

**Table 4.** Polychlorinated biphenyl content of imported foodstuffs in Hungary (1993 - 1998). Number of analysed samples: 478

Foodstuff	Range (mg/kg)	Residue limit (mg/kg)
Fish in oil (mackerel, tuna, sardines, herring)	< 0.01 - 1.16	2
Fish in sauce (herring, mackerel, sardines, sprottni)	< 0.01 - 1.79	2
Deep-frozen fish (pike, cod, burbot, heck, shrimps)	< 0.01 - 0.03	2
Cod liver	0.085 - 2.53	5
Cheese	< 0.01 - 0.42	1*
Dietary supplements	0.09 - 0.18	-
Pork fat	< 0.01	1*

\* calculated on fat basis

tamination even in the adipose tissue of the 1-month-old infant or the 85-year-old man. The PCB contamination can be detected in most of the mother milk samples.

As the result of prohibition of use since the end of the 60s, the total DDT and total-HCH content of Hungarian foodstuffs decreased close to the detection limit, but in some import foodstuffs even today significant levels can be detected. The organochlorine pesticide content in imported foodstuffs measured between 1996-1998 can be seen in Table 4.

Polychlorinated biphenyls occur in traces in foodstuffs of plant origin. In foodstuffs of animal origin their level is lower than that

**Table 5.** Imported foodstuffs with objectionable organochlorine pesticide content in Hungary (1996 - 1998)

Number of analysed samples: 1 964 Objectionable: 41 foodstuff	Range of total-DDT content (mg/kg)	Range of total-HCH content (mg/kg)
Cayenne pepper	0.224 - 0.352	
Tea (black, green, ginzeng, antiadipose)	0.324 - 0.810	
Chili	0.256 - 0.310	
Oregano	1.156	
Clove	1.924	
Allspice	0.325	
Cinnamon	1	
Nutmeg flower	1.2	0.236
Curcuma		0.268 - 2.236
Black pepper (grounded)		0.224 - 0.482
Black pepper		0.234 - 0.268
Curry		0.282 - 0.612
Ginzeng, red ginzeng capsule		12.33 - 18.93
Sesam		0.504
Hungarian legal limit (till 1998)	0.1	0.2

reported by highly developed industrial countries. The PCB content in foodstuffs imported to Hungary is evident from Table 5.

## EVALUATION OF CYTOTOXICITY, DIOXIN-LIKE ACTIVITY AND ESTROGENICITY OF COMPLEX ENVIRONMENTAL MIXTURES

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### SUMMARY

Complex organic extracts from soils and sediments collected in an industrial region of the Czech Republic were tested with in vitro recombinant cell lines for their potential dioxin-like and estrogenic activity and cytotoxicity. For dioxin-like toxicity tested on H4IIE-luc cells complete dose-responses were obtained with all extracts. The MCF-7-luc cell line used for determination of estrogen receptor-mediated activity was sensitive to the cytotoxic effects of the soil and sediment extracts. Dose dependent cytotoxicity was observed with most samples. Significant estrogenic activity was found in all samples. The toxic or estrogenic equivalents based on the analytically determined concentrations of organic pollutants with known dioxin-like or estrogenic potency were calculated and compared to the bioassay-derived estimates. Fractionation along with mass-balance calculation enabled identification of the most active fraction and classes of compounds. Polycyclic aromatic hydrocarbons were identified as the group of compounds responsible for most of the TCDD-like activity as well as for important portion of estrogenic activity.

**Key words:** cytotoxicity, estrogenicity, dioxin-like activity

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### INTRODUCTION

Contaminants are present in environmental media as complex mixtures. Therefore the determination of total toxic potency is complicated. Comprehensive methods that can assess the overall toxic potential, including interactions within mixtures are needed. A valuable approach is development of bioassay that can account for all compounds acting through specific mode of action, such as receptor-mediated effects. So far the most widely studied are effects mediated through aryl hydrocarbon (AhR) and estrogen receptor (ER). In vitro bioassays with recombinant cell lines where specific response gene is under control of dioxin-responsive or estrogen-responsive DNA enhancer element were shown to elicit higher sensitivity and lower

variability than endogenous receptor-mediated responses (1). In this study the applicability of in vitro recombinant cell line bioassay systems to characterize receptor-mediated activities of complex environmental mixtures was evaluated. Tested samples included organic extracts from soils and river sediments. Three major endpoints were measured: cytotoxicity, TCDD-like activity and estrogenicity. The extracts were analyzed for the compounds potentially causing the observed activity, such as PCDD/Fs, PCBs, PAHs and alkylphenols. The toxic and estrogenic equivalency factors were derived from both bioassays (TCDD-EQ and E<sub>2</sub>-EQ) and analytical results (TEQ and EEq). Mass balance calculation revealed the portion of the total activity that was accounted for by the analyzed com-

Table 1. Fractionation of sediment extracts into three fractions

Fraction	Fr. 1	Fr. 2	Fr.3
Polarity	nonpolar	moderately polar	polar
Eluent	hexane	20% DCM in hexane	100% DCM
Eluted	PCBs, PCDD/Fs	PAHs, alkylphenols OC-pesticides	polar metabolites, steroides
Analysis	GC-MS, GS-HRMS	GS-MS/FID, HPLC-FD	GC-MS

pounds. By means of fractionation of the sediment extracts and mass-balance calculations the major classes of compounds responsible for the observed activities have been identified.

## MATERIALS AND METHODS

Seven sediment and seventeen soil samples (20 g) collected from an industrial area in the Czech Republic were Soxhlet extracted for 16 hrs with dichloromethane. Sulphur was removed from all samples. Sediment extracts were fractionated on activated florisil column as described in Table 1, which also documents final analytical techniques for determination of different classes of pollutants.

Bioassays were conducted on 96 well microplates (1). Luciferase activity was assessed by luminometric measurement as a measure of binding of ligands present in the samples to Ah-receptor (in H4IIE-luc cells - standard TCDD) to evaluate TCDD-like activity or to estrogen-receptor (in MCF-7-luc cells - standard 17 $\beta$ -estradiol (E<sub>2</sub>)) to evaluate ER-mediated activity. Viability index was measured by fluorimetric method with calcein AM/ethidium bromide. TCDD equivalents (TCDD-EQs) and E<sub>2</sub> equivalents (E<sub>2</sub>-EQs) expressed as pg standard-equivalent/g sample were calculated from bioassays results based on EC<sub>50</sub> responses. Standard equivalents from analytical results (TEQs and EEQs) were calculated for all samples by multiplying the concentration of specific congener with previously published compound's relative potency (1-3).

## RESULTS AND DISCUSSION

Significant TCDD-like activity was found in all samples. Complete dose-response relationships were obtained with maximum responses 57-143 % of maximum response caused by TCDD. Cytotoxicity was not significant for any dilutions except of greatest concentrations of some samples. There were very good correlations between toxic equivalencies based on bioassay measurements (TCDD-EQ) and total analytical TEQ (Fig. 1) or TEQ based only on concentrations of PAHs, or total concentration of PAHs in sediment samples. Mass-balance analysis revealed that PAHs are the dominant group of compounds in soils (representing 65-100 % of total TEQs) as well as in sediment extracts (more than 99 % of total TEQ). Sediment fractions with different polarity were tested on the bioassays. Very low TCDD-like activity found in fraction 1 confirmed the data from chemical analysis with low contribution of PCDD/Fs and PCBs to the total TEQ. Fraction 2 was the most active fraction, maximal induction between 88 and 130 % of standard maximum, which confirmed high correlation of PAHs and their TEQs with total TCDD-EQs. Significant correlation in the fraction 3 suggested presence of unknown polar compounds with the ability to bind to the Ah receptor.

Significant estrogenicity was observed for all samples. The greatest induction reached from 15 to 69.6 % of the standard maximum. MCF-7 luc cells were sensitive to cytotoxic effects of extracts. Dose-dependent decrease in viability index was observed. Normalization of the luciferase induction data to viability index increased the linear range of response of samples and relative induction compared

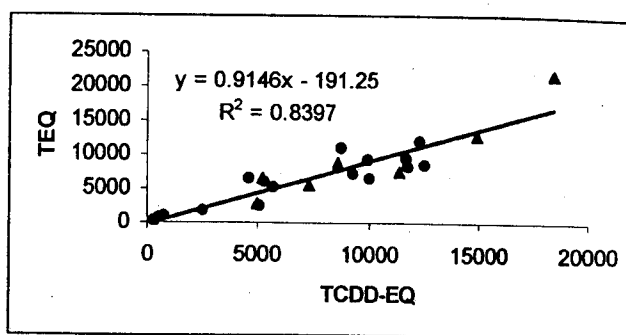


Fig. 1. Relationship of toxic equivalency factors (pg TCDD/g dw sample) derived from bioassay results (TCDD-EQ) and calculated from chemical analysis results (TEQ). triangles = sediments, circles = soils.

to standard maximum and did not increase the variability. Estrogenic equivalents relative to E<sub>2</sub> were calculated from the normalized results.

After fractionation of the sediment extracts low antiestrogenic effect was measured in fractions 1 and 3 of some samples. High induction was measured in fraction 2 for all samples. This fraction seems to be responsible for the majority of the ER-mediated effects observed with total extract. Only limited mass-balance for estrogenic activity was possible due to non-existing E<sub>2</sub>-equivalents for majority of compounds. The most active fraction 2 contains alkylphenols and PAHs. Contribution of alkylphenols to the total EEQ equivalent was less than 2 %. PAHs accounted for more than 98 % of the EEQ. The bioassay derived E<sub>2</sub>-EQs were in good agreement with EEQ, suggesting that PAHs and alkylphenols account for majority of the ER-mediated activity.

Complex mixtures extracted from soils and sediments contained both dioxin-like activity (ranging up to 23 000 pg TCDD/g dw) and estrogenic activity (up to 1 200 pg E<sub>2</sub>/g dw). Good agreement between toxic and estrogenic equivalents derived from bioassay and analytical results supports the assumption of generally additive interactions between compounds within mixture and documents that the analyzed compounds can account for the observed effects.

## CONCLUSION

Significant TCDD-like and estrogenic activity was identified in all complex organic extracts from soils and sediments. Both recombinant cell lines proved to be very useful tool for screening these types of activities, with MCF-7-luc cell line being more sensitive for cytotoxicity. When the cytotoxicity is significant, normalization to viability index enables to account for the cytotoxic effects and avoid misinterpretation of the data. The toxic and estrogenic equivalency factors derived from bioassay results agree very well with those calculated from analytical results. Mass-balance calculation and fractionation of the sediment extracts showed that PAHs are the class of compounds responsible for major portion of the TCDD-like and ER-mediated activity.

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