PCDDs/Fs in sediments from Morava river catchment area*

Ivan Holoubek[1], Miroslav Machala[1,2], Klára Štaffová[1,3], Jan Helešic[1], Alena Ansorgová[1], Karl-Werner Schramm[4,1], Antonius Kettrup[4,1], John P. Giesy[3,1], K. Kannan[3,1], Jiří Mitera[5]

[1] RECETOX – TOCOEN & Associates, Kotlářská 2, 611 37 Brno, Czech Republic
[2] Research Institute of Veterinary Medicine, Hudcova 70, Brno, Czech Republic
[3] Department of Zoology, National Food Safety and Toxicology Center, Institute for Environmental Toxicology, Michigan State University, East Lansing, Michigan, USA
[4] Institute for Ecological Chemistry, C GSF, Neuherberg, Germany
[5] AXYS Varilab Praha, Czech Republic

* Paper is Part XXXIV the presentation of Project TOCOEN

Introduction

The long-term research project „The Relationships between Environmental Levels of Pollutants and Their Biological Effects" is focused to the identification of ecological risks based on study of real environmental mixtures of persistent environmental pollutants (PEPs) and long-term effects on ecosystems. It is difficult to provide direct conclusive proof of a causative relationship between environmental levels of specific PEPs and adverse impacts on a wildlife population. Linkages between contaminant exposure and effects may nevertheless be identified by evaluating all available study data and applying a multiple statistical analysis, PCA etc.

These topics are studied from molecular and cell levels to ecosystem. Project has three levels of basic approaches:
(1) hazard identification vs. ecotoxicological properties of environmental compartments,
(2) hazard identification and assessment in the field without previous knowledge about the stress factors involved,
(3) risk assessment focused on sites (area) with known influence of stress factors.

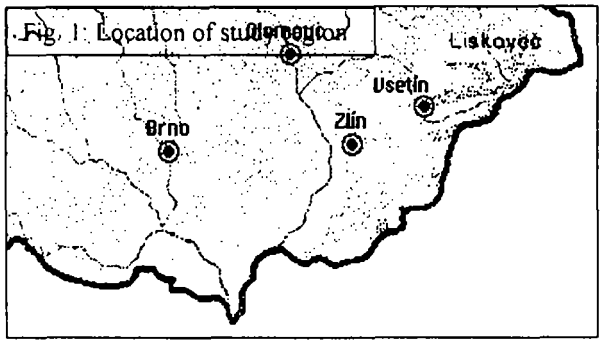
On the molecular and cell level, the effects of potential environmental pollutants on cell proliferation, differentiation, apoptosis and risk/safety assessment of their role in tumor promotion are studied. Ecosystem level includes the study of effects of anthropogenic and natural hazards on the population and communities in aquatic and terrestric ecosystems (study of biodiversity - aquatic toxicology, *in vitro* tests of toxicity, biochemical markers *in vivo* in fish liver, study of parasites).

This study of potential harmful effects which used very wide laboratory and field battery of tests, is focused to environmental/ecological risk assessment of various types of environmental mixtures of pollutants. Project is realised in present time in Czech Republic as

a example of „from molecular and cell levels to ecosystem type study", and the first example of results will be discussed in this presentation.

Methods

The project is realised as a model case study in region Zlín, east part of CR. Project includes very wide range of chemical and ecotoxicological laboratory and field methods and compares their results. This presentations is focused to the sediments from Morava river catchment area. The sediments were collected from river Dřevnice, tributary of river Morava and from river Morava. The sampling area is located in East Moravia in the surroundings of town Zlin (See Fig. 1). Sediments were collected in sampling period 1995-1998. Also samples from other moravian rivers, were collected (for comparison and study of contami-nation of Morava river catchment area – Project Chemical Time Bombs [1,2]) – see Table 1.



The level of contamination of these sediments by selected PEPs (mainly PAHs, Cl-PEST, PCBs, PCDDs/Fs, HMs) were determined and compare these values with their biological effects. The concentrations of PAHs, PCBs, PCDD/Fs, organochlorine compounds and heavy metals were determined in the extracts by conventional HPLC, GC/ECD, GC/MS, and AAS methods in order to relate the results of toxicity tests to the actual amounts of pollutants present in the samples. The clean-up procedure and PCDDs/Fs analysis were carried out according to Schramm *et al.* [3,4].

Table 1: Description of sampling sites

Number of samples	Locality
ZL001	River Moravská Sázava over town Zabřeh – background site
ZL002	River Morava above town Kroměříž, above confluence of river Dřevnice with river Morava
ZL009	River Dřevnice below Malenovice
ZL010	River Morava, town Spytihněv, below Zlín region
ZL011	River Svratka, below Brno agglomeration
ZL012	River Bečva, below Valašské Meziříčí (location of PAHs and carbon black producer)
ZL014	River Morava, below Uherské Hradiště (location of colour with contents of PCBs producer)

The major objective of the study was to develop and implement a set of *in vitro* tests suitable for the assessment of impacts of environmental contaminants on organisms at the cellular level. Dichloromethane and methanol extracts of river sediments were used as model environmental mixtures. The extracts were tested for their toxic and mutagenic effects in a set of assays of cell proliferation, cytotoxicity, apoptosis and differentiation in HL-60 cell cultures, in the bioassay of TCDD-like toxicity based on the Ah receptor-mediated induction of luciferase activity (CALUX assay), and in Mutachromoplate test. Dose-response curves were prepared for both the cellular and the biochemical parameters under study using serial dilutions of extracts. The last part of present phase of project is focused to using of *in vitro* bioassays in the assessment of AHR- and ER-mediated activity of sediments from model region. The responsiveness of different cell lines (mammalian vs. Fish cell lines and wild type vs. recombinant cell lines) were compared. On this base, the TCCD-like activity, estrogenic activity (with an estrogen-responsive cell line), potential antiestrogenicity (by addition of 17β estradiol to the cell system) in extracts from sediment samples, were determined. Part of study was focused to the determination which chemical sub fractions contain AhR and ER active substances.

Results and discussions:

Example of level of contamination of sediments from Morava river catchment area are shown in Figures 2-4. The present level of contamination will describe more in presentation.

The obtained results from set *in vitro* tests indicate the usefullness of the cellular markers of toxicity (enhanced cell apoptosis) for risk assessment in organic contaminants bound in river sediments. Therefore, the determination of the above-mentioned cellular parameters could be, together with the TCDD-like toxicity and mutagenicity tests, included among *in vitro* assays for the elucidation of mechanisms of adverse effects and toxic potentials of contaminants present in environmental samples. Continued studies will be necessary to elucidate in more detail the mechanisms underlaying the observed effects.

A suite of biochemical markers in liver tissue of various freshwater species was used for *in vivo* assessment of chemical impacts. Modulations of biochemical markers observed in the field were compared with data obtained in short-term laboratory studies in chub and rainbow trout exposed to prototypal xenobiotics. The covariance structure of the field data was subjected to multivariate principal component analysis in order to outline general patterns of biochemical responses to different types of pollutants and to reveal relationships among the biomarkers:

- CYP1A-dependent 7-ethoxyresorufin O-deethylase activity was clearly distinguishable from other biomarkers at sampling sites containing low concentrations of TCDD-like compounds.

- Testosterone 6b- and 16a-hydroxylase activities were considered an expression of CYP3A-like enzymes independent of CYP1A inducers. These activities were slightly increased at low-contaminated sites, but significantly decreased in samples from heavily polluted areas.

The interpretation of responses to contamination is complex and further investigations will be necessary.

- No considerable differences were found among responses of prospective biochemical indicators of oxidative stress, including. microsomal glutathione S-transferase activity towards 1-chloro-2,4-dinitrobenzene, cytosolic glutathione S-transferase with ethacrynic acid, and glutathione reductase. The levels of microsomal NADPH-dependent lipid peroxidation *in vitro* (as a biomarker of susceptibility to the oxidative damage of membranes) differed from the rest of oxidative stress parameters at some sampling sites; therefore this biomarker could be tested separately. The values of other biochemical markers of oxidative stress under study, including *in vitro* production of reactive oxygen species and *in vivo* lipid peroxidation, did not show good correlation with the concentrations of contaminants in sediment and fish muscle samples.

As concerning to cell comparison, all cell lines proved to be useful tools for evaluating the TCDD-like activity in sediment samples. There is a good correlation between results from different bioassays. The ED_{50} for standards and samples are cell line specific, different for fish and mammalian cell lines.

Acknowledgements

This work was supported by the Ministry of the Environment, Czech Republic (VaV 340/1/96).

References

[1] Holoubek, I,; Land Degradation and Rehabilitation, **1993,** 4, 333.
[2] Holoubek, I., Čáslavský, J., Helešic, J., Vančura, R., Kohoutek, J., Kočan, A., Petrik, J., Chovancová, J.; Toxicol. Environ. Chem. **1994,** 43, 203.
[3] Schramm, K.-W., Henkelmann, B., Kettrup, A.; Wat. Res. **1995**, 29, 2160.
[4] Bruckmeier, B. F. A., Juttner, I., Schramm, K.-W., Winkler, R., Steinberg, C. E. W., Kettrup, A.; Environ. Pollut., **1997,** 95, 19.

Fig. 2: Sum of PCDDs and PCDFs and sum of their TEQs in sediments from different sites of river Morava catchment area (calculated on dry weight basis)

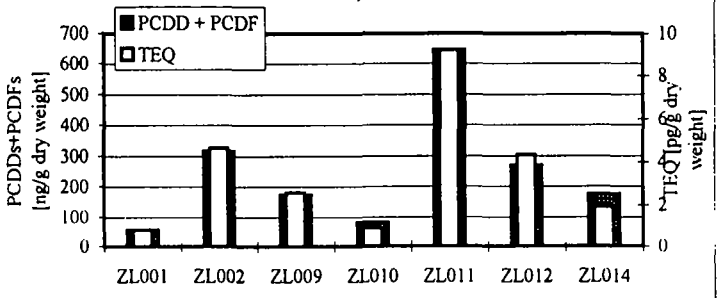


Fig. 3: Sum of concentrations of three non-ortho PCBs (77,126,169) in sediments and their TEQ (based on dry weight)

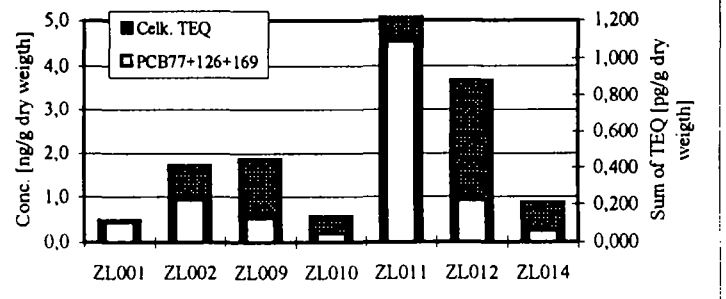


Fig. 4: Sum of PCBs + chl-pest. [ng/g] from different sites of river Morava catchment area