDs
BY IN VITRO REPORTER GENE BIOASSAY

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Abstract

Semi-permeable membrane devices (SPMD) are passive sampling devices suitable for concentration of trace contaminants from the environment. In this presentation we summarize results of pilot experiments with applications of the in vitro reporter gene bioassay (H4IIE.luc cells) for assessment of AhR-mediated ("dioxin-like") toxicities of SPMDs exposed in waters in localities in the Czech Republic. Observed AhR-mediated effects were in good correlation with the content of dioxin-like compounds (such as PCDD/Fs, PCBs and PAHs) and the results confirmed suitability of the reporter gene bioassay as a rapid and specific in vitro biomarker tool.

Introduction

Persistent organic pollutants (POPs) are a group of ubiquitous environmental contaminants. They are highly lipophilic, bioaccumulative and in spite of the trace environmental concentrations they represent significant toxic hazard. A critical part of any environmental analytical procedure, especially in water, is sampling. There are two principal approaches – passive or active sampling. The active technique lies in collection of discrete amounts of sample (water) for further extraction. However, results obtained by this method may be often not representative due to local distinct contaminant concentrations in water environment and questionable bioavailability of extracted contaminants.

Therefore, a passive approach is often used for assessment of water pollution. A relatively new method of passive sampling developed in early 1990s is use of semipermeable membrane devices (SPMDs; Huckins et al., 1990; Lebo et al., 1992). These devices consist of a low-density polyethylene membrane tube with pore diameter approx. $10^{-9}$ m and with the constant porosity all over the membrane filled with a relative non-polar liquid with large molecules – the most widely used fill is a synthetic triolein $1,2,3$-tri-[cis-9-octacenoyl]-glycerol (Kočí et al., 2003). The membrane is penetrable for small environmental contaminant molecules but not for triolein and due to high lipophility of the triolein the pollutants do not tend to penetrate back to the water environment.

This sampling arrangement allows a long-time sampling of a water body providing a weighted average of contamination levels values. Furthermore, lipophility of triolein is similar to that of living organisms. However, although it was supposed that the bioaccumulation potencial of contaminants is represented by SPMDs, Richardson et al. (2003) showed that SPMDs cannot dorectly replace use of bioindicator organisms.

On the other hand, SPMD has several advantages against bioindicators – they lack the variability among individuals, the analysis is much easier than that of a tissue and they can be used even in highly contaminated sites where are not good conditions for living organisms. A typical SPMD is about 90cm long, 2-2.5 cm wide with a LDPE membrane of thickness about 75-90 µm loaded with 0.5-1 ml of triolein in a thin layer. The contaminants concentrated in the SPMD are mined by multiply dialysis after exposure.

In this study we present pilot results of assessment of AhR-mediated toxicities ("dioxin-like" effects) of SMPDs exposed in Czech rivers and lakes. We employed in vitro reporter gene assay based on measuring of AhR-mediated luciferase expression (luminiscence) in stably transfected rat liver
hepatoma cells H4IIE.luc. The toxicity results were correlated with the chemical data and the applicability of the bioassay for screening of complex SPMD extracts is discussed.

Materials and methods

SPMD exposure. Several aquatic localities (rivers, lakes, ponds) in the Czech Republic were selected based on previous informations on organic contamination and considering possible contaminant sources. SPMDs were exposed in waters for different exposure periods and after the exposure the membranes were washed and dialyzed as previously described (Kočí et al., 2003). Dialysates (acetone/DMSO 1:1) were used for assessment of toxicity and parallel samples were analysed for content of POPs (PCBs, PCDD/Fs, PAHs). To integrate differing exposure periods, a variant of V(tox) parameter (Kočí et al., 2003) was used: \( V_{tox(50)} = \frac{1}{m \cdot EC_{50} \cdot d} \), where "m" = concentration of extracted membranes (pcs.ml\(^{-1}\)), "d" = sampling period (days), and EC\(_{50}\) - evaluated effective concentration.

Bioassay design. Stably transfected rat hepatoma cell line H4IIEluc - kindly donated by prof. J.P.Giesy, Michigan State University (MI, USA) were cultivated in Dulbecco’s modified Minimum Essential Medium (MEM) containing 10% of fetal calf serum. 60-70% confluent cells were resuspended and seeded in 100µl of MEM into 96-well microtitration plates. After 24h the 100µl of exposure medium was added containing dilution series of samples and of a reference compound 2,3,7,8-tetrachlorodibenzodioxin. After 24 h exposure, the medium was removed, the cells were washed and luminescence measured using Steady-Glo luciferase assay kit (Promega) in luminometer GENios (TECAN, Switzerland).

Results

Example of induction of AhR-dependent luciferase in H4IIE.luc cells after exposure to selected SPMD extracts is shown in Fig. 1. Apparent concentration-dependent luciferase inductions were observed in numerous samples and were significantly correlated with the concentrations of major dioxin-like POPs (i.e. PCBs, PCDD/Fs and PAHs).

![Fig. 1. Induction of AhR-mediated luciferase activity by SPMD extracts and reference toxicant (2,3,7,8-TCDD) in reporter gene assay with H4IIE.luc cells.](attachment:image)

The data in this proceeding contribution represent only a pilot study focused on the applicability of reporter gene bioassay for toxicity assessment of exposed SPMDs. Further examination of the data and discussion is necessary and is presented in the conference presentation. Our results revealed good correlation between chemical data and the toxic potencies and the suitability of the bioassay as a rapid screening tool for dioxin-like compounds concentrated by passive sampling techniques.

Acknowledgements

The research was supported by the Ministry of Education (VaV J07/98 - 141100003) and the results are presented under the auspices of the EC Center of Excellence "RECETOX" (EVK1-2002-00519, http://www.recetox.muni.cz).
References


