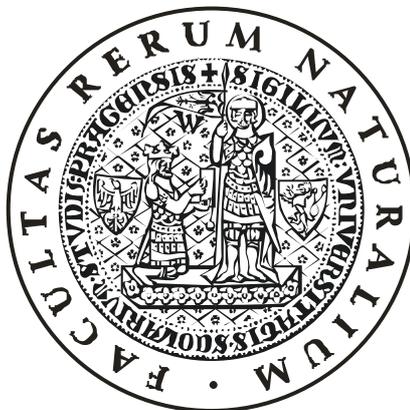


Charles University
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**STUDY AND DEVELOPMENT OF SAMPLING METHODS FOR PERSISTENT
ORGANIC COMPOUNDS
METHODOLOGY OF DATA ANALYSIS FOR POPS ASSESMENT**

by

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Under supervision: Prof. J. G. K. Ševčík

Doctoral Thesis

Prague 2010

Program of study:

Complexity of analytical sampling procedures of toxic compounds.

Water Sampling by Semipermeable membrane device and Data analysis.



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Poznání příčin přináší poznání výsledků

Marcus Tullius Cicero

Title:

A sampling strategy by Semipermeable membrane device as passive sampling method. An application of methodology for data analysis for assessment of persistent organic compounds contamination.

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Abstract

Monitoring and assessment of POPs is a very complex scope. Whereas analytical procedures passed through past 20 years by remarkable development and therefore many methods have been found as acceptable and validated, sampling analytical procedures are still of increasing importance. Parallel to conventional active sampling (grab sampling), passive sampling methods were found, based on biotic and abiotic accumulation of POPs. By wider use of those methods many distinctive features were found to increase their role in environmental monitoring in various applications, as well as screening tool. One common feature of passive sampling methods is the sampling only truly dissolved organic phase, having crucial toxicological relevance. Despite their worldwide use, recent tendencies are focused on abiotic systems, represented by (among others) semipermeable membrane devices (SPMDs), the applications of which demonstrate a strong potential for assessment of chemicals like POPs, as well as of overall toxicity, mutagenity, etc....

Results from environmental monitoring are considered as the first part of overall assessment; they are represented by *data*. As shown in many studies, the demand for any serious conclusion is the usage of complex methods. A mere graphical representation is only a halfway to a valuable extraction of data value to yield information. For large scale data sets, different *statistical approaches* for univariate and multivariate data analysis have been used in many studies. However, high costs of environmental surveys result in small data sets. Hence, both classical (e.g. Horn's) and alternative robust methods for small data sets are required. One of suitable methods is the *gnostic approach* that is well theoretically described and successfully applied in other scientific branches.

Main scope of this thesis was (i) demonstration of SPMDs capabilities for various environmental applications and identification of many findings for the broadest applicability, which are critical and fundamental for practical use in water protection and management, (ii) use of complex evaluating methods, a part for all applications, both classical marginal and multivariate statistical and not conventional gnostic approaches for revealing of hidden phenomena in the data, and (iii) development sampling device for chemical and toxicity tests for underground water assessment. All those key points were fulfilled. Moreover, some new systematic actions were initiated supporting increasing quality demands and data evaluation.

Keywords:

POPs, SPMDs, PCDD/Fs, PCBs, OCPs, PBDE, dioxins, pesticides, polychlorinated biphenyl's, accumulation, toxicity, data analysis, PCA, FA, CLU, GA, gnostic approach, statistical approach, passive sampling.

Prohlášení

Disertační práci jsem vypracoval samostatně s použitím uvedené literatury a na základě konzultací se školitelem disertační práce a ostatními odborníky v oboru.

Tato disertační práce ještě nebyla obhajována.

V Ostravě, dne 25. 5. 2010

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ACKNOWLEDGEMENT

Special acknowledgement is dedicated to persons, who played significant roles in this thesis: Jiří G.K. Ševčík, from Charles University, has stood as my supervisor and provided me with many great advices and leadership, in the concert with many critical comments by Milan Meloun. Pavel Kovanic kindly dedicated me hours of explanation of gnostic theory for possible application to environmental data; Vladimír Pekárek has stood as my “dioxin teacher”. In toxicity testing, many thanks to Vladimír Kočí, who was called to collaboration, with whom we brought together a series of common pioneering projects. Special acknowledgement must be expressed to my great friends Jim N. Huckins (called as Huck), Kees Boij, Per-Anders Bergquist, Hao Zhang, David Alvarez – all of them brought me great advices during development of SPMDs (as well as for DGTs and POCIS) and implementation in our laboratory into routine practise.

The director of Institute of Public Health Ostrava, Petr Hapala, is kindly acknowledged for his all-possible support of the National Reference Laboratory for analysis of POPs and for all international activities, which could become a part of this thesis.

The team from the lab has become my professional cradle and I am kindly obliged to all colleagues I professionally could work in the field of passive sampling. Mainly, Roman Grabic has brought a lot in excellent analytical work during pilot adoption of SPMDs, POCIS. It is not possible to forget the sampling group and persons providing technical support, leaded by Ladislava Vašutová, in the concert of excellent, systematic work by Martina Chmelová. Finally, great acknowledgement is dedicated to Jaroslav Žvak; his technical experience and technical cleverness are hidden in unique solutions of protective shrouds.

Much of work was supported by: The Grant Agency of Czech Republic, Ministry of education, Youth and Sports, under various grants. Routine data were used from CHMI’s support within the framework of bioaccumulation monitoring by the Ministry of Environment of the Czech Republic.

Finally, my recent and present family - my great children Anne and Vojta and my girlfriend Darina; all were giving me unprintable support, having much of patience during performance of all project phases, travelling, and starting of this thesis.

Tomáš Ocelka

1 INTRODUCTION

Human health and the global environment are the most discussed issues for the past 40 years. No doubt that special attention dedicates to chemicals, released into environment by direct input either as industrial chemicals or as unwanted by-products of industrial processes or combustion. Substances referred to as Persistent Organic Pollutants (POPs) play a significant role. There are hundreds of studies available about the fact that both humans and wildlife are exposed to these highly toxic chemicals.

The PCBs, PCDD/Fs, pesticides as well as HCBs, and PBDEs dominantly represent the group of chemical, manifesting the persistence, bio-accumulation and bio-concentration (with effect of bio-magnification), long-range transport in the environment. As a consequence of inputs by various pathways, their concentrations in fatty tissue can be magnified by up to 70,000 times¹⁻⁷ with respect to the background levels. Fish, predatory birds, mammals, and humans are high up the food chain and exhibit the greatest concentrations.

POPs could damage central and peripheral nervous systems, cause reproductive disorders and disruption of the immune system. Many of POPs are also considered to be endocrine disrupters, which, by altering the hormonal system, can damage the reproductive and immune systems of exposed individuals. Consequently, those compounds cause cancer or allergies.

Several actions were established within the UNEP program for international prohibition of POPs in 1998-2000, e.g. under UN agencies (UNEP, UNIDO, etc...). Nowadays, Stockholm Convention became one of the most important POPs global treaty acts to protect human health and the environment. This Convention was adopted at the Conference of Plenipotentiaries (held 22 to 23 May 2001 in Stockholm, Sweden) and opened for signature. The Czech Republic belongs to one of ratifying countries. As for the Czech Republic, there have been many studies realised from the time of first interest in POPs issue. Hand to hand, some of legislative rules have also been adopted. The Convention entered into force on 17 May 2004 in accordance with paragraph 1 of Article 26 by signature majority of countries. Stockholm Convention on POPs thus has become an international law, launching a global campaign to eliminate 12 hazardous chemicals: pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, and industrial chemical and unintended by-product), mirex and toxaphene, industrial chemicals – PCBs (unintended by-products) and unintended by-products – dioxins and furans.

Main goals of the force are possible to sum up into following tasks:

- reducing or eliminating the carcinogenic chemicals known as dioxins and furans, which are produced unintentionally as by-products of combustion,
- assisting to countries in malarial regions to replace DDT with the increasingly safe and effective alternatives,

- supporting efforts by each national government to develop an implementation plan of the Convention,
- measuring and evaluating the changes in the levels of POPs in the natural environment and in humans and animals in order to confirm that the Convention is indeed reducing releases of POPs to the environment,
- establishing a POPs review committee for evaluating additional chemicals and pesticides to be added to the initial list of 12 POPs,
- finalizing the guidelines for promoting BEP and BAT that can reduce and eliminate releases of dioxins and furans.

From this survey of priority tasks, the importance of data on high-priority organic pollutants in adequate quality is apparent. Data reliability is challenging; even from the point of sampling, analysis and data evaluation. Application of standard methods for sampling and analysis, proven laboratory experience and good documentation are pre-requisites for high information quality. No doubt, that sampling procedure plays an important role for data quality. Whereas, analytical methodology has been developed and transferred into accepted standard. However, many differences have been found among sampling methodologies, causing so many problems with both measurement and data comparison. Typically, for POPs, where levels are very low, some rigid criteria should be met. Therefore, many sampling methods, relevant for POPs were subjected to standardisation, however only for grab sampling in water⁸⁻¹⁸, sampling by biotic organisms or sediments¹⁹⁻²⁵.

Assessing of environmental pollutants exposure, especially targeting the POPs, is closely connected with applications of an *in-situ* passive sampling approach. Passive dosimeters are mostly applied to monitor air and water environment. Passive sampling technology offers a lot of advantages over the standard sampling methods^{23, 26-35}.

A new, integral, passive sampling technology for POPs reflecting aquatic exposure is based on the use of semipermeable membrane device (SPMD). This has been shown as highly effective dosimeter of hydrophobic, lipophilic organic contaminants (and their mixtures) in water of very low concentration due to bioaccumulation ability. This feature makes it suitable for sequestering previously mentioned contaminants for subsequent human and environmental risk assessment.

Due to rapid information's revolution, we have a lot of data available today from which, by modern methodologies (software databases and search engines) and powerful tools (hardware and internet connections) it is possible to yield information of particular interest. Univariate (marginal) data analysis brings useful information about quality of data, however related to single variables only. Multivariate data analysis (MVDA) is understood as the complex of methodologies for mining information and dependencies hidden in measured data; MVDA deals with extracting sample from large tables of data from diverse kind of information.

Many of persistent compounds are toxic, with various adverse effects. A standard toxicity and

genotoxicity assays are often used to assess the effects of POPs in environment ³⁶⁻⁴¹. This approach has some advantages giving complementary information to organic pollutants detected by analytical chemistry methods. However, it accounts to some extent for multiple interactions between components of complex mixtures. Moreover, the bioavailability of a particular chemical compound depends upon its hydrophobicity, molecular weight and dimensions and the “shape” of the molecule as well as other parameters ^{7,42-49}.

Nowadays, data analysis is mainly based on statistical principles. For a complex evaluation of multivariate data matrix distinctive for POPs, the usage of well described and robust methodology is a must, if classical statistics do not pass data normality. However, when using statistical approach, we are mostly limited to large data matrices. Main tool to prove statistical statements, The Central Limit Theorem, assuming randomness, independence and stationary conditions⁵⁰⁻⁵⁴ with an a priori required data model, makes complication in small scale and non-normal data. Complementary to statistical data analysis are methods based on gnostic approach⁵⁵⁻⁵⁸, with its own procedures suitable also for small data sets, with recent successful applications in economy and medicine⁵⁹⁻⁶². This methodology is filling gap between treatment of very small and medium set of data, namely when normality of data distribution is not met.

In this thesis, some applications were carried out to reveal basic objectives about POPs sampling. Both conventional (grab) and SPMDs (passive, long-term) monitoring method were performed, consequently introduced into routine monitoring. The comparison of commonly used organisms for assessment of POPs and SPMDs was also made. Moreover, the linkage among chemical parameters and battery of selected toxicity tests applied on extracts from SPMDs and there are also discussed. As a new fundamental approach, toxicity from the point of view of molecular structure and their planarity is discussed. Based on validated analytical background (coming from the laboratory where PS were accredited for sampling and analysis), some important conclusions to QA/QC were defined. Each data sample was subjected to data treatment, followed with marginal and multivariate data analysis was applied with the use of classical statistical and/or robust (statistic, gnostic) procedures.

2 THEORETICAL PART

A. Persistent organic compounds

Persistent organic compounds belong to a wide spectrum of compounds, characterised by bio-relevant properties such as lipophilicity, toxicity, etc...^{63,64}. The Stockholm Convention classifies as a most dangerous: pesticides (aldrin, endrin, dieldrin, chlordane, DDT, heptachlor, mirex, toxaphene, HCB), industrial products (PCBs) and unintended by-products (PCDDs and PCDFs). In this thesis, next to the compounds stated above, an attention is paid also to the following compounds and groups: PAHs, PBDEs, lindane, and chlorophenols. Below, there is the most important information about POPs. Extended information about their properties can be found in many others sources, e.g.^{50, 65-69}.

PCBs (polychlorinated biphenyls)

Polychlorinated biphenyls ($C_{12}H_{(10-n)}Cl_n$) are compounds with 1 – 10 chlorine atoms in molecule, having 209 positional isomers. Most of PCB congeners (under normal conditions) are colourless, crystalline solids at room temperature and atmospheric pressure, but some of the less chlorinated compounds are liquids. They have low water solubility ($0.01-0.0001 \mu\text{g.l}^{-1}$), good solubility in fat ($\log K_{OW}$ is in range of 4.3-8.26), non-flammability, limited volatility ($1.6-0.003 \times 10^{-3}$ Pa at 20°C), resistance to oxidation, hydrolysis; as the degree of chlorination increases, aqueous solubility and reactivity tends to decrease.

Due to their properties, PCBs were widely applied in industry, e.g. as coolants and lubricants in transformers, dielectric fluids in capacitors, hydraulic fluids and heat-transfer media, various additives, mainly into paints. Since their introduction (from 1929), they were manufactured in different countries under various trade names (e.g. Aroclor, Delor).

It is generally agreed, that the acute toxicity of PCB is relatively small. Acute (i.e. single-dose) oral LD_{50} values for a series of Aroclor mixtures with rats are reported of the order of 10 g/kg body weight. Put in context, this means that these mixtures are less toxic than DDT and other chlorinated pesticides. IARC has concluded that PCBs are carcinogenic to laboratory animals and probably also for humans. They have also been classified as substances for which evidence of endocrine disruption^{50, 65, 66, 68} exists.

PCDD/Fs (polychlorinated dibenzo-p-dioxins and furans)

Polychlorinated dibenzo-p-dioxins ($C_{12}H_{(8-n)}Cl_nO_2$) and polychlorinated dibenzofurans ($C_{12}H_{(8-n)}Cl_nO$) are compounds with 1 - 8 chlorine atoms in molecule, having 75 and 135 positional isomers, respectively. All those compounds have different CAS numbers.

Those compounds have very low solubility in water, within range from 2×10^{-4} to 0.43 ng.l^{-1} at normal conditions, low volatility (vapour pressure $2-0.007 \times 10^{-6}$ Pa at 20°C), and good solubility in fat ($\log K_{OW}$ in the range 6.6-8.2 for tetra- to octa- substituted congeners).

PCDD/Fs have never been produced intentionally and have been recognized as by-products of numerous industrial activities only, e.g. from production of pesticides (Spolana Neratovice). Almost all of the 210 individual congeners have been identified in emissions from thermal and industrial processes. Consequently, PCDDs/Fs were found as mixtures of individual congeners in environmental matrices, mostly strongly adsorbed on particles and surfaces. Concentration in water is very low.

Among these 210 compounds, 17 congeners can have chlorine atoms at least in the positions 2,3,7,8 of the parent molecule, considered as toxicologically and biologically significant. Toxicity of various congeners is related to the strength with which it binds to the AhR. It depends on the molecule shape; it is greatly influenced by degrees and pattern of chlorination. Toxicity of individual dioxins and furans varies by several orders of magnitude. Dioxins and furans are environmental contaminants detectable in almost all compartments of the global ecosystem in trace amounts. Some of them, especially, the 2,3,7,8-chlorine substituted PCDDs/Fs are extremely stable in the environment and bio-accumulated in fatty tissues (high K_{ow}) of animals and humans.

Organochlorinated pesticides (OCPs)

Most of used pesticides fall into five categories: (1) DDTs and analogues, (2) hexachlorocyclohexanes (lindane and its isomers), (3) cyclodienes and related compounds (aldrin, chlordane, dieldrin, endrin, heptachlor), (4) toxaphene and related compounds, and finally, (5) mirex and chlordane. Special group is formed by chlorobenzenes, especially hexachlorobenzene that is on the list of UNEP Stockholm Convention. Attention within this thesis is paid only to first two groups and HCB.

DDTs and analogues

DDT (dichlorodiphenyltrichloroethane) is a pesticide once widely used to control insects in agriculture and insects that carry diseases such as malaria. DDT is a white, crystalline solid with no odour or taste. This compound is partially soluble in water ($140 \mu\text{g}\cdot\text{l}^{-1}$, vapour pressure: 1.78×10^{-7} Pa at 20°C). DDT and its metabolites are well soluble in fat ($\log K_{ow}$ 3,69-6.2).

Technical product is a mixture of about 85% p,p'-DDT and 15% o,p'-DDT isomers⁷⁰⁻⁷⁵. Its use was banned in 1972 in the U.S., as well as in Czech Republic⁷⁶ because of damage to wildlife, but is still used in some countries. DDE and DDD are chemicals similar to DDT that contaminate commercial DDT preparations. DDE (similarly like DDD) enters the environment as contaminants or breakdown product of DDTs; DDE have no commercial use. DDT has been shown to have an oestrogen-like activity, and possible carcinogenic activity in humans.

Hexachlorocyclohexanes (lindane and its isomers)

Hexachlorocyclohexanes are compounds with general formula ($\text{C}_6\text{H}_6\text{Cl}_6$). These compounds are produced as an isomeric mixture by the photochlorination of benzene. Technical-grade

hexachlorocyclohexane (HCH) consists of 65-70% α -HCH, 7-10% β -HCH, 14-15% γ -HCH, and approximately 10% of other isomers and compounds⁷⁷. Lindane (as γ -isomer) contains > 99% γ -HCH. It is a solid, with a low vapour pressure (3.3×10^{-5}), and is poorly soluble in water (7 mg.l^{-1}), but very soluble in fat (log Kow 3.8). CAS number: 608-73-1.

The acute oral toxicity of lindane is moderate: the LD50 for mice and rats is in the range 60-250 mg/kg body weight. The dermal LD50 for rats is approximately 900 mg/kg body weight. HCHs are classified as possibly carcinogenic to humans (group 2B) by the IARC. Chronic health effects can occur some time after exposure to lindane and can last for months or years.

Hexachlorobenzene

Hexachlorobenzene (HCB) is one of products from the group (total 12) of chlorinated benzenes. This aromatic hydrocarbon has moderate volatility (vapour pressure 1.09×10^{-5} Pa at 20°C). It is practically insoluble in water ($50 \text{ }\mu\text{g.l}^{-1}$). All chlorinated benzenes are highly lipid-soluble and bio-accumulative (log Kow 3.9-6.42). CAS Number: 118-74-1.

HCB is toxic to aquatic life, plants, animals, as well as humans. It is listed by the IARC as a Group 2B carcinogen, with possibly carcinogenic effect to humans.

Polybrominated diphenyl ethers (PBDEs)

Those compounds do not belong to UNEP 12 list of the most targeted chemicals, but along with other substances⁷⁸⁻⁸¹, it falls into interest for long-term assessment. Their importance is increasing as in the consequence of increasing their common usage. They have the general chemical formula ($\text{C}_{12}\text{H}_{(10-n)}\text{Br}_n\text{O}$), with the sum of H and Br atoms always equal to 10. The congeners are numbered from 1 to 209 using the same IUPAC scheme used for PCB.

All of the PBDEs present in commercially available products have (under normal conditions) low water solubility ($<1 \text{ ng.l}^{-1}$), and high log Kow values (4.4-9.9), low volatility 3.85×10^{-3} - 1×10^{-7} Pa at 20°C. The toxicology of PBDEs is not well understood, but PBDEs have been associated with tumours, neurodevelopmental toxicity, and thyroid hormone imbalance^{69, 82-86}. It is presumed that PBDEs are endocrine disruptors, but research in this area is scant. The neurotoxic effects of PBDEs are similar to those observed for PCBs^{87, 88}.

All previously mentioned POPs can be found in many ecosystems. Their presence in water, found by SPMDs within this thesis is described further. Some applications are related to source places of POPs (Spolana Neratovice of PCDD/Fs and OCPs).

B. Sampling of POPs

In general, the quality of chemical information depends on the adequacy of the sampling strategy, the effectiveness of sampling techniques (sampling devices, capability of persons, supporting technical equipment, etc....) and methods for *in-situ* sample treatment⁸⁹⁻⁹⁴. The objective of representative sampling is to ensure that a sample or a group of samples accurately characterizes site

conditions in a representative manner. Sampling procedures should be designed to minimize the sampling error and to document an estimate of the overall error, which includes (i) sampling error, (ii) sample-handling error, (iii) analytical error, (iv) data treatment error. Planning for sampling and assessment of sampling methods should be given the same care as analytical data validation. Active sampling techniques is the most widely used approach for the collection of representative portion of sampling object, and those are subjected to wide standardisation, e.g. ^{95, 96}.

Active sampling technique for water has distinctive features of the total content of chemical in all waterborne phases, e.g.: dissolved phase, various inorganic and organic particles, micro-organisms and algae. Toxicological evaluation of POPs should distinguish between generally less bio-available residues in other waterborne phases and waterborne residues represented in dissolved phase. Many states and environmental protection authorities adopt only general rules for grab sampling methods of POPs, mainly PCDD/Fs, PCBs dioxin-like, PBDEs. However, no special attention is put to sampling with respect to POPs. A standard protocol for sample collection and on-field sample treatment, of particular samples, needs with respect to POPs a special attention of each laboratory responsible for analysis. Due to lack of general rules of sampling strategies for sampling of POPs, special attention must be given to preparation steps, reflected in sampling plan.

Due to very complex phenomena of active sampling, only general rules for passive sampling by SPMDs of POPs are given in following parts.

Passive sampling methodology

In recent years, *in-situ* passive sampling techniques have been developed, in parallel to conventional techniques, mentioned above. Main advantage of passive sampling systems (PS) is an integration of chemicals over exposed time as a result being at the time weighted average (IWA) level^{19, 21-25, 29, 35, 97, 98}. Moreover, during exposure of PS by a contaminant, the total concentration reflects integral amount of analyte and thus reduces problems arising from its short time concentration peaks. Passive samplers provide reliable sampling efficiency at very low concentration level, even for application in glacial or mineral water assessment^{99, 100}. Assessing of POPs is generally based on risks evaluation. For this purposes QSARs methodology have also been developed. This methodology attempts to predict the toxicity of compounds based on physicochemical properties and descriptors of compounds, in both aquatic and terrestrial species¹⁰¹⁻¹⁰³. The standardisation and result comparison, e.g. in the form of intercalibration studies means upcoming challenge¹⁰⁴⁻¹⁰⁹.

The evaluation of contamination levels, risk estimation, interrelation among observations and parameters of truly dissolved phases by means of different statistical methods^{110, 111} (from exploratory to marginal analysis and Principal Component Analysis (PCA), FA (Factor Analysis), Cluster Analysis (CLU), or alternative robust methods (gnostic analysis^{112, 113}) is discussed on practical results later.

Development of SPMDs

Passive sampling methodology for POPs operates on the principle of partitioning of particular contaminant with sampling device, called Semipermeable Membrane Device (SPMD). Application of SPMDs is dominantly based on those factors ^{59, 114}:

- physicochemical properties of the chemical of interest (e.g. molecular weight, size, polarity),
- environmental conditions (e.g. temperature, velocity of the medium),
- physicochemical properties of assessed system and its total composition (e.g. phase compositions, TOC, DOC),
- properties and behavioural characteristics of sampling system (e.g. construction for synthetic system, anatomical character for biotic systems).

Applicability of SPMDs

Main characteristic of partitioned non-polar organic compounds (POPs), expressing high tendency to partition from the aqueous phase to natural organic phase, is the partition coefficient K_{OW} , which quantifies concentration of compound in n-octanol (C_O) and water (C_W). As example, K_{OW} defined as 1000 means that the total amount of the contaminant in the octanol phase is 1000-times higher than in an equal amount of the contaminant in the aqueous phase. K_{OW} are often given as $\text{Log } K_{OW}$. This value is one of critical parameters, which enables prediction of capability to sample (sequester) a given compound^{99, 100, 115-121}.

First passive sampling devices (like SPMDs) were being made from cellulose, vinyl chlorides, polyvinylidene fluoride, and polytetrafluoroethylene, since the introduction of SPMDs in. As a sequesterant, a non-polar solvent was used. Similar construction, based on cellulose membrane and filled up with hexane was developed later¹²²⁻¹²⁵. This device was limited for narrow scale of sequestered chemicals, mainly PCBs and OCPs. By further research problematic differences were shown in their uptake rates, given, most probably by (i) uncontrolled dissipation, (ii) not well specified thickness and structure of membrane wall, (iii) mass transfer resistance. Until now, cellulose membranes have not found too much practical applications for routine monitoring of POPs. Next generations of sampling membranes were introduced by nonporous polymeric membranes based on LDPE, PP, PVC, silicone, polyacetate. As major sequesterant, hexane, ethyl acetate, dichloromethane, isooctane were used. Best results, from the point of comparability, were exhibited by LDPE and PP. Further development and testing on various membranes and sequesterants optimized the design, which was introduced in 1989, and consisted of 99% triolein as sequesterant, filled in layflat LDPE ^{99, 126}. Just triolein has been selected as a standard sequesterant for SPMDs design. However, fish lipid and silicone fluids exhibited good results for sequestering non-polar compounds ¹⁰⁰.

The application potential of the SPMDs is quite broad: various authors demonstrated their

applicability for various applications, e.g. ¹²⁷⁻¹²⁹. Stockholm Convention pollutants (PCBs, OCPs, PCDD/Fs), PBDEs, PAHs, other pesticides (such as pyrethroids, endoulfan, diafon), PCNs, PCBs, PCP.

The SPMDs technology has been *roughly* standardised in those aspects: design, manufacture, general procedure for various applications, and application of conventional analytical procedures. The SPMDs are subjected to US Government^{101, 130-137}. The US Department of Commerce granted a private company Environmental Sampling Technologies (abbreviated as EST; 1717 Commercial Drive, St. Joseph, MO, USA, www.est-lab.com) for exclusive license to manufacture and sell SPMDs in the USA. The applications related to an aquatic systems are described below.

Biomonitoring organisms

Nowadays, various organisms are routinely used for assessing waterborne bio-available chemicals. However, the concentration found after their exposure has certain variability due to their characteristics (anatomy, physiology, behaviour, migration, etc. . . .¹³⁸⁻¹⁴⁰. Due to potential variability in BMOs characteristics, the exposure (expressed as BCF and BAF) can be found as site- and kind-specific. To minimize such variations, those BMOs require a special care^{119, 141-147}. If such conditions are met, the comparison of various sampling organism can evaluate similarity and suitability of sampling methods. Various studies shows ¹⁴⁸⁻¹⁵¹, that environmental exposure does not represent thermodynamic equilibrium, where residues in tissue are proportional to environmental contamination. On the contrary, this feature can help to estimate the lethal dose, approximated by application of gnostic approach^{99, 101, 122, 125, 126, 132, 148, 152-157}. Despite to these features, which seems to be limiting for wide applications, BMOs still have applicability for monitoring studies, together with newly adopted synthetic passive sampling devices. The comparison of BMOs and SPMDs – both groups of PS, involved into biological monitoring of surface water from 1999 and 2003 respectively to the present, evaluated by analytical methods (statistics, gnostics)^{158, 159}, is given below, as presented recently, and ready for publication.

Toxicity testing on SPMDs

Nowadays, testing organic solvent extracts from any environmental compartment plays a very important role. It is often used to assess the total toxic effect of hydrophobic POPs. This methodology was also applied for aquatic environment in various studies abroad¹⁵⁸⁻¹⁶¹. This approach is advantageous over the risk assessment of organic pollutants based on their concentrations detected by analytical chemistry methods as it accounts to some extent for multiple interactions between components of complex mixtures. For SPMDs, various bioassays and toxicity on extracts were applied within this thesis, too. Some of them were published recently^{156, 162-168}.

C. Data analysis (univariate, multivariate)

When all results are received with given QA/QC, the final step must meet requirements of proper data treatment. Generally, either statistical or alternative approach can be used. Statistical approach is the most commonly used tool. In laboratory, univariate statistical methods are mostly

used to support validation of measuring methods, in the form of exploratory data analysis (EDA) or marginal analysis¹⁵⁸⁻¹⁶¹. Particular cases are distinctive by number of replicated analyses, analyses of standards to estimate calibration of analytical system, made to provide quality. The concept of statistical approach was theoretically well described^{111, 169, 170} and applied^{112, 113}, to be used as example for complex analysis of POPs and toxicity responses. However, despite its wide applications, this approach is generally usable whenever one has a sufficient amount of data required by the theory of larger sets of data, resulting from paradigm of statistics (the central limit theorem, randomness of data, independence and stationary conditions¹⁷¹⁻¹⁷⁶).

The philosophy of Gnostics is a counterpart to agnosticism¹: the existence of an individual data's true value is taken for certain and an optimal theoretical path to estimation of its value is derived. Proper measuring of information born by individual data and maximization of its yield is the important point. Gnostic characteristics can be thought of as path integrals over Riemannian spaces. It is of fundamental importance that the metric of these spaces (the way of measuring distances, angles and curvature) do not result from some *a priori* (and subjective) assumptions; metric is solely determined by the data to be treated. Detailed description of gnostic theory is exceeding the scope of this work and can be found in literature^{55-58, 112, 113, 177}. However, basic assumptions, on which applications have been realized, mainly for economy and accounting^{59, 114, 178-180}, followed demonstration of the usability potential for environmental data^{61, 114}.

Within the framework of this thesis, there was no attention put to univariate statistical analysis yielding descriptive parameters. Due to complexity of POPs, main attention was put to multivariate statistical analysis, like CLU, PCA, FA, revealing relations between variables and observations ^{158, 160, 161}. As an alternative, the gnostic approach was taken into account.

¹ An agnostic is one who thinks it is impossible to know if there is a God or anything behind the material phenomena. (In the context with the gnostic substitute "a true data value" instead of "God".)

3 EXPERIMENTAL RESULTS, DISCUSSIONS AND OUTCOMES

D. Methods, procedures and data analysis

Semipermeable membrane device (SPMDs)

Since the development of SPMDs, there were various arrangement used. Applications described in this thesis used triolein based SPMDs as the best choice for all applications due to:

- (i) Its commercial availability,
- (ii) Low LDPE permeability due to high molecular weight,
- (iii) Presence of triolein in many organisms as a storage lipid,
- (iv) Well correlated and comparable results among other applications world-wide,
- (v) Sequestration ability up to 0°C (due to freezing point about -4°C),
- (vi) Applicability to toxicity tests on the same SPMDs, exposed for chemicals.

For all experimental work considered below, following standard design of SPMDs was used: the lay flat thin-walled tube of nonporous (with transient cavities) material LDPE, filled with 1 ml of synthetic lipid – triolein, neutral triglyceride (1,2,3-tri-[cis-9-octaceno]glycerol) of high purity (>97%), which makes a thin film in membrane. Exploded view of overall SPMDs is given in Figure 1. The SPMDs as a whole as well as quality of used LDPE is protected by patent pending (see above). The selection of nonporous LDPE layflat tubing for SPMDs was based on its resistance to organic solvents, abrasion and defined surface. Transports of contaminants were through transient pores, with specific diameter approx. 10^{-9} m (similar to postulated size of transient cavities in biomembranes is $9.8 \cdot 10^{-9}$ m)^{112, 113, 177}. The SPMDs had following dimensions: width 2.5 cm (lay-flat), overall length 91 cm and wall thickness 75 -90µm, overall sampling area is about 460 cm², total mass is about 4.5 g.

Since then, after SPMDs postulated as a *standard membranes* designed with previously mentioned parameters, the SPMDs were commercially manufactured, with own QA/QC, which made them possible to compare measured results. Since introduction of SPMDs, majority of presented applications (as well as those within the framework of this thesis) have been provided on the standard design. However, some applications were found on non-standard membranes, with limited reproducibility and applicability of calibration data, despite some attempts for calibration^{100, 118, 120, 181, 182}, using own membranes or as a tool for effective method of separation of organic contaminants from lipids^{183, 184}, based on SPMDs.

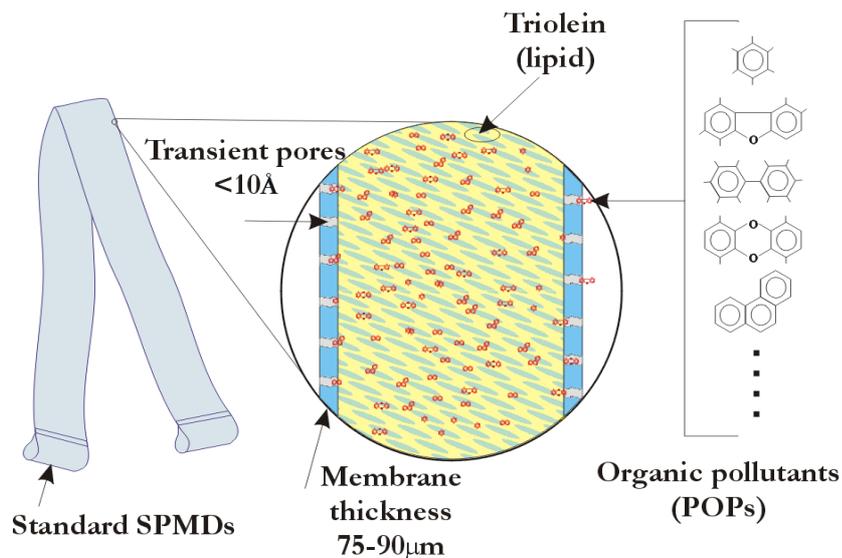


Figure 1 Exploded view of standard SPMDs

Uptake modelling and ambient concentration evaluation

In general, there are two types of models available for uptake of hydrophobic organic chemical in SPMDs: (i) chemical reaction kinetics (based on chemical reaction principle), (ii) mass transfer coefficient (based on Fick's law of diffusion). For the objective of given list chemicals, mainly POPs, the chemical reaction uptake kinetics was used precisely described below. This modelling is derived from the assumption that the uptake process obeys first-order kinetics (see Figure 2).

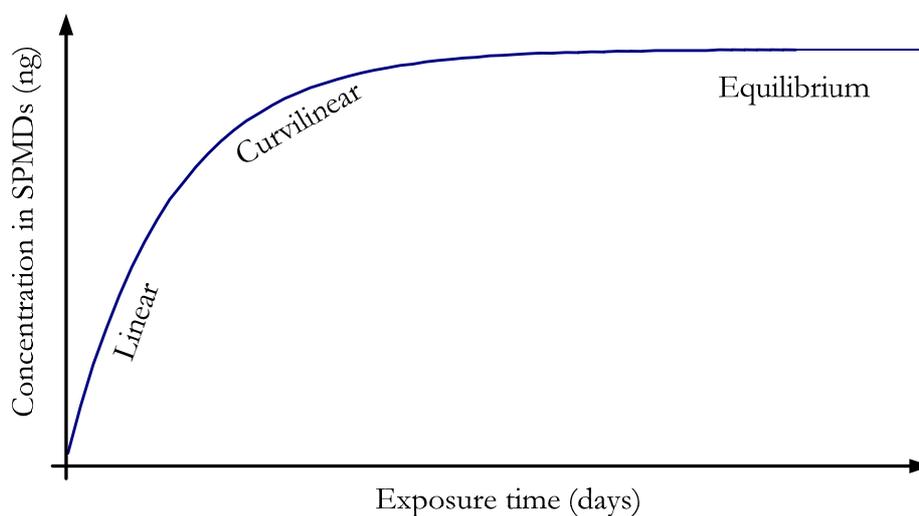


Figure 2 Typical uptake of sampled POPs in SPMDs
 This uptake has three phases. Time on each phase depends on physicochemical properties of POPs to be sampled and on other conditions (mainly: SPMDs design, linear velocity, temperature, TOCs)

As in reaction kinetics, there are two directions (both first order kinetics): contaminant (i) uptake (forward), given by uptake rate constant (k_U), and dissipation (backward), given by dissipation rate constant (k_D). The rate of change of the contaminant (i) concentration in the SPMDs (C_S) by time (t), from its concentration in water (C_W) is given by:

$$\frac{dC_{S,i}}{dt} = k_U C_{W,i} - k_D C_{S,i} \quad \text{Eq. 1}$$

By integration at initial conditions, $C_S = 0, t = 0$, by substitution by SPMD-water partitioning coefficient, $K_{SW} = k_U / k_D$, it is possible to write:

$$C_{S,i} = K_{SW} C_{W,i} [1 - \exp(-k_D t)] \quad \text{Eq. 2}$$

Equation 2 describes basic uptake of contaminant (i) in SPMDs. From this equation, there are two marginal situations derived mostly applied in practical use:

Integrative sampling (linear uptake model)

This is the case of short exposure time and/or highly hydrophobic compounds, where $k_D t \ll 1$, where the $C_{S,i}$ is given by Eq. 3. It holds:

$$C_{S,i} \cong K_{SW} C_{W,i} k_D t \quad \text{Eq. 3}$$

In this case the $C_{S,i}$ increases linearly with the time and exposure is called as „linear uptake model“ and sampling is integrative.

Equilibrium sampling (equilibrium model)

This is the case of long-term exposure, when concentration in SPMDs gradually increases with time until equilibrium is attained, where $k_D t \gg 1$, and it holds for the $C_{S,i}$:

$$C_{S,i} = K_{SW} C_{W,i} \quad \text{Eq. 4}$$

In this case, the exposure mode is called as “equilibrium sampling”.

As for curvilinear phase, there is a transition state from *linear* to *equilibrium* model. This case is not practically used for evaluation $C_{S,i}$ or $C_{W,i}$, respectively.

If substituted for $C_{S,i} = n_{S,i} / V_S$, where $n_{S,i}$ is the amount of contaminant (i) in SPMDs and V_S is volume of SPMDs, then we can rewrite the Eq. 4 into following form. It holds:

$$n_{S,i} = R_{S,i} C_{W,i} t \quad \text{Eq. 5}$$

where $R_{S,i}$ is given by:

$$R_{S,i} = V_S K_{SW} k_D \quad \text{Eq. 6}$$

The calculations of ambient concentration were usually performed from the following equation, derived from equation 5 and 6. It holds:

$$C_{W,i} = C_{S,i} V_S / R_{S,i} t \quad \text{Eq. 7}$$

The term $R_{S,i}$ is the *sampling rate* parameter of contaminant i and provides a conceptual link between classical extraction techniques and passive sampling with SPMDs. Term $R_{S,i} t$ can be understood as a volume of water, in which contaminant $C_{W,i}$ is dissolved. Sampling rate is in L.d⁻¹, which can be interpreted as the volume of water (L), dialysed per day, for particular contaminant (i) and time(t).

Sampling rates were used for calculation of ambient concentration of contaminant $C_{W,i}$. They have been determined for a large number of compounds, given in application survey below: PAHs^{115, 116}, PCDDs/PCDFs^{130, 136, 149, 185-197}, PCBs^{100, 136, 148, 195, 197-203} and a number of polar pesticides^{136, 197, 198, 203-205}. Their values were determined for various temperatures, practically within the range from 2°C to 30°C. Those sampling rates were also used for calculation of ambient concentration within applications given below. For temperature correction, Arrhenius equation were used; R_S can also be estimated as a function $\log K_{OW}$, found in publication for $15 \pm 4^\circ\text{C}$. All those approaches are available in detailed description in publications^{100, 150, 201, 206}. This approach was practically used, until PRCs (Performance Reference Compounds) approach was not used. Within this thesis, both approaches were used.

The PRCs approach

The method has been generally applied to assess behaviour of SPMDs and of a contaminant during real exposure, under given condition in a water ecosystem. This approach was mostly applied within this thesis, data with temperature based R_S were not all recalculated. If compared on selected data sets, data were well comparable. Principle of this approach is similar to use of an internal standard, uptake rates of contaminants in SPMDs devices were calibrated *in-situ*. Such standards were added to SPMDs triolein prior to an exposure. All those compounds were introduced into standard SPMDs device in given quality and concentration level prior deployment. Using PRCs approach, temperature logging were not further required.

Dissipation of the PRCs was derived from equation 8. It holds:

$$n_{PRC} = n_{0,PRC} \cdot \exp(-k_D t), \quad \text{Eq. 8}$$

where n_{PRC} is the amount of the PRC in SPMDs at time t , and $n_{0,PRC}$ is the amount of the PRC in the SPMD at the beginning of the sampling. Both parameters (n_{PRC} , $n_{0,PRC}$) were measured and evaluated. Solution of Eq. 8 results in equation for dissipation rate constant k_D . It holds:

$$C_{W,i} = C_{S,i} V_S / R_{S,i} t \quad \text{Eq. 9}$$

Assuming that the uptake in the SPMDs was linear and integrative, the estimated k_D were used for calculation of $C_{W,i}$, according Eq. 9. It holds:

$$C_{W,i} = \frac{n_{S,i}}{k_{D,PRC} K_{SW} t V_S} \quad \text{Eq. 10}$$

More detailed descriptions of PRCs and various approaches are given in ^{123, 136, 149, 186, 207-210}.

Arrangement of deployment devices for SPMDs

All arrangements described below, were adopted from various applications. Practical arrangements using standard membranes can be seen in the Figure 4. General requirements about conditions for deployment apparatus were recently described^{100, 126, 192, 211, 212}. Mostly applied sampling

arrangements for surface and underground water are shown in the Figure 3.

For *surface water*, SPMDs were inserted and stretched in sampling racks (by means of hooks and spiral springs), put in amount of maximally five into protective shrouds.

For *underground water*, SPMDs were inserted and stretched in a sampling holder (also by means of hooks and spiral springs). A new protective shroud was developed for *two* SPMDs sampling devices (e.g. one for chemical parameters and one for toxicity). This system is subjected to patent pending (on the time of submission of this thesis).

Because uptake rate (R_s) depends on temperature (see Eq. 7 above), temperature was measured during whole deployment before using SPMDs in the most recent studies. Temperature measurement for applications described below, has been carried out by means of Tiny Talk® (by INTAB Interface-Teknik AB, Sweden). This data-logger continuously registers the temperature at set up intervals².

Fully equipped sampling system was mounted into secured place, by rod or rope (see Figure 4). The sampling equipment was placed in such a way that a flow of contaminants from sediment was omitted. Under a high water flow (see WWTP application), protective shrouds were inserted into special stainless-steel box having function of flow-splitter.

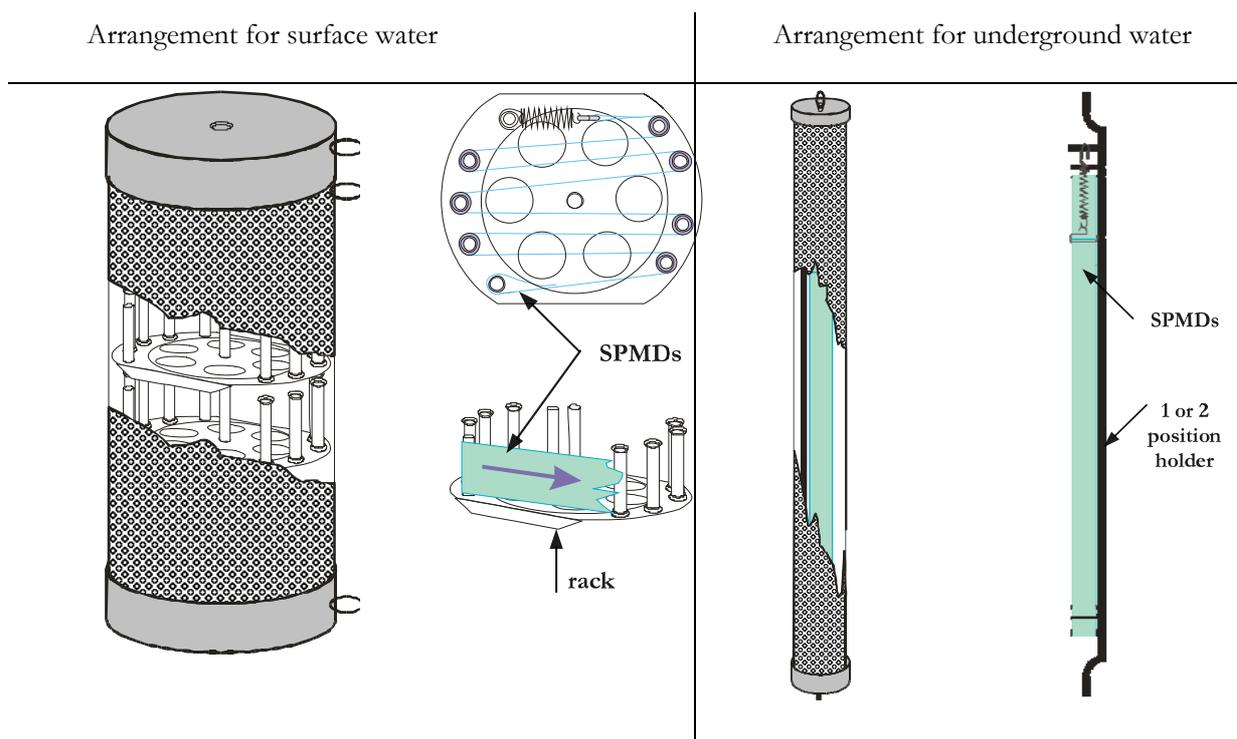


Figure 3 Sampling arrangements – protective shrouds for SPMDs
For surface water: SPMDs are inserted and stretched in sampling racks (left); for underground water SPMDs they are inserted and stretched in sampling holder (right)

² Mostly 1-2 hours. In some cases (see application *Assessment of POPs in Rivers in the Czech Republic* below), passive sampling devices for heavy metals and polar organics were used (such DGTs or POCIS respectively), and mounted to protective shroud as well.

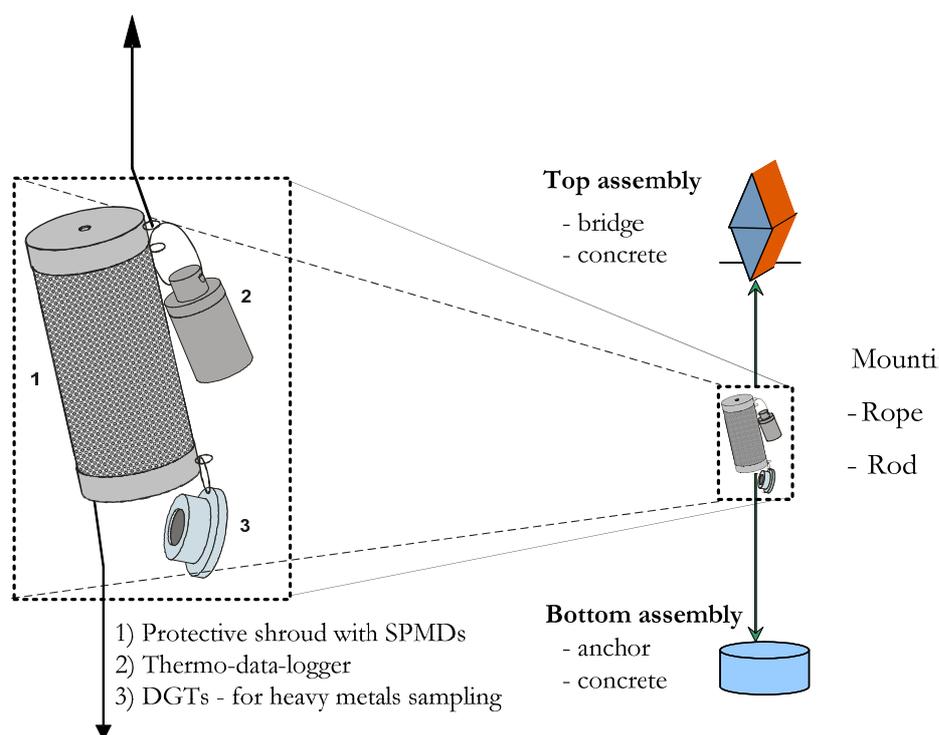


Figure 4 Overall configuration for SPMDs deployment

Used sampling strategy and quality QA/QC for SPMDs

All published studies of passive sampling by SPMDs were derived from generally accepted strategies for active (grab) sampling, and compared with other studies²¹³⁻²¹⁸. Due to special character and complexity of passive sampling of POPs, judgemental and random strategies are mostly applied and published. This strategy was also applied within this thesis.

Used biotic organisms

Dreissena polymorpha used for sampling was collected in sand-pit with very clean water. Small shells were placed in polypropylene cages (100 pieces/cage) and fixed at the same float as SPMDs. *Dreissena polymorpha* were exposed to sampled water for two months. Both SPMD dialyzates and *Dreissena* extracts were analyzed by GC/MS/MS using isotope dilution and internal standard; analytical methods were validated in accordance with EN ISO/IEC 17025 standard. For PCBs, over 95 PCB congeners were detected and quantified.

Toxicological responses from SPMDs; used toxicity tests

First applications within this thesis were realized to assess chemical contamination. However, due to broad potential of SPMDs to assess toxicity after exposure, the whole SPMDs were also used in various unique studies within this thesis¹³². Even the first application for SPMDs was performed at WWTP in Ostrava, and on the contrary, at clean environment^{103, 219, 220}. Their selection was based on

the particular sensitivity of SPMDs to exposed POPs³. The toxicological response was based on EC₅₀, expressed as the V_{TOX} parameter that was developed during our research recently¹⁶⁴.

General method for SPMDs pre-treatment was established as follows: an extract after dialysis was transferred into an acetone: DMSO (1:1) mixture. Samples prepared in this way were used for grounding of the dilution series for bioassays. Conventionally applied aquatic bioassays were applied. With respect to a limited amount of obtained extract, only small volume toxicity tests (microbiotests) were usually carried out. This approach had been successfully used in following cases: test of inhibition of light production of bioluminescent bacterium *Vibrio-fischeri*, acute toxicity test with cladophora *Daphnia pulex*, test with rainbow trout *Oncorhynchus mykiss* and test of inhibition of algal growth of *Scenedesmus subspicatus* and/or *Selenastrum capricornutum*^{40, 103, 132, 161, 164, 167, 168, 220-222}.

Toxicity of POPs contaminated effluents depends on the amounts and types of the individual compounds present; however, even for pure compounds, concentration-toxicity relationships are generally nonlinear. Toxicity of mixture of compounds is not linearly additive with individual toxicities of components in the mixture and it causes a problem for prediction of the impact of a contamination on the ecologic system.

For toxicity evaluation, the SPMD extracts were tested on chlorococcal alga *Desmodesmus subspicatus* (earlier *Scenedesmus subspicatus*) and luminescent bacteria *Vibrio fischeri*.

Algal bioassays were provided with *Desmodesmus subspicatus*, strain BRINKMANN 1953/SAG 86.81 (obtained from Culture Collection of Autotrophic Organisms, Institute of Botany, Czech Acad. Sci., Trebon). The alga was kept and cultivated in suspension condition and medium recommended in ISO 8692 guideline. Due to a small amount of SPMD dialysate 96 wells microplates with 0.3 ml of suspension in 6 replications were used. Monospecific algal cells were cultured for several generations in a defined medium containing a range of concentrations of the tested SPMD aliquot, prepared by mixing appropriate quantities of nutrient concentrate, demineralised water and an inoculum of exponentially growing algal cells. The amount of 10⁴ cells per millilitre as initial cell density were used. The test solutions were incubated for a period of at least 96 hours, at a light intensity of 60 μ E.m⁻².s⁻¹, fluorescent tubes-day, and temperature 27°C. Cell density in each suspension was measured every 24 hours, microplate reader BIOTEC was used to measure the optical density at 750 nm. Cell density determination was based on optical density – cell density calibration curve. Inhibition was measured as a reduction in growth and growth rate, relative to control cultures grown under identical conditions. The results, values of 96hEC₅₀, were counted for inhibition of algal growth rates.

Test with bioluminescent bacterium was carried out following the standard procedures (ISO 11348)^{132, 223}. The samples were tested in a medium containing 2% of NaCl and about 10⁷ cells of

³ It is noteworthy that only selected POPs were determined. No finite range of chemical parameters were observed, neither relevant toxicological response (individual or mixture).

bacteria reconstituted from the lyophilised reagent (Bruno Lange, *Vibrio fischeri* NRLL-B-11177). Control samples (i.e., bacterial suspensions to which 2% NaCl was added instead of a test sample) were always run parallel to the test sample. Tests were performed at 15°C, pH of all dissolved samples in this study was 5-8; it was not adjusted. Each test was run in duplicate 6 to 10 sample concentration and a negative control. The luminescence was measured with the LMZ II tube luminometer (Immunotech, A Beckman Coulter Company) at 5-, 15- and 30- min exposure times. The concentration of the original SPMD triolein (mg/L/day), which caused a 50% reduction in light production after exposure for 5 (or 15) minutes, was designated as the 5 (15)-min EC50. Potassium dichromate ($K_2Cr_2O_7$) was used as a reference toxicant, as corresponded with the ISO 11348 guideline. As for EC50 (30min), the inhibition caused 4 mg/l of dichromate that was within recommended range as specified in the test guideline.

The bioassay with crustaceans is based on the immobilization of *Daphnia magna* STRAUS, following the procedures recommended by ISO 6341, which is in turn compliant with national and international standard methods (e.g. OECD, EEC, US EPA, ASTM guidelines). Female individuals of *Daphnia magna*, up to 24 hours old, were exposed to the samples for 24 and 48 h at a temperature of +20°C. All animals originated from laboratory breed were kept and fed (with algae) accorded to ISO guideline. Because of small amount of tested SPMD membranes, miniaturized test design was applied. Tested solutions were 20 ml and reduction in number of treated animals was provided; minimum volume for one crustacean was 5 ml.

The parameter V_{TOX} allows direct comparison of sample toxicity by SPMDs with different duration of its exposition, different sites, projects and laboratories to be performed. In general, V_{TOX} represents a volume of media which is theoretically needed for dilution of all toxicants absorbed in one membrane during one average day of deployment to obtain solution with chosen effective concentration, for example EC50. The higher is V_{TOX} , the larger volume of toxicants was absorbed and thus the higher contamination of sampled site results. The following formula defines V_{TOX} , where (m) is the concentration of extracted membranes in solvent mixture expressed as number of membranes in ml of solvent mixture (pcs.ml⁻¹), (d) is duration of deployment of membrane during a sampling (days) and ECXX is an effective concentration of extract on chosen organism, for example EC50 (ml.L⁻¹) – see equation 11.

$$V_{TOX}(50) = \frac{1}{m \cdot EC50 \cdot d} \quad \text{Eq. 11}$$

Similarly, like toxicological unit TU, one of the benefits of V_{TOX} is its property (L.d⁻¹) of easy demonstration of contamination level – the higher V_{TOX} , the higher ambient contamination.

Strategy for identification source of contamination

In a study noticed below (see application *Assessment of POPs in Rivers in the Czech Republic*), the sampling strategy was also suited for identification of POPs' contamination sources. For this application, judgemental sampling strategy played important role. For proper selection of reasonable,

cost-effective strategy, all sources and migration pathways were considered before the sampling locations were chosen. The strategy was developed for various typical applications (see Figure 5), with (i) pre-identification of all potential sources, (ii) description of migration of targeted contaminants, and (iii) design steps of detailed identification from the main stream, including the tributaries. For proper design of strategy for POP source, following fundamentals were important to be accounted for:

- Current status of each contaminant in selected profiles, including the recent trends (if available). For POPs, it was also important to consider sampling and analytical methods used. The older data, the less reliability was attached to the strategy design.
- Site topographic features (possible sources, water flows of the streams of concern, and their changes upon various climatic conditions).
- Upcoming meteorological conditions during identification were taken in account (mainly the temperature; occasionally wind direction, wind speed, humidity), as common parameters registered during sampling.
- Human/wildlife activities on or near the site.

A basic proposal for practical identification of relevant sources of POPs by SPMDs was designed and applied:

- General description of the system (depth, width, flow, possible sources),
- Identification of the main stream (toxicity and all chemicals of interest),
- Identification of the tributaries (of the main stream); toxicity and indicating chemicals, relevant POPs based on fingerprinting from the point above.

If a positive response in identification of some contamination at downstream was obtained, then identification of the rest of sub-tributaries was proposed with toxicity parameters and selected (indicative/fingerprints) POPs parameters. When a source was found, all its chemicals of interest had to be identified.

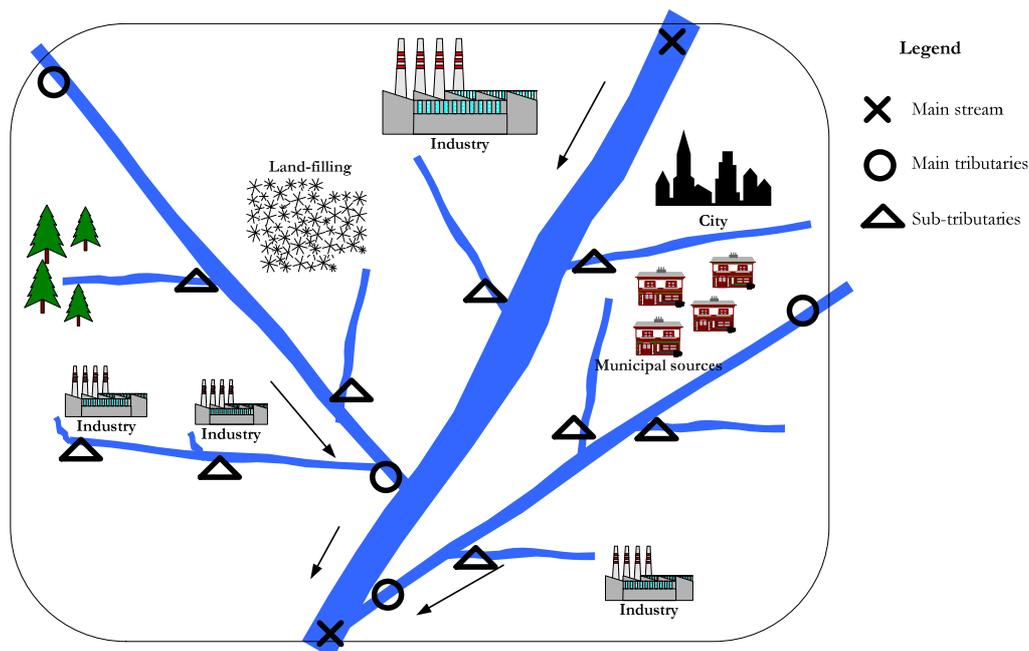


Figure 5 Identification of sources of POPs by SPMDs

Identification of sources is an expensive, time consuming process. For that reason, the methodology was realized stepwise. Such approach requires preliminary requirements and format for data comparability to be defined. Some results of this methodology can be seen in application below.

Handling, sampling and analysis – the protocol for SPMDs

Standard method of sampling, handling and analysis was considered as the main prerequisite of producing comparable results of appropriate quality. A general scheme for overall proceeding with the SPMDs has been recently proposed as a base for particular adoption in analytical laboratories²²⁴, on which the method was adopted in NRL, where all SPMDs within this thesis were analysed. The scheme showing application relevant for quality data production can be seen in following Figure 6, with detailed description in the Table 1.

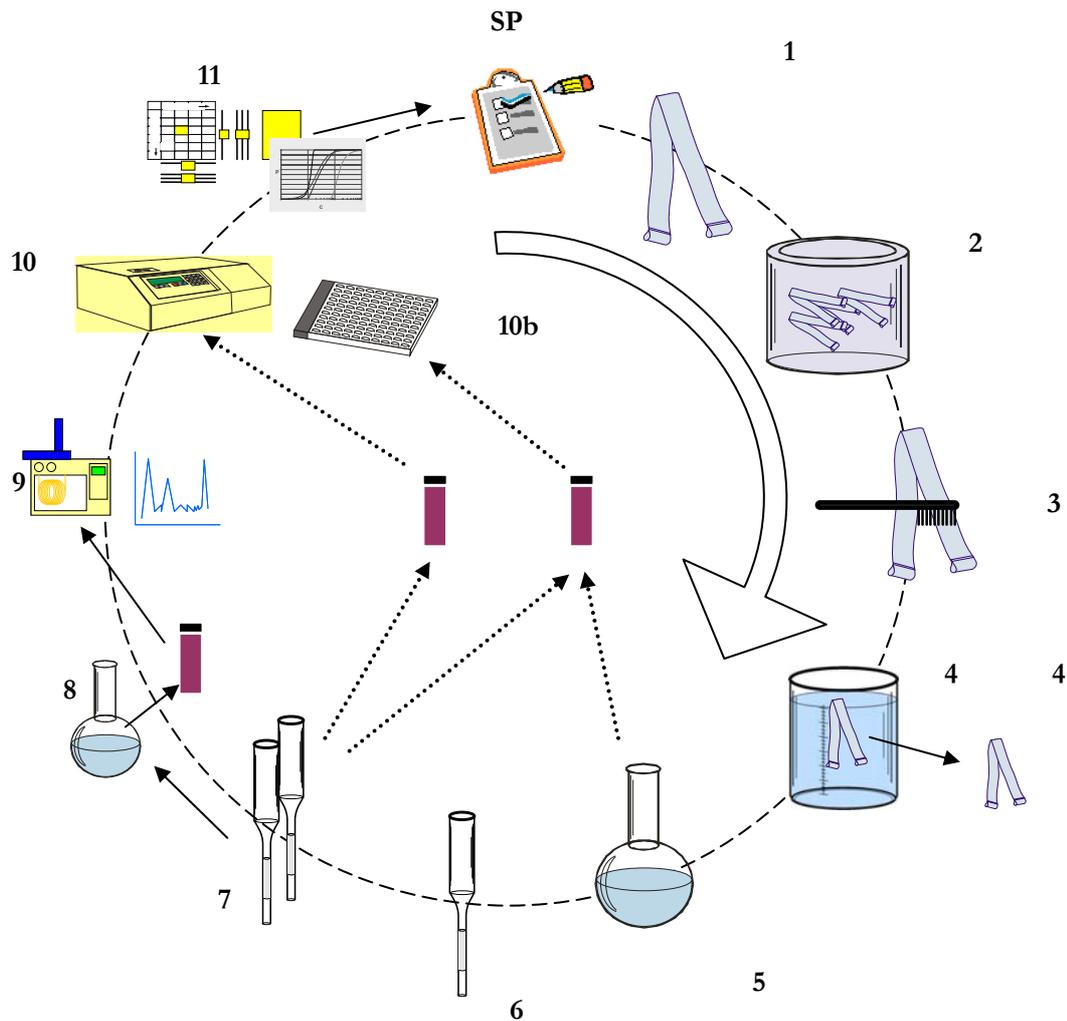


Figure 6 Overall SPMDs processing (the “SPMDs clock”)
Legend: SP=sampling plan; 1) manufacture, storage before using; 2) transport to deployment; 3) exterior cleaning after retrieval; 4a) dialysis; 4b) discard; 5) dialysate; 6) clean-up; 7) fractionation; 8) pre-concentration for chemical analysis; 9) chemical analysis; 10a) immunoassay, toxicity; 10b) bioassay; 11) reporting, data analysis

Each laboratory has to pass through each analytical step with the aim to provide: (i) appropriate validation, (ii) uncertainty description, (iii) adopting the appropriate QA/QC (blanks). Within all applications, the QA/QC protocol was developed, and used for accreditation. It can be seen as a recommendation, as a result from practical use in various water ecosystems, as described and applied (see below).

Ref	Operation	Description/ Requirements /Blank (frequency) site/project/lot/sample set <i>*= if applicable; see details in the Appendix ()</i>
1	Fabrication of SPMDs	Defined QA/QC conditions / FaBl (0/1*/1*/0)
1	Storage	--/Air-tight can, about -10°C/PrBl (0/1*/1*/0)
2	Transport	--/Air-tight can, ≈0°C/ TrBl (1/1*/0/0)

Ref	Operation	Description/ Requirements /Blank (frequency) site/project/lot/sample set <i>*= if applicable; see details in the Appendix ()</i>
2	Deployment/Retrieval	SPMDs into or from the rack or holder/short time/FBI (1/1*/0/0)
2	Exposure	Up to 30 days/security of sampling site, temperature recording/No blanks required
3	Exterior cleaning	Removal of all external impurities/rinsing by water, fast operation/ReBl (0/1*/0/0)
4a	Dialysis	250 ml solvent, 2 exchange/24 hour per one solvent/FaBl*, PrBl (0/1*/1*/0)
5	Dialysate (chemicals) Dialysate (bioassay)	Collection of all solvent/spike by isotopic standards/see 4a Collection of all solvent/no spike, taking of aliquot/ see 4a
6	Clean-up	Oligomers, impurities removal/by analytical method/see 4a + ReBl (0/0/0/1)
7	Fractionation (chemicals) Fract. (immunoassay)	Isolation of analytical fractions/by analytical method/see 6 Isolation of analytical fractions/by analytical method/see 6
8	Pre-concentration	10µl/nonane solvent/see 6
9	Chemical analysis	Quantification of chemicals/by analytical method/see 6
10	Immunoassay/bioassay	Toxicological response/by analytical method/see 6
11	Data analysis, reporting	Ambient concentration by Rs/PRCs; PCA, FA, GA/data pre-treatment/Evaluation of QA/QC, including blanks
SP	Sampling plan	New definition of sampling plan based on new foundations/given from 11 and new project purposes/given from 11

Table 1 Abbreviated protocol for handling, sampling and analysis of SPMDs

SPMDs' quality rules

In general, quality requirements for sampling are given by following points^{23, 98}:

Precision: measurement of variability in the data collection process,.

Accuracy (bias): measurement of bias in the analytical process.

Completeness: percentage of sampling measurements which are judged to be valid.

Representativeness: degree to which sample data accurately and precisely represent the characteristics and concentrations of the site contaminants.

Comparability: evaluation of the similarity and conditions under which separate sets of data

were taken.

To ensure that the analytical samples are representative of site conditions, quality assurance measures must be associated with each sampling and analysis event.

The data quality objectives (DQOs) state the level of uncertainty that is acceptable for data collection activities and define the data quality necessary to make certain decisions. As the general conditions for POPs assessment, following question must be answered by the data:

- Why analytical data are needed and how the results will be used.
- Time and resources of data collection.
- Descriptions of the analytical data to be collected.
- Applicable model or data interpretation method used to arrive at a conclusion.
- Detection limits for analytes of concern.
- Sampling and analytical errors.

Within this thesis, the *sampling plan* (SP) with these QA measures and DQC and this strategy has been incorporated as a general guideline into (accredited) methods for passive sampling realized in NRL.

All applications were performed upon accredited system – see below in part of applications. Despite of already emphasized importance of uncertainty of all the parts of environmental measurements, calculation of uncertainty has not been finished yet. Results will be published later.

Handling with SPMDs ; analytical procedures

Sampling and materials

Both standard and performance reference compounds (PRC) SPMDs were purchased from Exposmeter AB. SPMDs have a standard dimension (2,5x91,4cm, filled by 1 ml of triolein). Since 2005 SPMDs with spike of PRC were used for in situ calibration of the sampling rates. The mixture of the 68 native PCBs (BP-GC Wellington laboratories) is used for quantification of the PCBs. Those PCBs, which are not present in the quantification standards, are quantified on the RF of PCBs with the most similar MS/MS spectrum. Developed method was validated using certified reference materials with wide range of PCB congeners NIST 1944 and NIST 1588a. 4 ¹²C₁₃ labelled PCBs (PCB 3, 8, 37 and 54) were used as PRC. Standard sampling arrangement was used, if used for surface water^{99, 125, 126}, adopted as a combination of several SPMDs in one cage (standards not spiked, PRC and SPMD designed for toxicity tests). The samplers were installed at float and sunk approx. 0.5-1 m in water.

Two PRC SPMDs were exposed to air and light at the same conditions as sample SPMDs. These field controls were used as reference (starting) values of PRC.

After collecting and sampling, all of samples were stored at -18°C until analysis started.

SPMD sampling was performed according to recommended good SPMD practice, compiled

from publications discussed above.

Clean-up SPMDs

If SPMDs were used not pre-cleaned, there were prepared in following procedures: there were immersed in hexane to remove monomers and others impurities for 24 hours, then placed in clean airtight steel cans and transported to sampling places with transport-trip and field blanks.

Placement and exposure SPMDs

On the sampling point, SPMDs were placed in a perforated stainless steel container to protect the membranes against mechanical damage and to restrict water flow velocity at the membrane. Numbers of exposed SPMDs per one site were given according to tested parameters and QA/QC aspect; in this research, 5 membranes per a site were used. With the SPMDs set deployed, other SPMDs were exposed to ambient air during the deployment (trip/field blanks) at the sampling places to monitor possible contamination from the air. Each container was equipped with a temperature logger (Tiny-Loggers, Intab, Stenkullen, Sweden), which registered water temperature every 15 minutes.

SPMDs after exposure

Membranes were transported in airtight metal cans. The membranes were cleaned and dialyzed with hexane in accordance with instructions in tutorial, as mentioned above. Combined dialyzates were adjusted to volume of 10 ml. Aliquots were used for different analysis: 0.3 ml aliquot was used for analysis of PAHs and their deuterated analogues; D10 labelled PAH were added to aliquot, samples were evaporated to volume of approx. 100 μ l and analysed by GC/MS in full scan mode.

Aliquot of 4 to 6 ml was used for determination of mono to deca-PCBs. Samples were cleaned on the column with 5g of H₂SO₄ deactivated silicagel. The PCBs were eluted by 50 ml of hexane. Then the standard was added and volume of the samples was adjusted to 100 μ l. Analysis was performed during one GC/MS/MS run.

Fish, fry and Dreissena samples were let to melt at room temperature. Homogenised muscle filet with skin was used as fish sample. Fry was homogenised as well. Muscle of Dreissena was separated from the shell and homogenized. The homogenized samples were dried by lyophilisation. 10 – 20 dry biota samples were Soxhlet extracted by hexane:acetone 3:1 mixture. Extract was evaporated and its volume adjusted to 10 ml. Appropriate aliquot of the sample was spiked by ten ¹²C₁₃ labelled PCBs. Clean up and analysis have been carried out in the same way as described above.

After sampling, each sampler was rinsed by drinking water; the SPMDs were placed in a clean airtight steel can. Periphyton, minerals and rough particulates were removed from membrane surface with clean cloth and then rinsed by clean water. Exposed membranes were preserved at temperature –18°C until being analyzed. SPMD membranes were exposed in steel basins into what ground water of constant flow from 5 to 40 litres per hour during whole exposition was introduced. This flow of ground water was sufficient to provide proper concentration of POPs in SPMD. Duration of

sampling was about 25-35 days.

Exposed SPMDs were dialyzed with hexane (suprapure quality, MERCK) for 3 days resulting in two fractions of 200 ml each. After dialysis the ¹³C-labelled isotopic internal standards (PCDD/Fs, PCBs – Wellington laboratories) or deuterated (PAHs) were added to the extract and analyzed in laboratory by accredited methods in accordance with ČSN EN/ISO IEC 17025 standard.

Analytical procedures

Determination of PAHs was carried out by HPLC-FLD method, using methanol as solvent. PCDD/Fs, PCBs and OCPs were analyzed by GC/MS/MS on GCQ or PolarisQ (Thermoquest). Clean-up method and optimisation of MS/MS detection were described elsewhere²²⁵.

PAHs and PRCs were analysed by GC/MS in full scan mode at PolarisQ (ion trap) and DSQ (quadrupole) instruments (Thermo Scientific). An DB-5m column (30m x 0.25mm x 0.25 µm) was used for separation of PAHs under following instrument set up: splitless injection of 1µl for 1 min. at injector temperature 260°C, helium 6.0 was used as carrier gas with constant flow of 1.1 ml/min, GC oven was programmed as follows: 65°C for 1 min. then 10°C/min to 260°C, then 20°C to 300°C and 5 min. isothermally. Ion source was operated at 200 °C, transfer line at 280°C. Data were collected in full scan mode in the range 100 – 280 m/z.

Multi-ortho PCBs were analyzed in 2% DCM in hexane fraction from Al₂O₃ column. Monortho PCBs and PCDD/Fs were analyzed in 50% DCM in hexane fraction from the same column after clean up on activated carbon column. PolarisQ™ operating in MS/MS (tandem mass spectrometry) mode was used for PCB analysis. DB-5ms column (30 m x 0.25 mm x 0.25 µm) was used for separation of PCB congeners under following instrument set up: splitless injection of 1µl for 1 min. at injector temperature 260°C. Helium 6.0 was used as carrier gas with constant flow of 1.1 ml/min. Oven program: starting temperature 75°C for 1 min, first ramp 20°C/min to 180°C, second ramp 2.5°C/min to 240°C, third ramp 10°C/min to 300°C hold for 2 min. MS interface was heated to 275°C. Ion source temperature was 200°C, flow of dumping He to ion trap was 1,05 ml/min.

All procedures used were validated in accordance with EN ISO 170125 standard.

Statistical and gnostic methods

It must be stated that many of our environmental data, especially those measured in ultra-trace concentrations, exhibit some distinctive features:

- Data set could contain extremely large values that are of questionable origin.
- Some measured values are very *low* or *not detected*, requiring treatment as low-censored.
- Range of data can be either narrow or extremely broad.
- Data sets are of a *very low size* (rarely more than 10 repeated measurements).
- Distribution of data is seldom *normal* or *log-normal*.
- Data can tend to *polymodality (non-homogeneity)*.

- Data are usually *skewed* to larger values.
- Data analysis of such data requires very robust and reliable procedures based on the data transformation for both evaluation of relevant quantitative characteristics and interpretation.

Those important key factors were also well described^{59, 112-114, 160}. Statistical methods have been applied in environmental analysis very broadly. The list of all applications would be enormous in spite of the fact that a few of such methods could completely satisfy the specific needs mentioned above. Unlike these, gnostic analysis, based on novel fundamentals^{59, 114, 160} (the theory of individual data), has already been successfully applied in economics and elsewhere²²⁶⁻²²⁸; due to rigid fundamentals it hides strong potential for treatment and evaluation the environmental data ^{114, 158, 160, 161}.

Nowadays, the multivariate data analysis plays a very important role, alongside univariate data analysis. Some of multidimensional analysis is based on latent variables that are linear combinations of original variables. Source matrix of data is arranged in columns (variables) and rows (observations). Main goal of this analysis is to identify clusters of observations with similar properties (variables). The similarity is based on a distance of objects: the closer distance, the closer similarity. One frequently used method for identification of model and variables structure with reduction of the number of variables is the Principal Component Analysis (PCA) and Factor Analysis (FA), in combination to Cluster Analysis (CLU), commented below. The insight and various applications are published.

Within the frame of this thesis, both statistical multivariate and gnostic univariate and Multivariate methods were used for supporting evaluation of POPs and toxicity, from various monitoring data.

Descriptive statistics

Many statistical programs offer a list of the estimates of various point parameters of location and spread but rarely help the user to choose the statistically adequate parameter for an actual sample batch. Only the exploratory data analysis and an examination of sample assumptions will find an answer to this question. Therefore, a rigorous procedure of the statistical treatment of univariate data with the exploratory data analysis was first carried out to evaluate the actual sample distribution. A curve of the probability density function for each analyzed parameter was examined and the analysis was based on histogram, symmetry plot, halfsum plot, Box-and-whisker plot, quantile plot and rankit Q-Q plot while the coefficient of skewness measures the asymmetry of the observations. Jarque-Berra test ($\alpha = 0.05$) was preliminary used to test the normality of concentration distribution

within each element. This test mostly revealed the lack of normal distribution. Moment coefficients of skewness and kurtosis describe how the shapes of sample frequency distribution curves differ from the ideal Gaussian curves, showing asymmetry of the upper and lower halves of the curve around the mean. The data from ultra trace monitoring were not ideal and did not fulfil all basic assumptions about a sample. Original data were then transformed to improve the symmetry of data distribution and variance stabilization. The proper data transformation led to a symmetric data distribution, stabilizes the variance or makes the distribution closer to normal. Furthermore, possible outliers were identified because strongly skewed distributions and outliers can contribute to biased conclusions in statistical analyses. For transformation, a Box-Cox^{112, 113, 176, 229} was used, as the most frequently applied statistical method.

Transformation leading to approximate normality may be carried out by the use of the *Box-Cox transformation family*, defined as:

$$y = g(x) = \begin{cases} (|x|^\lambda - 1) / \lambda & \lambda \neq 0 \\ \ln |x| & \lambda = 0 \end{cases} \quad \text{Eq. 12}$$

where x is a positive variable and λ is real number. The Box-Cox transformation can be applied only to positive data. To extend this transformation means to make a substitution of x values by $(x - x_0)$ values which are always positive. Here x_0 is the threshold value $x_0 < x_{(1)}$. To estimate the parameter λ in a Box-Cox transformation the method of profile likelihood may be used, because for $\lambda = \hat{\lambda}$ the distribution of the transformed variable y is considered to be normal, $N(\mu_y, \sigma^2(y))$. The logarithm of the profile likelihood function may be written as:

$$\ln L(\lambda) = -\frac{n}{2} \ln s^2(y) + (\lambda - 1) \sum_{i=1}^n \ln x_i \quad \text{Eq. 13}$$

where $s^2(y)$ is the sample variance of the transformed data y . The function $\ln L = f(\lambda)$ is expressed graphically for a suitable interval, for example, $-3 \leq \lambda \leq 3$. The maximum on this curve represents the maximum likelihood estimate $\hat{\lambda}$. The asymptotic $100(1 - \alpha)$ % confidence interval of parameter λ is expressed by $2[\ln L(\hat{\lambda}) - \ln L(\lambda)] \leq \chi^2_{1-\alpha}(1)$, where $\chi^2_{1-\alpha}(1)$ is the quantile of the χ^2 distribution with 1 degree of freedom. This interval contains all λ values for which it is true that

$\ln L(\lambda) \geq \ln L(\hat{\lambda}) - 0.5 \chi^2_{1-\alpha}(1)$. This Box-Cox transformation is less suitable if the confidence interval for λ is too wide - and if the sample size is small then the confidence interval for the parameter will be wide. When the value $\lambda = 1$ is also covered by this confidence interval, the transformation is not efficient.

After an appropriate transformation of the original data $\{x\}$ has been found, such that the transformed data give an approximately normal symmetrical distribution with constant variance, the statistical measurements of location and spread for the transformed data $\{y\}$ are calculated. These include the sample mean \bar{y} , the sample variance $s^2(y)$, and the confidence interval of the mean $\bar{y} \pm t_{1-\alpha/2}(n-1) s(y)/\sqrt{n}$. These estimates must then be recalculated for the original data $\{x\}$. More correct re-expressions are based on the Taylor series expansion of the function $y = g(x)$ in a neighbourhood of the value \bar{y} . The re-expressed mean \bar{x}_R is then given by:

$$\bar{x}_R = g^{-1} \left\{ \bar{y} - \frac{1}{2} \frac{d^2 g(x)}{dx^2} \left(\frac{dg(x)}{dx} \right)^{-2} s^2(y) \right\} \quad \text{Eq. 14}$$

The variance is then expressed as follows:

$$s^2(\bar{x}_R) = \left(\frac{dg(x)}{dx} \right)^{-2} s^2(y), \quad \text{Eq. 15}$$

where individual derivatives are calculated at the point $x = \bar{x}_R$. Because almost all the Box-Cox transformed data sets follow the normal distribution, they were used for further multivariate statistical analysis as described below.

Principal component analysis (PCA)

In many ways, this analysis is denoted as one of important statistical multivariate techniques. The method was applied to analyses for simplifying data matrix of large number of observed variables to set of linear combinations of the original variables, especially for POPs, where number of variables exceeded 100 (including congeners of PCDD/Fs and PCBs). Application of PCA enables (i) to *reduce* the number of variables and (ii) to *describe structure* in the relationships between variables. Results are described in the application part below.

The PCA can be modelled according to the equation 16. It holds:

$$X = 1\bar{x}' + TP' + E \quad \text{Eq. 16}$$

where the X is a model for source data. The first part of the right hand side ($\overline{1x'}$) represents the variable averages. The T represents the column of score vectors (graphical representation as score plot). This parameter characterises the relation between objects (samples, experiments). The P is denoted as the loading vectors (graphical representation as loading plot) that reflects relation between variables (compounds). It means that TP is the model's structure. The E is the residual matrix (errors or noise).

Approximation for a particular variable in the data matrix can be interpreted as it holds:

$$x_{ik} = \bar{x} + \sum_{i=1}^M t_{im} p'_{mk} + e_{ik} \quad \text{Eq. 17}$$

where the x_{ik} are descriptors of particular compounds compiled in the multivariate characterisation (i are compounds, k represents experiments/observations). The score t_{im} describes the weight of compound's contribution to the m -th PC (principal component). The loading p'_{mk} reflects how much the k contributes to PC. All PCs are ordered with respect to their variance: PC1 describes the largest part of variance; the latest PC reflects the smallest variance of source data. The notion of *explained variance* ranged from 0 (no explanation) to 1 (complete explanation of variable) provides information, how a combination of particular variables fits to analyzed data. In applications described below, only the first two principal components were used to explain the X% variance of considered data. This has been an important conclusion with the trade-off between goodness of fit and predictability. The PCA procedures are based on linear combinations of the variables and their correlations. PCA was based on the correlation matrix, with no factor rotation. For simplicity, graphical method of projection was used to reveal "important" PC, called Cattell's scree graph.

Factor analysis (FA)

The method was carried out to determine the basic latent data structure using the following settings: the analysis was based on the correlation matrix and the obtained *factors* were rotated using Varimax normalized algorithm, which allows an easier interpretation of the factors loadings and the maximization of the variance explained by the extracted factors. FA creates a new set of uncorrelated variables, which are the linear combination of the original ones with the same amount of information. Since the FA is conducted if the original variables have significant linear intercorrelations, the first two factors will include the largest part of the total variance. Elements belonging to a given first factor F1 were defined by factor matrix after Varimax rotation, with those having strong correlations grouped into factors. Factor analysis was performed by evaluation of principal components and computing the eigenvalues higher than 1 (Kaiser criterion). Afterwards,

the rotation of factors was carried out by Varimax normalized algorithm which allows an easier interpretation of the principal component by both maximizing the variance of the extracted factors and reducing uncertainties that accompany initial unrotated factor loadings.

Cluster analysis (CA)

This technique was considered as an exploratory data analysis technique for solving classification problem. It comprises an unsupervised classification procedure that involves measuring either the distance (or the similarity) or the correlation coefficients between objects to be clustered. The information obtained from the measured variables is used to reveal the natural clusters existing between the studied soil samples. Soils are grouped in clusters in terms of their similarity so that the degree of association is strong between members of the same cluster and weak between members of different clusters. The initial assumption is that the nearness of soils in the space defined by the element contents reflects the similarity of their properties. The similarities in this case were quantified through the Euclidean distance measurement. The CA is complementary method to PCA and FA. Results are shown in a dendrogram where steps in the hierarchical clustering solution and values of the distance between clusters are represented.

Correlations of vectors/variables

An attention with respect to further application in multivariate techniques (like PCA, FA) was paid to analysis of association between two samples of data expressed by correlation coefficients. Four methods were applied for estimation:

1. Pearson's correlation of classical statistics,
2. Kendall's correlation of robust statistics,
3. Spearman's correlation of robust statistics,
4. Gnostic correlation based on data probabilities taken from the distribution functions.

The first one was used as an implicit method. For comparison, the first three statistical methods and gnostic estimate of the correlation coefficient of the data samples was used, as the use of the theoretically based linear relation between probability and data irrelevance (data error). In this way, robustness with respect to outliers and to the censored data was enabled.

Gnostic analysis in Environmental Assessment

It is the first time that gnostic theory was applied to environmental data mentioned below. Results achieved demonstrate a strong potential for evaluation of data from environmental monitoring, as well as for applications in Human Risk Assessment evaluation. The basic rules of

advanced data analysis (in general and within this thesis) are as follows:

- Avoiding transformation and “violation” of the data by subjecting them to unjustified *a priori* models or distribution functions (despite mathematical rigorosity by transformation in statistical approach),
- Using of all available data, including censored data, as well as adequately weighted *outliers* (exclusion of data is allowed *only* after proving their negligible impact on results).
- Respecting data limitation by estimating the bounds of data supports, with testing of outliers/inliers (the ‘membership’ problem) and of sample’s homogeneity while considering the structure of inhomogeneous data.
- Using distribution functions, as a complex approach to the point estimations of data characteristics.
- Comparing only objects which behave in accordance with the same mathematical model.
- Explaining causes of behaviour of data instead of reference to randomness.
- Preference of robust estimation and identification methods over no robustness.

Comparison of statistical and gnostic approach

The original mathematical background of gnostics^{59, 114} was first applied in pilot environmental studies within this thesis¹⁵⁸⁻¹⁶¹, further extended and completed into the practical guide²³⁰. The main goal of gnostic development was to make an expansion of alternative methodology for practical applications to small data sets. Nowadays this method is supported by the Web-based Open-Source system (R-project based on the R-language²³¹).

The differences between statistical and gnostic approaches can be summarised in Table 2.

Problem/aspect	Approach	
	Statistics	Gnostics
Quantity of data to be treated	Mass data, large data samples	Individual data and small samples
A priori given statistical model of data	Required	Not used. Data assumed to satisfy algebraic requirements of measurement theory. Model to be applied results from data only.
Main theoretical tool	Additive measure over a sigma-algebra	Non-additive measure over two bi-algebras
Axiomatic	Formal, based on the Central Limit Theorem	Based on laws of Nature
Notion of probability	Formally defined	Derived mathematically from the Clausius’ deterministic data entropy
Notion of information	Formally introduced	Derived mathematically from the Clausius’ deterministic data entropy
Inherent geometry	Euclidean geometry	Riemannian geometry determined by data
Optimality criteria of estimation	Formal (e.g. least squares or max. likelihood)	Minimum information loss or entropy increase determined by variation principle

Problem/aspect	Approach	
	Statistics	Gnostics
Variation features	Non-existent	Variation theorems for data errors, information and entropy
Bounds of a data sample	Ambiguous, dependent on a subjective decision	Uniquely and objectively determined by data
Robustness of an estimate	In classical statistics – not available In robust statistics achievable by means of an artificial superstructure over the basic theory	Resulting from the basic theory as its inherent and natural feature
Convergence of the two theories	Unknown	Proved for the case of high quality data
Ties with existing theories of Nature	Not defined	Proved close relations to classical thermodynamics, relativistic mechanics and both classical and robust statistics.

Table 2 General comparison of statistical and gnostic approach

Some fundamentals of gnostic approach

Gnostic analysis is concentrating on *real data*: as numerical structures mapping the structures of real (existing) quantities. The paradigm of *real data* accepted by the classic theory of measurement represents the measured data as elements of a numerical structure with the same strictly defined algebraic features as the structure of their originals (natural quantities mapped by the measurement). However, the measurement theory is dealing only with precise quantification, leaving the data uncertainty to statistics. In gnostic theory, the data uncertainty is not something random; it is a result of lack of knowledge of unfound factors that influence the observed values. It is not surprising that these (uncertain) factors are considered as (some others) real quantities that could be also quantified as the true quantities and which have the same structure. In gnostic, *real data* are represented by points in a two-dimensional plane (true value, uncertainty) observed only as a one-dimensional projection. Important result of this theory is the isomorphism of its model of uncertainty of an individual uncertain data item with the energy-momentum tensor of the Einstein's special relativity theory. Gnostic formula of information born by an individual data item derived from the Clausius' classical entropy can be viewed as an independently derived and far-reaching generalization of Boltzmann's and Shannon's entropy formulae. Convergence of gnostic characteristics of uncertainty to statistical ones in special cases of very weak uncertainties is also proved.

Within this thesis, gnostic methodology was used to evaluate data from various studies, using passive sampling tools, as well as biotic monitoring of water contamination and POPs in blood (see below).

One-dimensional analysis

Gnostic marginal (one-dimensional) analysis is based on a consequent usage of the program for estimation of four types of gnostic distribution functions, upon those conditions:

No *a priori* assumptions on the distribution function.

- Application to both additive and multiplicative data.
- Applicability to three types of censored data.
- Application to compressed data (such as a histogram).
- Applicability to homoscedastic and heteroscedastic data.
- Robust estimation of scale parameters.
- Robust estimation of bounds of the data support.
- Test of data homogeneity and classification of outliers.
- Robust estimation of the location parameter (mode).
- Robust estimation of probabilities and densities of probability for arbitrary data.
- Robust estimation of quantiles to given probabilities.
- Objective robust estimation of membership bounds of a homogeneous sample.
- Robust monitoring, filtering and prediction of time series.
- Robust cross-section filtering of data.
- Robust marginal cluster analysis.
- Classification of data sub-samples.
- Evaluation of the degree of similarity between two samples.
- Robust estimation of covariance and correlations.

The problem of membership bounds deserves a special comment because of its relation to diagnostics.

Quantitative diagnostics

A frequently used method of establishing the bounds of the membership interval for a data set d to accept values is based on the equation:

$$BMI = \phi(d) \pm K(p) \times STD(d) \quad \text{Eq. 18}$$

where BMI are the bounds, $\Phi(d)$ is the mean and $STD(d)$ the standard deviation of the set of data d . A crucial role is played by the multiplier $K(p)$, which depends on the probability of rejected candidates for membership (significance of the test). Such a method is subjective not only because of the hidden assumption of normal (Gaussian) data distribution but also due to a “freedom“ in choosing the multiplier. *The outcome of such a test is not what data say but what the user wishes.*

Gnostic membership intervals are based on the unique feature of the global distribution function of a homogeneous data sample: its density function has only one maximum. If one considers a fixed data sample extended by additional data x , then there exist exactly two x 's values (the lower and upper bound) at which inflexions appear in the density of the extended sample. The procedure

includes: extraction of a homogeneous sub-sample of the data sample, optimization of its data support bounds and of its scale parameter and finally finding the values of the fictitious extension x causing the density's inflexions. Results of these operations are determined only by data, they are *objective*. Both found bounds are *unique characteristics* of the data sample.

Multi-dimensional analysis

As already mentioned, the use of gnostic criterion functions opens the way to robust estimation of parameters of both linear and non-linear multi-dimensional models. Gnostic programs have been developed for solving the most frequently tasks of MD-analysis:

- Robust estimation of parameters of an MD linear regression of four types:
 - Explicit regression of data.
 - Implicit regression of data.
 - Explicit regression of data probabilities.
 - Implicit regression of data probabilities.
- Robust estimation of correlation matrices.
- Robust extraction of homogeneous kernels from non-homogeneous MD objects applicable to the cluster analyses in MD space. Robust cluster analysis of inhomogeneous objects.
- Cross-section analysis and monitoring of MD objects.
- Robust monitoring and prediction of MD time series.
- Robust ordering of MD objects.

The ordinary (explicit) form of the regression model was based on a (hidden) assumption: it is possible to choose one variable (that is to be explained) and which is “only dependent“ on the other variables assumed to be explanatory ones. Such an assumption can rarely be applied to real multi-dimensional objects or processes because of feed-back existing between all variables. For such cases the implicit form of the regression is suitable: all variables are explanatory jointly explaining a constant (eg 1). Solution of the equation system reveals the relative contributions of individual variables to the constant.

The probabilistic regression is also worth attention: if data are interdependent, so their probabilities do as well, but in a non-linear way. Gnostic probability p can be defined such as:

$$p = (1 + i) / 2 \qquad \text{Eq. 19}$$

This is a simple linear function of the estimating irrelevance i that can be interpreted as a non-linear (Riemannian) generalization of the error measured by the deviation of the data item's irrelevance from zero. Recall that estimating irrelevance is a non-linear function of a datum, which is robust with respect to outliers). As shown in gnostics, the robust estimate of a covariance can be obtained as a product of irrelevances (in an analogous manner like in classical non-robust case of

errors). It is well known that the classical solution of the linear regression task is determined by covariance. It was therefore natural to use the gnostic method of the regression in probabilities instead of traditional data regression. An additional advantage is obtained in that all variables as well as the model coefficients are dimension-less. This makes the interpretation and usage of results easy.

All four mentioned regression models are robust when a gnostic criterion function is applied to calculate them instead of the classical applications of the least squares method.

Used software packages for statistical and gnostic computing

Statistical computing was realised in combination of following software:

- Adstat™, v. 1, 25 (Trilobyte®, Ltd.),
- QC.Expert™, v. 2.51 (Trilobyte®, Ltd.),
- Statistica Cz™, v. 9.0, (StatSoft® CR, Ltd.),
- S-Plus®, v. 6.2, (Insightful® Corp.),
- NCSS 2007™ (Dr. J. L. Hintze).

For gnostic computing, following programs were used²³⁰:

- S-Plus™ ver. 6.2 (Insightful® Corp., Seattle) – at the stage of first applications,
- R-project®⁴ - since 2007, within the EU project 2-FUN (Full-chain and UNcertainty Approaches for Assessing Health Risks in FUTURE ENvironmental Scenarios).

For data handling and preparation of some graphical outputs, MS Excel™ and Corel Quattro Pro™ packages were used.

E. Applications

Experimental work within this thesis falls into the beginning of applications of passive sampling technique, mainly SPMDs, since 1997. At first, wide spectrum of application of SPMDs was realised to verify this system, to assist analytical methods for determination of POPs in fat tissue; for sampling, it make appropriate validation²³², followed by accreditation, with the aim to prove it for practical monitoring in the Czech Republic. Experimental studies were realised to prove applicability to: (i) samples pre-treatment (isolation of analytes from fat samples) and (ii) sampling in a clean environment, revealing geogenic background (glacial water, mineral water), as well as identification of key pollutants in drinking water^{101, 135, 219, 233, 234}. Industrial applications are represented by assessment on contamination from runoff waster, flooding episode around hot-spots and assessment of waste water treatment plants^{102, 134, 164, 220, 233, 235}. In the latest time period, application of SPMDs on toxicity parameters (for POPs) was realized, as complex, long-term studies, having transnational effect for adoption on international scale²³⁶⁻²³⁸. These applications fill the gap between

⁴ The R-project® is an open-source version of the mathematical and statistical environment using the R language. This project was started in the 1980s and has been in widespread in the statistical community. Environment R is implemented on wide variety of UNIX platforms (Linux), MacOS X and also Windows. There is also a base documentation and links to other resources as the best known CRAN (Comprehensive R Archive Network). The R-language is the interpreter.

chemicals and ecotoxicological effects. As a application within remediation and identification of underground water sources, in concert with Integral Pumping Test²³⁹. In all applications, data collected were subjected to data appropriate data analysis: statistical (EDA, PCA, FA, CLU) and gnostic one, where censoring and interpretation of LOD values are addressed²⁴⁰.

NOTE: All source data from applications are included in digital form only. However, due to complexity of all results from each data treatment and operation (produced from presented used statistical packages), all outputs are not included to avoid overwhelming. Presented results within this thesis incorporate only those outputs, supporting general conclusions. Though any output as a part of the complex results is of available on demand.

Application of SPMDs for sample pre-treatment

Selection of correct pre-treatment method is the crucial analytical step in analysis of organic compounds on ultra-trace concentration level as relevant for POPs. Methods for lipid reduction were developed recently^{115, 116, 241}, and extended in NRL-POPs and validated for all POPs mentioned above. A recovery of this operation, related to mostly found important parameters, like (i) kind of

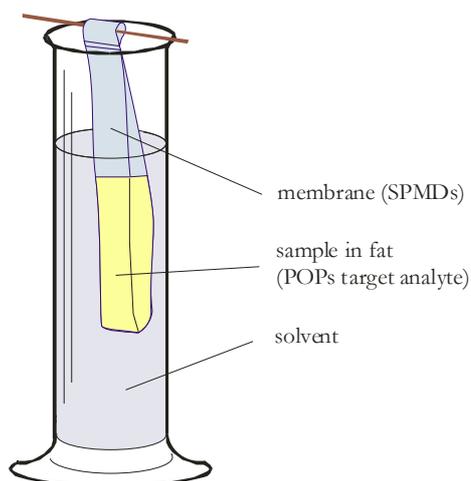


Figure 7 Basic scheme for lipid reduction from samples with fat content. Sample is in the form of extracted fat, spiked with isotopic-labelled compounds.

samples, (ii) fat portion and (iii) concentrations, are given in Table 3. Behaviour of those samples reflected in PCA projection (see Graph 1), where similarities can be found among various biotic organisms. Arrangement of membrane in analytical procedure is in Figure 7. Apart from sampling SPMDs, membrane for dialysis had length 21 cm, on one site sealed.

Samples were homogenised, weighted (about 10 g), placed into membrane and spiked with isotopically

labelled compounds. Then, membrane with fat was placed into 100 ml hexane (Merck - suprasolv) and hooked on the graduated cylinder. Dialysis was performed three times, with new portion of solvent. Collected portions were combined, evaporated and subjected to clean-up and fractionation, followed with analytical procedure, as described earlier.

Analytical procedures was established in following steps, and incorporated to the SOP (and subjected to accreditation in NRL for POPs):

1. Identification of sample to be treated,
2. Sample homogenisation,
3. Sample lyophilisation (if applicable),

4. Sample extraction,
5. Fat reduction (by SPMDs),
6. Further clean-up, fractionation,
7. Analysis, reporting.

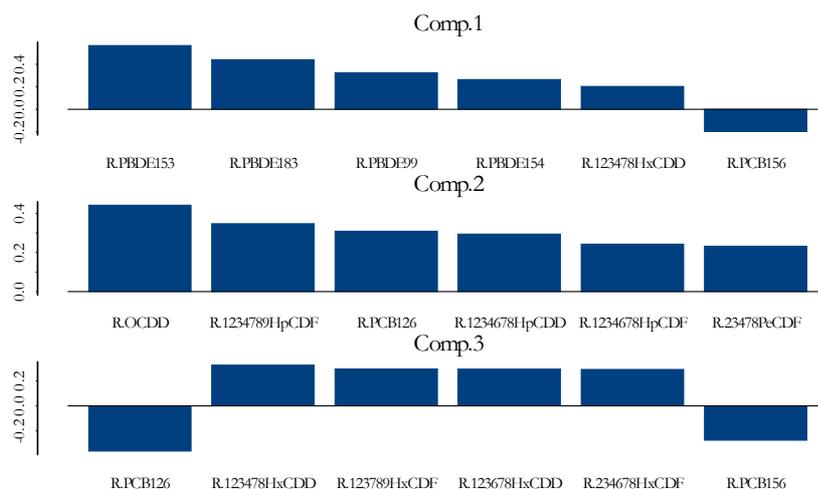
Within the framework of our analytical work, following samples were used, to isolate fat from: pork, fish, butter, various oils, meats, rendering fat. From the total number of samples, 90% samples fulfilling criteria defined above.

Congener	S 1	S 1'	RSD	RS 1	RS 1'	RSD	S 2	S 2'	RSD	RS 2	RS 2'	RSD
2378TCDD	1,37	1,35	1%	0,57	0,60	2%	1,44	1,60	11%	0,66	0,60	4%
12378PeCDD	2,58	2,40	13%	0,52	0,58	4%	4,14	4,15	1%	0,70	0,64	4%
123478HxCDD	1,03	1,12	6%	0,55	0,58	2%	1,49	1,36	9%	0,78	0,72	4%
123678HxCDD	4,90	4,95	4%	0,57	0,58	1%	10,3	11,1	57%	0,64	0,56	5%
1234678HpCDD	9,76	9,32	31%	0,47	0,44	2%	4,34	4,24	7%	0,62	0,56	5%
OCDD	47,4	48,7	92%	0,41	0,43	2%	51,9	52,7	57%	0,57	0,47	7%
2378TCDF	1,30	1,27	2%	0,59	0,61	2%	0,381	0,271	8%	0,65	0,64	1%
12378PeCDF	0,762	0,861	7%	0,57	0,63	4%	0,150 ^{a)}	0,200	4%	0,70	0,65	4%
23478PeCDF	15,6	16,0	28%	0,59	0,61	1%	22,3	22,3	0%	0,68	0,62	4%
123478HxCDF	5,06	4,94	8%	0,59	0,57	1%	6,26	6,26	0%	0,69	0,61	6%
123678HxCDF	3,95	4,00	4%	0,58	0,56	2%	5,87	5,41	33%	0,64	0,57	5%
234678HxCDF	1,13	1,10	2%	0,59	0,57	1%	0,523	0,429	7%	0,66	0,59	5%
123789HxCDF	0,098 ^{a)}	0,092 ^{a)}	0,4%	0,67	0,69	2%	0,19 ^{a)}	0,12 ^{a)}	5%	0,68	0,58	7%
1234678HpCDF	2,19	2,05	10%	0,48	0,43	4%	2,09	2,13	3%	0,61	0,55	4%
1234789HpCDF	0,130 ^{a)}	0,130 ^{a)}	0,0%	0,66	0,63	2%	0,22 ^{a)}	0,15 ^{a)}	5%	0,73	0,56	12%

values*) = LOD, S1, S2 = samples content (pg/g fat), SR1, SR2 = recoveries of samples (w/w), 1', 2' – duplicate analyses/ results. E

Table 3 Results from replicate dialysis, recoveries and RSDs

Data sets were subjected to PCA, to identify groups of compounds, to reveal similarities among their groups. The group PCDD/Fs yield correlation in Comp. 1, the PCBs form correlation of the Comp. 2; no correlation was found between those groups, revealing structure of those variables. Clusters can be explained by various abilities to recover those chemicals from fat, which is probably given by chemical structure (molecule sizes), as well as by log Kow. Biplot reveal information that no correlation to any congener group was found to the amount of treated sample (all about 10 grams).



Graph 4 Loading plots for fish data
the first component is dominantly represented by PBDEs, the second one (and others) by PCDD/Fs and PCBs

Similar results are available for other POPs, however their discussion would exceed the frame of this thesis. However, all the experience was used to come to conclusions.

Conclusions

The PCA was used for the insight into variable structure. From all findings, following general sub-conclusions can be accepted for use SPMDs as a tool for fat reduction:

1. based on the results, the method of fat reduction can be applicable for the fat content higher than 1%(m/m), which corresponds to *feeding stuffs* (premixes), *food* (feeding oil, raw animal (pig) fat, butter, natural and dried eggs, sausages, salami, chocolate, whipped cream, cheese, beef, pork, fish, poultry, kidney, liver, dried milk), *human adipose tissue*,
2. For those samples, recoveries of added isotopic standards can be expected within 70-95% interval. This is in a good agreement with recently published results^{115, 116}.
3. When the SPMDs method is used for any (fat) sample, validation must be taken into account for similar kind of sample to identify separation capability (denoted as recovery of internal standards).
4. PCA methodology can be used for identification of similarities in behaviour of various samples and variables.
5. PCA can be also used for identification of goodness of fit, if one group is considered as reference (the cluster “core”) and other as comparable (the cluster “scatter”), e.g. as for general evaluation of capability of handling with SPMDs, for complex list of POPs. The higher scattering from the core is, the less agreement can be found for given samples.

Identification of POPs as identification of environmental background

Following applications were realized with the aim to accomplish various tasks, mainly: (i) testing of capability SPMDs to accumulate ultra-low concentrations, at atypical environmental conditions, (ii) description of the background values for priority targeted contaminants, called “geogenic” response and finally (iii) to compare data with respect to chemical parameters and ecotoxicological response. Those applications were based on two, sequential applications: 1) assessment of POP contamination in glacial water in Wieliczka (with close connection to drinking water assessment of POPs based only on chemical parameters), 2) assessment of POPs contamination in mineral water, around the Podębrady region (based on chemical parameters and set of ecotoxicity tests).

Assessment of POPs in Wieliczka salt mine

This work was initiated together with direct studies run for assessment of POPs in drinking water. Due to expected extreme sensitivity to POPs independently on background contamination, it was necessary to reveal unknown facts on level of contamination around Silesia region, having similar historical formation and changes of stratum.

Up to realisation of this study, no similar study was realised in Wieliczka and adjacent area. No publication data were found, allowing adoption both methodology and found values enabling the data comparison.

Wieliczka salt mine is situated about 10 km away from Krakow city (Poland), at the south-east direction (rough GPS: N 49°59, E 020°03). The map is given in Figure 8. Sampling was performed in depths of –237 to –250 m above sea level, used only for occasional, geological work. Sampling places are described in Figure 9. For deployment (for 30 days), there were standard SPMDs used (no PRCs added, with processing, transporting and on-field blanks), put in standard protective shrouds (see description above), with measurement of temperature (T-dataloger TinyTalk™) in 2 hours interval. The age of water was received from data available from monitoring of isotopic composition by methodology based on assessment of *an absence of radioactive carbon*.

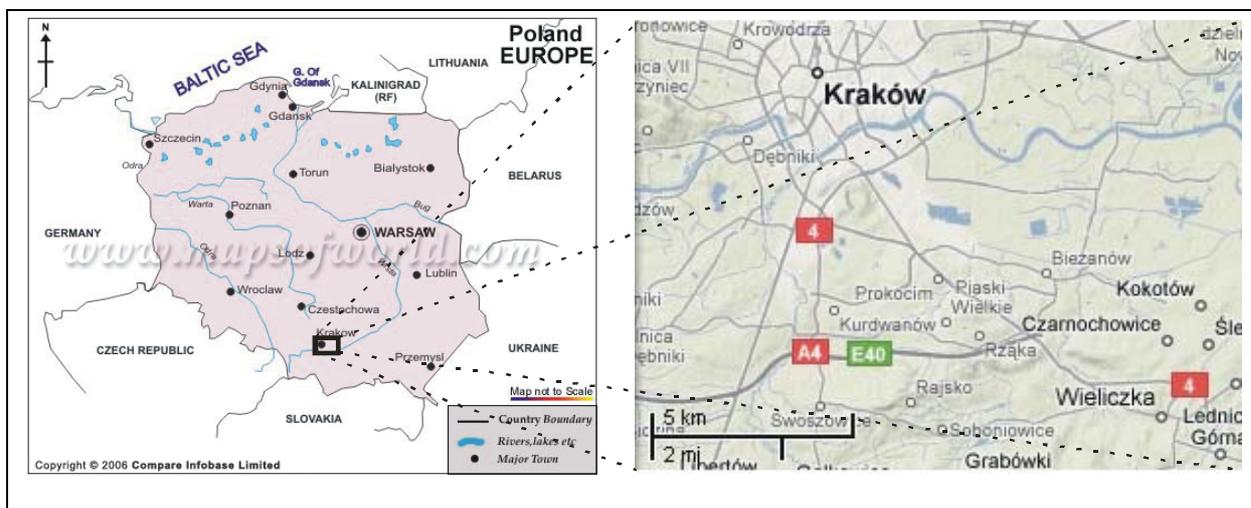


Figure 8 Wieliczka: Map of salt mine in the south-eastern part of Krakow

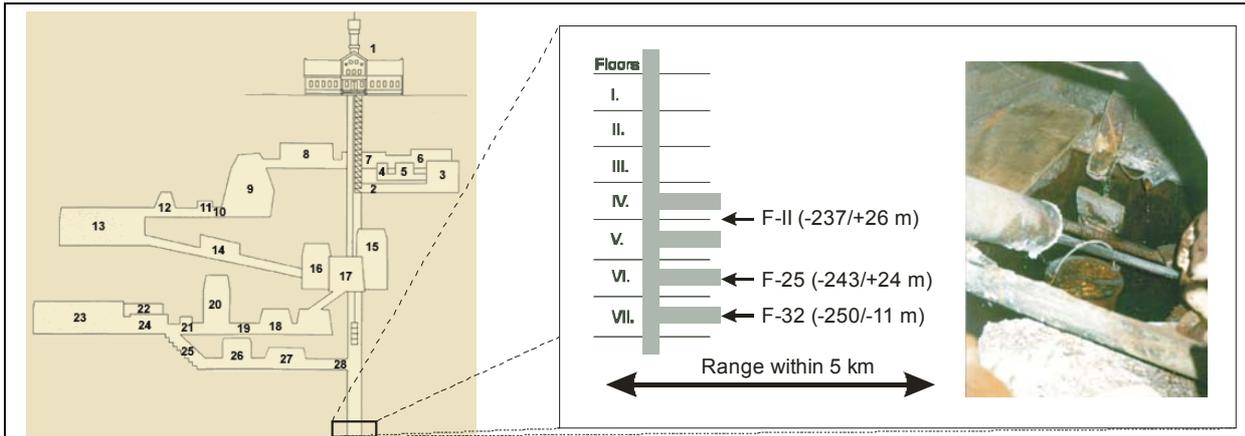


Figure 9 Wieliczka: placement of sampling places
Depth express both terrain level and elevation to the sea level. Picture on the right side describes placement of protective shroud in F-II sampling place.

Sampling places were selected as follows:

Sample F-II (-250 m): Glacial water from Chodenice series layers, without a relationship of discharge water with recent waters,

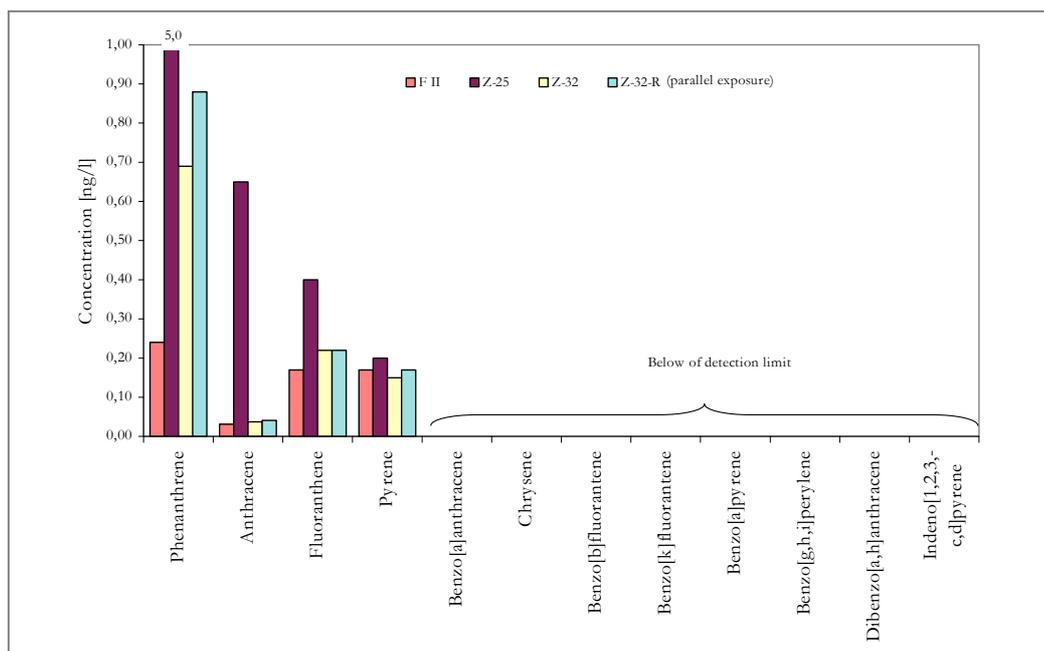
Sample Z-25 (-243 m): Waters formed at the end of last glacial period in Chodenice series layers,

Sample Z-32 (-237 m): Recent water that is percolating through marl rocks (exposed as duplicate).

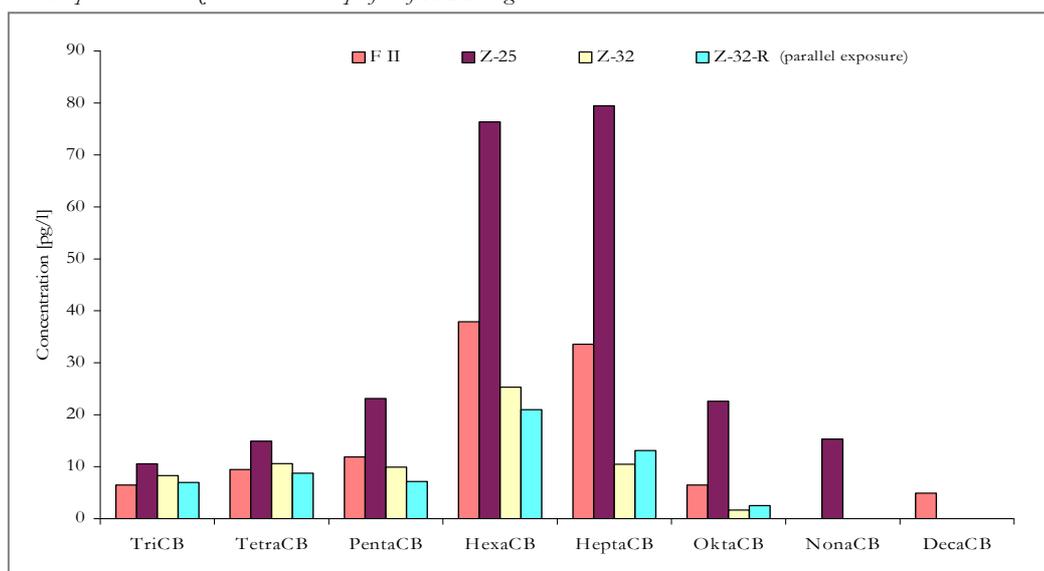
Treatment of SPMDs was done in a standard way, as described previously, with no PRC approach⁵. Following parameters were assessed: PAHs (12 from 16 US EPA), PCBs, PCDD/Fs (within the range of I-TEF, WHO 1998).

The most important results can be seen in the Graph 5 and Graph 6.

⁵ This study belongs to the one of the earliest among many others.



Graph 5 Wieliczka: concentration profile of PAHs in glacial water



Graph 6 Wieliczka: concentration profile of PCBs in glacial water

The profile and quantitative content reflect geogenic background. Results of exposed SPMDs in parallel exhibit RSD within the interval 1 to 13%; this RSD includes analytical procedure uncertainty. Results show excellent comparability (repeatability).

It is apparent, that only few from monitored PAHs were detected. Mainly, those having 3 aromatic rings; only one having 4-ring structure was identified (pyrene). Both applicability for low concentration and fingerprinting are important features of SPMDs. No carcinogenic PAHs were detected, like benzo(a)pyrene. On the other hand, surprisingly, measurable concentrations of PCBs were found, with profiles slightly different to measured profiles of technical mixtures, frequently originating from recent use (Z-25 and FII). For each sample, OctaCB were the latest isomer group

detected. At first, it was difficult to explain the causality. However, based on communication with Wieliczka mine administrators looking in historical records, information have been found about usage of paintings on the pipes recently used. This is apparent from Graph 6, where finger-prints feature for Delor 106 (or alternative, with domination of Hexa-CB) appear. Detectable concentrations were at the levels of 5- 80 pg/l of the sum of PCB congeners.

From all available data, the F-II and Z-25 profile can be considered as anthropogenic background, representing at least one group from Nona-CB and Deka-CB. On the contrary, the Z-32 can be considered as a geogenic background, due to absence “higher” isomers and the lowest concentration found.

For PCDD/Fs concentrations, none detectable amounts were found in exposed samples neither by using the GC-MS/MS technique.

Comparison of found results to profiles Podebrady (mineral water) and Sance (drinking water) are mentioned below.

Conclusions

1. SPMDs techniques was used for identification of PAHs geogenic background, revealing presence only few of monitored parameters, with following concentrations found (for the cleanest profile F-II): phenanthrene (0,25 ng/l), anthracene (0,03 ng/l), fluoranthene (0,18 ng/l) and pyrene (0,18ng/l).
2. It is apparently difficult to estimate anthropogenic background of PCBs on selected sampling places due to probable contamination outlet from historical residuals PCBs, added to paintings as protection of pipe inlet.
3. SPMDs have confirmed usability for extreme conditions (high salinity) and low concentrations of monitored POPs including PAHs.
4. Knowledge of geogenic background opens possible assessment of POPs in drinking water for evaluation of historical deposition of various contaminants.

Assessment of POPs in Poděbradka mineral waters

The scope of this application has been derived from Wieliczka salt mine, with extension for applicability of SPMDs for selected ecotoxicity tests.

Middle Bohemian Cennoman bedrock (Mesozoic, Cretaceous) on its southern part, near Poděbrady town, the Czech Republic, is a source of high quality drinking water, which is used for spa purposes and for treatment of commercially harnessed drinking mineral water. This Middle-European area is one of main sources of high quality mineral water. The direction of groundwater flow is from north to south and the direct length of the underground flow is 50 km. The Cennoman bedrock is approximately 100 millions years old; age of the underground water is estimated on 1000 years according to flow and geological conditions,. For assessment of groundwater contamination, four underground bore holes of depth 350 meters, were selected. A pressure of ground water in

selected bore holes was constant and sufficiently high, ensuring constant flow of sampled groundwater. Denomination of sampled bore holes in Czech geological system is (in north to south order): SK7C – Buda (most northern site); SK6C – Plazy; BPV 1 – Malý Vestec and BPV3 – Poděbrady (most southern site) near the bank of Elbe River. Affiliation after a dash is a name of municipality where the bore hole is located. The north to south order of bore holes corresponds to the direction of groundwater flow. The sampling site Buda is approximately in 10 km south direction to beginning of Cennoman bedrock. Low concentration of POPs in groundwater was expected.

Methods described above were used. No PRC approach was applied. SPMD sampling was performed according to recommended good SPMD practice: immersed in hexane to remove monomers and other impurities for 24 hours, then placed in clean airtight steel cans and transported to sampling places with transport-trip and field blanks.

Following chemical parameters were monitored: all detectable tri-deca polychlorinated biphenyls (PCBs), 12 of 16 US EPA monitored polyaromatic hydrocarbons (PAHs): phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluorantene, benzo[k]fluorantene, benzo[a]pyrene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene and group of organochlorine pesticides (OP): hexachlorobenzene (HCB) and isomers of hexachlorocyclohexane (HCH), DDT and their metabolites (DDE, DDD).

<i>Sampling site</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Distance to northern site</i>
Buda	50°29′	14°59′	10 km to north end of Cennoman bedrock
Plazy	50°24′	14°58′	8.8 km to Buda
Malý Vestec	50°13′	15°08′	23.7 km to Plazy
Poděbrady	50°08′	15°07′	9.3 km to Malý Vestec

Table 4 Poděbrady: Coordinates and distance of sampling sites

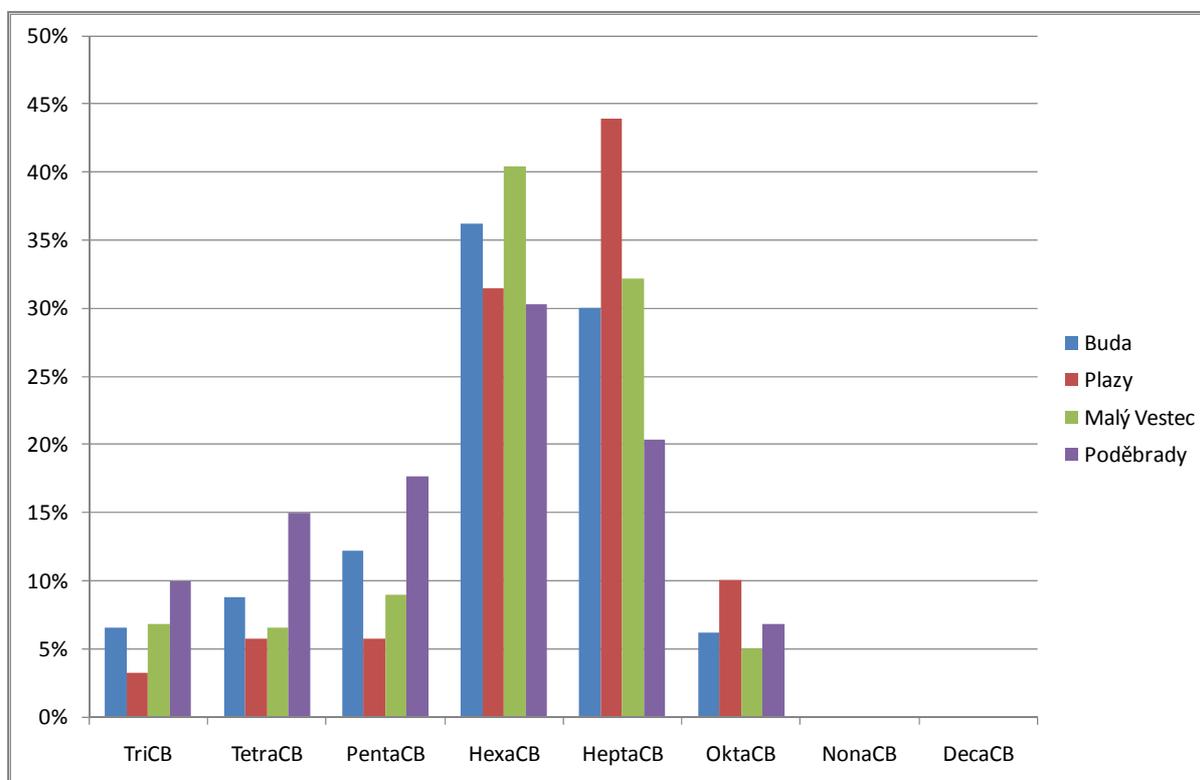
The level of contamination of groundwater is very low, if compared to similar studies and limit values for drinking water. The following table summarizes obtained data for PAHs, PCBs and selected OCPs. Table 5 shows approximate sums of main contaminant groups. These values are rough as the concentrations of a high number of assessed compounds were below the detection limit. In such cases the value of detection limit was used for counting of sum of contaminant group.

pg/L	Buda	Plazy	Malý Vestec	Poděbrady
Sum of PAHs	16159	4380	2807	179
Sum of PCBs	95	168	72	22
Sum of HCH	220	140	220	470
HCB	7.9	8.3	8.3	2.2
Sum of DDT	4.2	4.1	3.9	26.7

Table 5 Poděbrady: concentrations of selected contaminants (pg.l⁻¹)

The most frequent contaminant of all analyzed substances was phenanthrene with the highest concentration of 13.9 ng.l⁻¹ in Buda bore hole. The concentration of phenanthrene was higher at Buda sampling site, near the entrance of surface water into underground Cennoman bedrock. On the other sampling sites the concentration of phenanthrene dropped down to value 0.094 ng.l⁻¹ at Poděbrady. Similar overall trend in decrease of concentration was observed on all other evaluated PAHs. Decrease of concentration is probably caused by sorption to solid surfaces together with partial degradation.

No such evident decrease in concentration like with PAHs was observed in the case of PCBs. The highest value in sum of all congeners was only 168 pg.l⁻¹, while the lowest observed value in Poděbrady sampling site was only 22 pg.l⁻¹. The interesting question is, why the value of sum of PCBs does not homogenously drops down like PAHs do. In Plazy sampling site is the value higher contrary to initial value at Buda, from where the underground flow is coming. The answer can be found in congener profiles. Graph 8 demonstrates that composition of PCB mixture is similar in sites Buda, Malý Vestec and Poděbrady. In Plazy sampling site different congener profile was observed. This means that different source of contamination into groundwater is apparent, concluding that PCB surface contamination can enter groundwater watershed even in deep Cennoman bedrock.



Graph 7 Poděbrady: relative pattern of PCB isomers.

Contrary to both groups of POPs discussed above, different course of contamination was

observed on organochlorine pesticides. Concentrations of pesticides were almost constant at Buda, Plazy and Malý Vestec (with exception of γ HCH at Plazy), but importantly different at Poděbrady site. The most abundant compound was γ HCH with starting value of 220 $\mu\text{g}\cdot\text{l}^{-1}$ at Buda site, dropping lower to 140 $\mu\text{g}\cdot\text{l}^{-1}$ at Plazy, increasing later at Malý Vestec back to 220 $\mu\text{g}\cdot\text{l}^{-1}$ and finally reaching the concentration 470 $\mu\text{g}\cdot\text{l}^{-1}$ at the end of groundwater flow at Poděbrady. Important increase of total DDT concentration at Poděbrady site was observed. DDT concentration achieved, after almost constant value 4 $\mu\text{g}\cdot\text{l}^{-1}$, the final level of 26.7 $\mu\text{g}\cdot\text{l}^{-1}$. Opposite situation was observed with HCB. This contaminant diminished its concentration at Poděbrady site to value 2.2 $\mu\text{g}\cdot\text{l}^{-1}$ after almost constant value 8 $\mu\text{g}\cdot\text{l}^{-1}$. The phenomenon that after almost constant value the concentration of pesticides rapidly changed at Poděbrady site can be explained by presence of Elbe River near the sampling bore hole. Elbe River watershed comprises the largest agriculture areas in Czech Republic, where the use of pesticides is very common and pesticide pattern is not surprising. There γ HCH dominates, HCB are about one order of magnitude lower, together with DDT and their metabolites. It is noteworthy, that as to the level of contamination, found concentrations are negligible from the point of view of toxicological evaluation. On the other hand, it provides basic overview for future evaluation of POPs.

The results of toxicity tests are presented by EC50 of aliquot from one whole membrane, its hill slope and 95% confidence intervals in this study. These intervals show how well experimental data fits the sigmoid curve chosen as a standard course of toxicity in a relationship dose (concentration) – response. The final interpretation of toxicity in assessed groundwater is presented by using the V_{tox} ; the V_{tox} has an opposite ability to express toxicity in comparison with EC50. Values of V_{tox} and toxicity of evaluated sample are in direct proportionality. This means the higher V_{tox} , the higher is the toxicity.

The course of toxicity during groundwater flow is apparently similar in bioassays with algae and crustaceans. From starting value of V_{tox} at Buda site it increases at Plazy site, drops down at Malý Vestec to value similar like in Buda to further decrease at Poděbrady site. Course of toxicity to bacterium is similar in first two steps. Comparing with Buda site, the toxicity is higher at Plazy, to drop down again (more significantly in comparison to algae and crustaceans) at Malý Vestec. Contrary to earlier bioassays the test with bacterium demonstrates an important increase of toxicity at Poděbrady site.

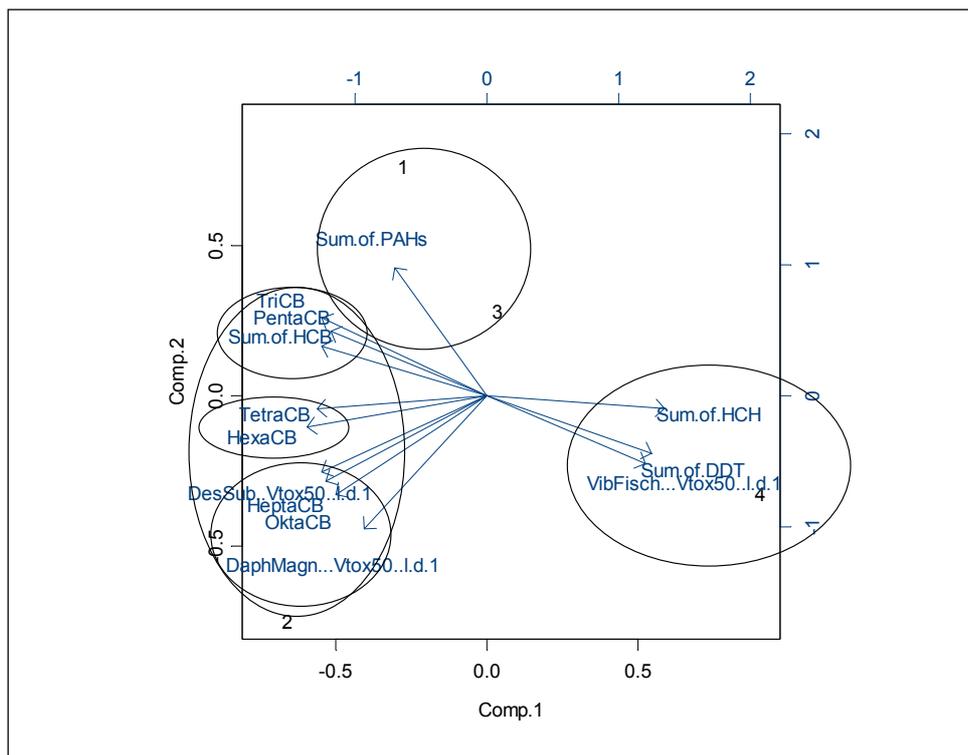
It is fundamentally not possible to correlate toxicity with chemical composition of mixture sample, such as aliquots from SPMDs containing POPs. Response of organisms to complex sample is more complex than addition and/or elimination. It is necessary to count with synergism and antagonism varying with changes in chemical and physical condition of environment together with changes in the state of every organism. Keeping this on mind, we compare course of main contaminant groups with course of toxicity expressed like V_{tox} .

The PCA analysis was additionally performed on summary data (PCB isomers, OCPs, toxicity

parameters), where score plot explains as much as 92,9% of total variations of analysed samples; the first component (Comp. 1) explains 74,6% variations, the second one (Comp. 2) 18,3% - see Graph 8. The loading vectors form three variable clusters: one for PAHs, and one for HCHs, DDT, together with *Vibrio* Fisheri. Statistical EDA and marginal analysis has shown limited possibilities, due to small data scale. Therefore

The course of toxicity to algae and crustaceans from Buda site through Plazy and Malý Vestec to Poděbrady site is similar in comparison with the course of concentration of sum of PCBs in the scale of tens of pg.l^{-1} . This similarity can be found in course of toxicity to bacterium except Poděbrady site. Bacteria were apparently more sensitive to sample from Poděbrady site, where pesticides were more abundant. No similarity of toxicity of all three testing organisms to course of contamination with PAHs is found, resulting in a presumption that contamination of water with PAHs concentrations under 16 ng.l^{-1} is of no toxic significance.

Although the toxicity at all sampling sites was determined, it is not possible to understand that the ground water was toxic. Concept of V_{tox} is able to express value of toxicity even if the SPMD membrane was exposed in natural clean and apparently non-toxic site. If the value of V_{tox} increases above critical level, then the site is toxic even for onsite living organisms¹⁶⁴. Values of all V_{tox} presented in this study are under critical level. This means that at all evaluated sites no toxic conditions occur. Benefit of V_{tox} is in its ability to rank the sites based on bioassays even in the case, that the sites are principally non toxic. On the other hand, V_{tox} based on SPMD sampling can be used for ranking of sites of extremely high toxic properties, where no living organism can be expected.



Graph 8 Poděbrady: the PCA graph of measured groups of contaminants, with toxicity parameters PC1 and PC2 were used for analysis (S-Plus)

The practical potential of V_{tox} was appreciated in comparing the contamination of sites with low contamination; there is useful to complete a rank of different sampling sites by chemical analysis by bioassays (because many chemical parameters were near or under detection limits). Bioassays marked effectively the sampling sites, where important contamination occurs: increased toxicity of all organisms of aliquot from SPMD exposed at Plazy site correlate with higher contamination by PCBs; the sensitivity of bacterium bioassay to aliquot from SPMD deployed at Poděbrady borehole refers to increased contamination with pesticide like compounds. Other information offered by bioassays was, that although toxic contaminants are present and their toxic level was determined, no acute toxicity can be expected on sampling site because the values of V_{tox} did not exceed critical level.

Conclusions

1. SPMD sampling system was used for monitoring the primary and secondary groundwater contamination of Cennoman bedrock. Chemical parameters like PAH, PCB and OCP concentration together with toxicity to *Desmodesmus subspicatus*, *Daphnia magna* and *Vibrio fischeri* were determined.
2. Although very low level of contamination was observed, secondary contamination of PCBs through bedrock was observed. PCBs sink down along the bedrock and secondarily contaminate sources of the water thousand years old in ground watershed. Secondary contamination of PCBs was observed at Plazy sampling site. Organochlorine

pesticides were found at a sampling site near a mouth of ground watershed.

3. Applied toxicity test affirmed presence of toxic substances and marked the site of higher contamination. Application of toxicological parameter V_{tox} enabled to rank underlying sites by their level of contamination even in cases, where concentrations of pollutants were near or under detection limits preventing thus the ranking the sites on a basis of chemical parameters.
4. Used bioassays showed no dependence on varying concentrations of PAHs under 16 ng.l-1. Changes in PCB concentration from 22 to 168 pg.l-1 induced homogenous changes in toxicity response of all used organisms. HCH Concentration 470 pg.l-1 together with DDT concentration 26.7 pg.l-1 caused significant response of bacterium, although crustaceans and algae showed no sensitivity to this level of pesticides.

Assessment of POPs at UNESCO protected area (Křivoklátsko)

The application focuses on PCDD/Fs (WHO I-TEF 1998), PCBs (toxic and all detectable) and OCPs (hexachlorobenzene and isomers of hexachlorocyclohexane, DDE, DDD and DDT), as well as toxicity parameter assessment, in surface water of middle-European natural reserve Křivoklátsko protected by UNESCO programme Man and Biosphere . Results of this application were recently published¹⁰³.

The natural reserve Křivoklátsko covers 630 square km, where no important industrial activities exist. The method of semipermeable membrane devices (SPMDs) was applied as described earlier, in two small Creeks and one water reservoir in the central part of this reserve was selected. No similar study about POPs contamination was realised before.. Watershed of these water bodies is industrial and agricultural activities free. The landscape relief of assessed area consists of up to 200 meters deep narrow valleys with a phenomenon of temperature inversion, preventing number of localities from direct imissions of pollutants. Due to previously mentioned facts, possible contamination by POPs was estimated as low and shall serve for further estimation of POPs background.

The exact allocation of sampling sites with GPS was determined: Oupor Creek sampling site coordinates are N49 58.103, E13 47.740; Miza Creek sampling site N49 56.235, E13 49.041 and coordinates of Klicava reservoir are N50 03.883, E13 56.027. Location of sampling places are given in Figure 10.

Handling with SPMDs was in accordance with methods described above. For ambient concentrations, no PRCs were used. Exposed SPMDs were analyzed both for chemical parameters of POPs. Toxicity bioassays on alga *Desmodesmus subspicatus*, bacteria *Vibrio fischeri* and crustacean *Daphnia magna* were performed as well. No extraordinary conditions were observed (e.g. turbulence), which would seriously affect sampling and make unfeasible usage of conventionally available calibration data.



Figure 10 Outline map of Krivoklatsko, adjacent to Prague and Pilsen. Locations of the sampling sites.

As there are no sources of pollution in the reserve, all detected concentrations of organochlorine pollutants are a result of transport from outside emission sources.

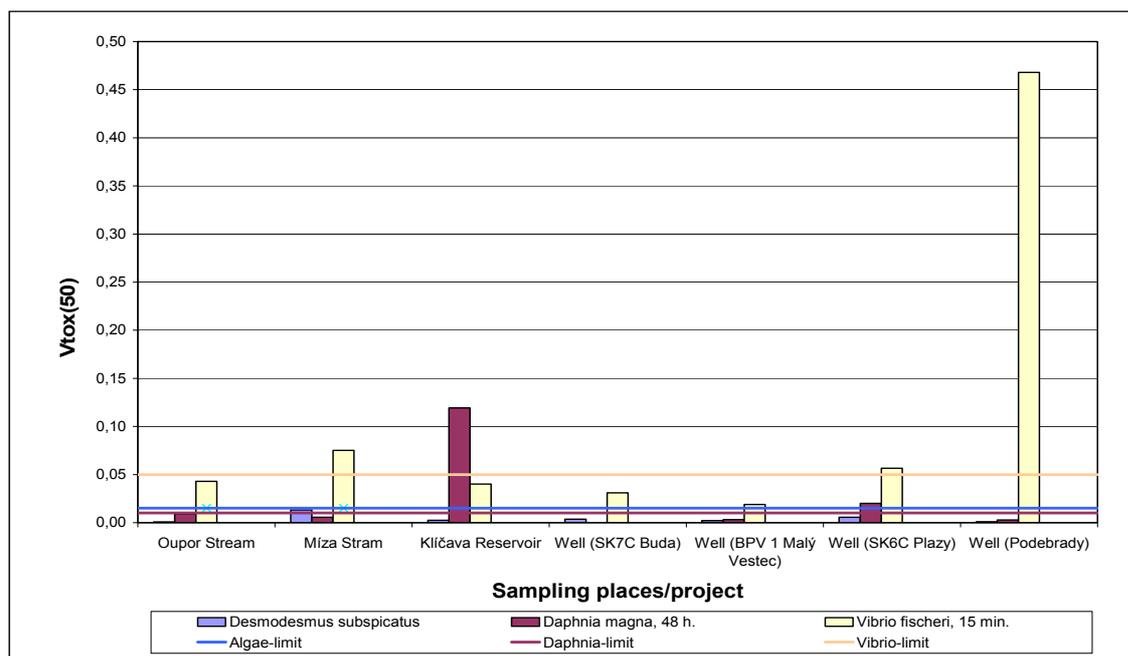
The highest concentrations were observed in gamma HCH (from 120 to 169 pg.l^{-1}) which was present in all assessed profiles. Contamination with HCB varied from 21 to 48 pg.l^{-1} and the level of DDT congeners and/or metabolites did not get over 45 pg.l^{-1} . Although in comparison based on dioxins, furans and PCBs the Oupor Creek was with the lowest level of contamination, from the point of view of pesticides this profile showed the highest contamination. The reason is its closer proximity to agricultural activities. Klicava reservoir watershed is larger than that of Oupor Creek, but its location is better protected to anthropogenic pollution.

Parameters found below the detection limit were taken into account as values of LOD. This approach was chosen to obtain the maximum value that can be found (with respect to toxicological relevance). These values are only approximate as the concentrations of many assessed compounds were below the detection limits. In such cases the detection limit value was used for counting the sum of a contaminant group. A concentration below detection limit is marked with the symbol “<”. Differences in LOQ are caused by different sampling times and temperatures. Extracted aliquots were the same.

As it was expected due to low level of contamination, the toxic response of applied toxicity test was very low. The results of toxicity test were expressed as effective volume V_{tox} . It is noteworthy that, as to the level of contamination, the found concentrations are negligible for toxicological evaluation with acute toxicity test. On the other hand, this data can be used for future reference regarding background POPs levels. This proved that present level of contamination has no direct

adverse effect on biota. The V_{tox} parameter was used for evaluation of the toxicity. From all results, no significant response in toxicity was observed. On the contrary, V_{tox} based on SPMD sampling could be used for ranking the sites by their toxic properties on such high levels, on which no organism could survive. If the value of V_{tox} increases above an empirically determined critical level, then the site can be considered toxic even for organisms living on site. All V_{tox} values presented in this study were under this empirical critical level, thus, no toxic conditions occur at any of these sites. The closer specification of empirical critical level of V_{tox} is a task for future research. Few sets of comparable data are available at present, so necessity of further research is warranted.

Toxicological results are presented in Graph 9.



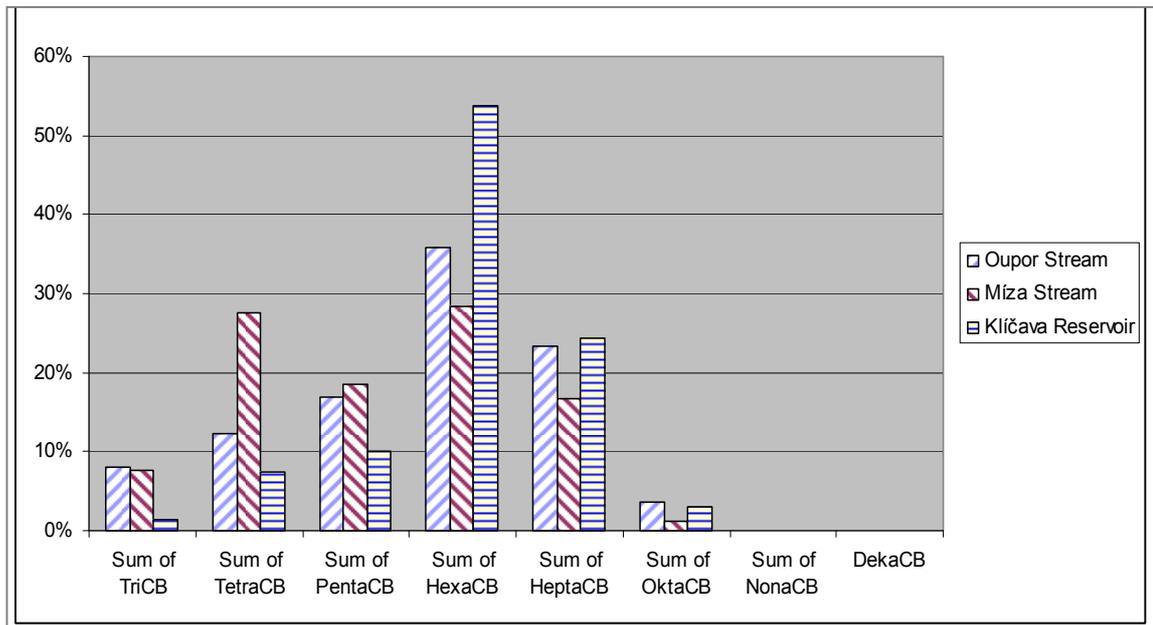
Graph 9 Krivoklatsko and Podebrady – toxicity, the comparison and proposed limits

The response of an organism to a complex sample is somewhat more complicated than the addition and/or elimination of toxic influence of individual compounds.

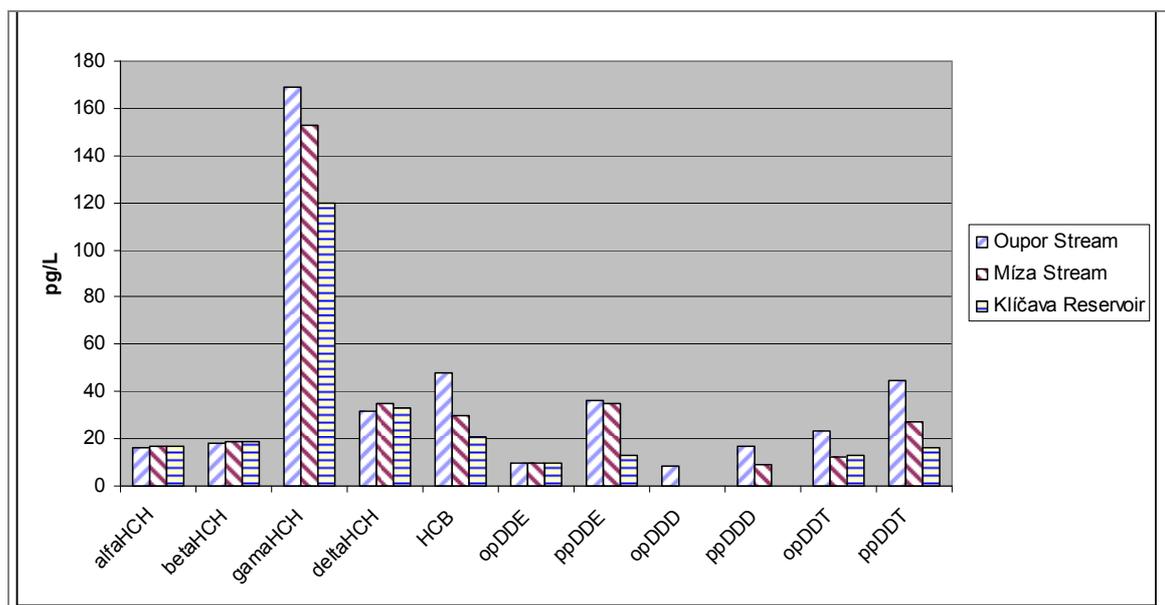
Found concentrations profiles are shown in Graph 10 and Graph 11.

At present the concentration of monitored contaminants reached the values up to tens of fg/L, except for Klíčava reservoir, where concentration of dibenzofurans reached 3.4 ng.l-1. No detectable concentration of toxic dioxins was observed for 1234678-HpCDD. From the group of non toxic dioxins okta and hepta congeners were detected. Higher contamination, in comparison with dioxins, is by dibenzofurans, where tetra and penta furans get on the level of nanograms per a litre. The highest concentration from PCB congeners was detected with PCB97, PCB37, PCB104, PCB54, PCB169 and 180, although even toxic congeners PCB169, PCB118, PCB 77, PCB126 and PCB81 pass the detection limit. The highest concentration detected for the organochlorine pesticides were HCHs, particularly gamma-HCH, followed by sum of isomers and metabolites of DDT. Tens of

femtograms of HCB were detected, too. Even if no detectable concentration was detected, this study is of value because very low detection limits were reached and presented.



Graph 10 Křivoklátsko: relative congener profile of PCBs



Graph 11 Křivoklátsko: OCPs profile

All those contaminants were described by point estimates of individual compounds. However, to make a good description, it would be necessary to determine them with varying synergism and antagonism, changes in the chemical and physical condition of the environment along with changes in the state of every organism. Even more, all chemicals affect as mixtures, as apparently found at this study. Keeping this in mind, the course of the main contaminant groups with the course of

toxicity expressed as V_{tox} was compared. Obtained concentrations of chlorinated organic pollutants can be used as an estimation of background values for Central European region.

The specific question of toxic effect caused by other than analysed hydrophobic substances, which can be found during sampling into SPMD, is not yet answered. Future research in this field is needed. The substances in question are PAHs. Based on our previous experience, the PAHs express toxicity after metabolic activation, thus their toxicity to bioassays used here is low. Thus, one of the possibilities how to distinguish a toxic effect caused by other substances is to use specific receptor biochemical assays. Special attention belongs to comparison of these results to those found in Poděbrady study, related to glacial water.

Conclusions

1. SPMDs were used for assessment of contamination of surface water with chlorinated organic pollutants, together with toxicity assessment. The objective of this study was to detect a real regional background of assessed compounds in area which is not yet industrialized and the ecological value of which is high with no damage caused by anthropogenic pollutants. It is the final step for the definition of background values for Central European region. Because Křivoklátsko area is declared as a healthy ecosystem, it is important information for future monitoring to know what level of contamination of organochlorine compounds is here at present time causing no evident decrease of ecological quality. Because of location of this region is located in Central Europe and relatively close to industrial activities, an increase of chlorinated compounds pollution in future can be thus expected,
2. PCDD/Fs, PCBs and OCPs were monitored in two creeks and one water reservoir in highly protected natural reserve.
3. Results of toxicity tests showed detectable response of toxicity, the values of which are under empirical threshold level. The applied bioassays showed no correlation with results of chemical contamination. This proves that level of contamination is low, causing no direct adverse effect to aquatic environment. This fact is in coherence with the good ecological status of assessed sites in natural reserve Křivoklátsko. Values of V_{tox} were under empirical critical value – showing no statistically significant toxic effect.

Comparison of SPMDs and biotic sampling systems

PCBs from the point of view of the ortho- side chlorinated congeners

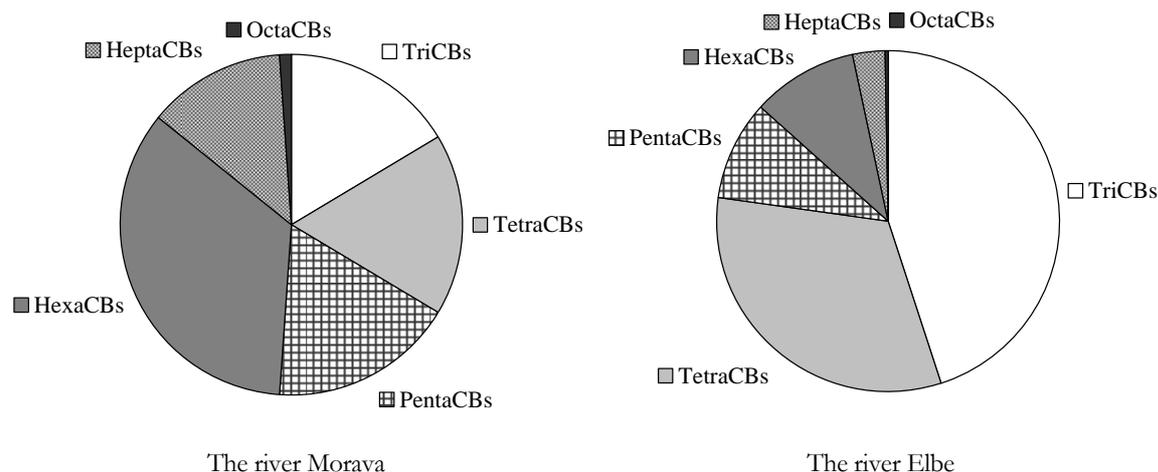
The polychlorinated biphenyl seems to be even at the present times the most serious contaminant in the Czech Republic. It is well known that in the former Czechoslovakia the intense production of different kinds of PCBs took place and the products used for different purposes as industrial additives. They were widely applied in the industry, e.g. as coolants and lubricants in transformers, dielectric fluids in capacitors, heat-transfer media, hydraulic fluids, additives mainly

into paints, sealing liquids, impregnating liquids, sizing materials, burning inhibitors etc. Their industrial production started in the year 1929 under various names (e.g. Delor 103, 103S, 104, 105, 105T, 106, 106/80X; Delotherm DH, DK; Hydolor 103, 104, 30, 137; Delofet and O-2; Delorit). The most applied Delors 103 and 106 correspond to the most applied chlorinated biphenyls Aroclor A1016 and Aroclor A1260. More than 11000 tons of Delors was produced and applied in the former Czechoslovakia within the period of 1959 to 1984^{77, 242-244}. These facts are the main reason why some parts of the Czech Republic are still contaminated with PCB²⁴⁵⁻²⁴⁷. This contamination of the fields and rivers by Delors is up to now the main effect of 25 years PCBs production and their use in the Czech Republic. Pollutants from industrial sides and from municipal sewage sludge treatment works entered into rivers and led to serious pollution problems. From the point of contamination view it is noteworthy to say that industrial PCBs mixtures, like Delor or Aroclor brands contain both PCDD/Fs, but also PCNs. The 2,3,7,8-TCDD was detected especially in the presence of mono- and non-ortho PCBs¹⁸. Those findings are results of recent systematic monitoring studies in environmental compartments, like air, soil, water, as well as sediments, biotic organisms, and human biotic material^{77, 248, 249}. In 2002, the Czech Republic was devastated by flooding episode, influencing both contamination of rivers and soils, but also of suspended particulate matters, sediments and fluvial soils^{77, 233, 248-251}.

The objectives of this study was to determine the contamination of the Czech (Elbe, Vltava) and Moravian (Morava, Oder and Dyje) rivers by PCBs in years 2001 and 2006. The status before and after flooding is also presented. The concentrations of polychlorinated biphenyls in the organisms of *Dreissena polymorpha*, *Leuciscus cephalus* (chub) and juvenile stadium of fish (fry) were determined and discussed from the point of view of the ortho-side chlorinated biphenyl structures.

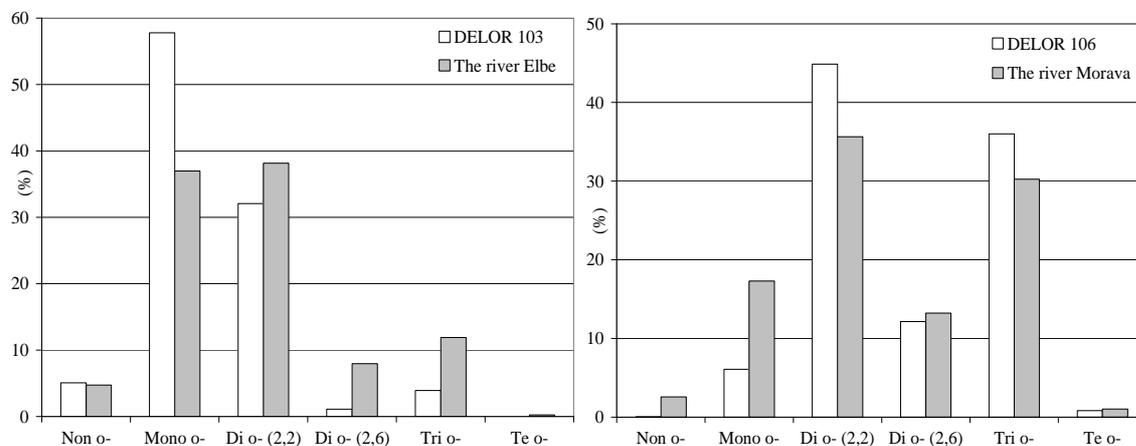
The waters of rivers Morava and Elbe were analysed for 95 PCBs congeners by using the SPMDs sampling method in May and June 2001. The sampling places are as follows: the river Morava - Lanžhot (near the town Břeclav upstream the confluence with the river Dyje); the river Elbe - Hřensko (between the town Děčín and the outflow to Germany). Used methods are described in methodology presented above.

At least 78 of 209 possible PCB isomers can exist as rotational isomers that are enantiomeric to each other. The percentage of the chlorinated biphenyl homologues is presented in Graph 12.



Graph 12 Relative ratios of PCBs homologues in the rivers (tri- to deca-chlorinated biphenyls)

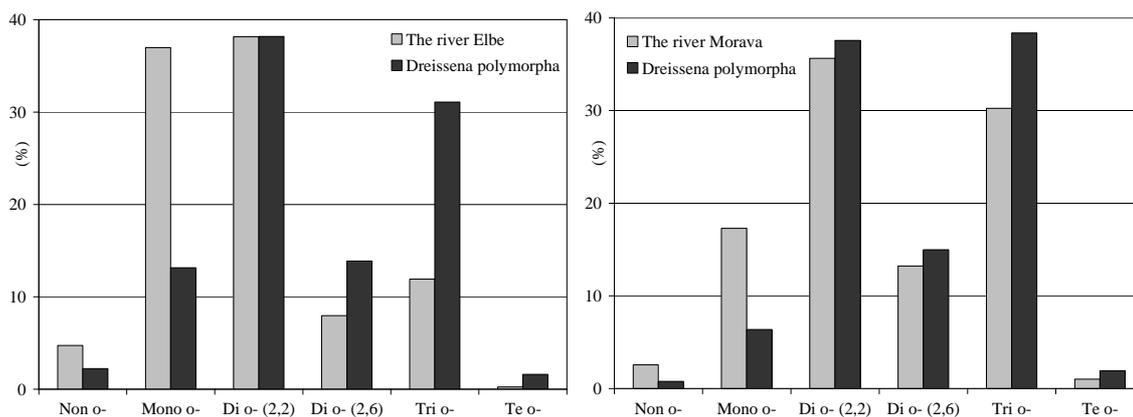
It is evident from these results that distribution of PCB homologues in the rivers differs significantly. In the river Elbe congeners of tri- and tetra-chlorinated biphenyls (CBs) are highly prevailing, however, the main contaminating components are highly chlorinated penta- to hepta-CBs in the case of the river Morava. It is evident from our analyses that Delor 103 (Aroclor A1016) contains mainly the mono-ortho and 2,2'-di-ortho-chlorinated biphenyls. The highly chlorinated Delor 106 (Aroclor A1260) contains not only the highly chlorinated tri-ortho-chlorinated congeners, but also the 2,6-di-ortho-chlorinated and mono-ortho-chlorinated biphenyls.



Graph 13 The ortho- side chlorinated PCB congeners in the rivers
As compared with congeners in Delor 103 and Delor 106 (non-ortho to tetra-ortho biphenyls)

In the Graph 13, the comparison of non-ortho- to tetra-ortho-chlorinated biphenyls distribution in the rivers Elbe and Morava with Delors 103 and 106 is presented. We can conclude from these results that the river Elbe is contaminated first of all by Delor 103, whereas the river Morava by Delor 106. The river Morava flows through Uherské Hradiště where production of paints existed. The total contamination of the rivers Morava and Elbe with polychlorinated biphenyls corresponds to the value of 1302 pg PCBs/l and 6475 pg PCBs/l, respectively, which means that contamination

of the river Elbe as compared with river Morava is approx. 5 times higher.



Graph 14 The ortho side chlorinated PCB congeners in the rivers
The comparison with contamination in *Dreissena Polymorpha* (non-ortho to tetra-ortho biphenyls)

The effects of contamination level in the rivers Elbe and Morava on the accumulation of the polychlorinated biphenyls in the whole dry mass of *Dreissena polymorpha* after two months exposure in rivers is presented in the Graph 14. It is evident from the results that the concentrations of non-ortho and mono-ortho-chlorinated PCBs in *Dreissena polymorpha* are considerably lower compared with the content in rivers, while the 2,2'-di-ortho-chlorinated PCBs are practically the same. Dominating accumulation of tri-ortho chlorinated biphenyls as well as the pronounced accumulation of 2,6-di-ortho- and tetra-ortho-chlorinated PCBs has been found.

There are different opinions for understanding the reasons of these accumulation phenomena. From our point of view, very high thermodynamic stability and rigid structure²⁵² of compounds mentioned above might be the possible and serious reason for accumulation in the biota. It is obvious from the Table 6 that values of dihedral angles for tri-ortho and tetra-ortho biphenyls are about 88 to 90° which means that the aromatic rings are perpendicular to each other. However, for certain structures of 2,6-di-ortho-chlorinated biphenyls, the perpendicular structure might exist as well. It is well known that the aromatic rings of biphenyls which contain three or four and even also two large groups in the ortho sides of biphenyl structures cannot rotate about the central bond because of the steric hindrance²⁵³⁻²⁵⁷. From the Table 6, it is evident that for PCB congeners with perpendicular aromatic rings the internal energy barriers of rotation are extremely high and correspond to hundreds kJ/mol, which is more than two orders of magnitude higher value in comparison with PCB congeners with non-ortho- and mono-ortho-chlorinated structures. These facts might explain the above mentioned facts. Highly different properties of chlorinated biphenyls (especially with respect to their structures and thermodynamic stabilities) might be a serious reason for different health effects for humans. Polychlorinated biphenyls elicit a diverse spectrum of toxic responses which were stated for Delors 103 and 106^{252, 258-264}.

Ortho positions	ω , dihedral angles ^{252, 265} (°)	Internal barriers of rotation ²⁶⁶ (kJ/mol)
non-ortho-	38 - 48	8.33 - 9.79
mono-ortho-	51 - 57	28.5 - 34.9
2,2'-di-ortho-	67 - 89	60.2 - 73.9
2,6-di-ortho-	77 - 90 (prevailing 89 - 90)	66.4 - 190
Tri-ortho-	88 - 90	177 - 224
tetra-ortho-	89 - 90	373 - 483

Table 6 Dihedral angles and internal barriers of rotation for different ortho-side chlorinated PCBs

It is evident from this table that the majority of 2,6-di-ortho and all tri-ortho and tetra-ortho PCBs are characterized by dihedral angles of about 89-90° where the aromatic rings are perpendicular to each other. Their internal barriers of rotation are higher as compared with non-ortho-, mono-ortho and 2,2'-di-ortho PCBs. These facts prove the idea that these compounds will be very stable from the thermodynamical point of view. It follows from our stepwise dechlorination experiments of decachlorobiphenyl²⁶⁷ that the last pathways configuration before ortho chlorines dehalogenation was the formation of the most stable tetraortho-chlorinated congener PCB 54, which means that the chlorines in ortho- positions seem to be the most chemical stable as compared with meta and para- positions, that comply with recent findings in PCB dechlorination²⁶⁸⁻²⁷². This might be the reason for bioaccumulation and biodegradation phenomena. It is well known that the aromatic rings of biphenyls which contain three or four and even also two large groups in the ortho-sides of biphenyl structures cannot rotate about the central bond because of the steric hindrance. There are different opinions for understanding the reasons of these accumulation phenomena. From the author's point of view, very high thermodynamic chemical stability and rigid structure of the compounds mentioned above might be the possible and serious reason for accumulation in the biota.

It was predicted²⁷³ that 19 PCBs will exist as stable atropisomers at room temperature. Atropisomeric PCBs are considered to be an important class of chiral organic pollutants, analytically separated and found in water environment²⁷⁴⁻²⁷⁸. They contain three or four o-chlorine atoms and exist as pairs of stable enantiomers. These compounds have been categorized as environmentally important compounds. Chiral compounds are powerful markers for biological processes such as enzymatic degradation and preferential membrane transport because biological pathways can be enantioselective²⁷⁹. It can be generally stated^{278, 280-285} that enantiomers of chiral organochlorine compounds exhibit varying biological responses such as activity, metabolic rates and toxicities. Chiral environmental chemistry is a rapidly growing area of research providing enhanced insights into environmental biological processes According to Kaiser's statement, it can be confidently predicted that individual PCBs isomers will exist in optical antipodes under following conditions: The two ortho chlorines in the first and one ortho chlorine in the second aromatic ring of

chlorinated biphenyl has to be substituted (tri ortho-chlorinated congeners) and fulfil the general requirements for optical activity (i.e. absence of either a plane of symmetry, a symmetry axis or of a centre of inversion and/or combination of). Nineteen polychlorinated biphenyls are optically active with stable conformation. The activation energy of their racemisation was determined and found to be dependent on their structures. The determined activation energies of polychlorinated biphenyls having in their structures the basic chlorine substitution in positions 26-2 (PCB 45, 88, 91, 95, 139, 144, 149, 183), 236-23 (PCB 84, 131, 135, 149, 171, 174, 175, 196) or 236-236 (136, 176, 197) correspond to the values of $\Delta E(20^\circ\text{C}) = 25, 29$ and 58 kcal.mol^{-1} , respectively. These activation energies required for the racemisation reactions seems to be generally greater than the thermal energy usually available in the ecosystems²⁷³.

	River (%)	Trend	Organism (%)	Change
Non o-	2.6 - 6.9		0.8 - 3.0	1.8 to 5.5
Mono o-	17.3 - 39.9		6.4 - 15.9	10.9 to 28.2
Di o-(2,2')	30.2 - 38.2		36.2 - 44.7	-1.8 to +14.6
Di o-(2,6)	7.7 - 14		13.2 - 16.1	1.1 to 5.9
Tri o-	9.4 - 30.2		21.3 - 38.4	8.0 to 19.2
Te o-	0.1 - 1.0		0,7 - 1,9	0.3 to 1.2

Table 7 Accumulation potential of selected PCBs isomers

These general ideas which were concluded might be supported by the following facts. It can be generally stated that fish have relatively low metabolism ability for high chlorine content of PCB congeners. This is in agreement with the fact that these compounds are in fish rapidly accumulated with very high biomagnification factors as found²⁸⁶ for PCB 95 and 136. As stated²⁸⁷, the congeners which are characterized by higher number of ortho chlorine atoms are indicating slower mobility and lower biotransformation ability. The enzyme induction²⁸⁸ in the biotransformation of PCBs to hydroxylated OH-PCBs metabolites by rainbow trout has been found to be dependent on the chlorine substitution pattern. Biotransformation appears to be limited to congeners with tri- and tetra-ortho chlorinated positions. Lesser chlorinated PCBs are more easily biotransformed. It is generally accepted that bioconcentration of PCBs in aquatic organisms correlates with the degree of chlorination, the stereochemistry and also with lipophilicity^{148, 288, 289}.

Though the TEF concept is still the most plausible and feasible approach for risk assessment of PCBs, it was concluded, that it was insufficient in vivo evidence to continue to use TEFs for some

di-ortho PCBs²⁹⁰. Up to now only the TEFs values are used for the determination of PCBs contamination for toxicity limits determination taken into account the low chlorinated PCBs, where the three- and tetra-ortho chlorinated PCBs are missing.

Until recently toxicologists paid very little attention to PCB with multiple orthochlorine atoms. The classic view was that the more toxic PCBs were those with no or few ortho chlorines. However, an entire spectrum of adverse effects has been associated with PCBs with multiple ortho chlorines. Enantiomers of chiral organohalogen compounds exhibit varying biological responses such as metabolic rates, activity and toxicities (hepatocarcinogenesis, neurotoxicity, (anti)estrogenic and calcium signal effects). The presence of chiral compounds in the environment is therefore of emerging significance, because many biological processes can be enantioselective and many of these compounds are released into environment as racemic mixtures^{291, 292}. Many components for agrochemical applications such as herbicides, insecticides and fungicides possess chiral molecules forming racemic mixtures or racemates²⁹³. However, the producers may wish to be assured that none of the applied components of a racemic mixture generates side effects for living nature. The existence of optical isomers between congeners of polychlorinated biphenyls has a bearing on the toxicity and metabolic interactions of these compounds. The tri-ortho-chlorinated PCBs (PCB 177, 178, 183 and 187 are associated with neurone-developmental effects²⁹⁴.

The author therefore fully agrees with the statement²⁹² of that the consensus on actual rise of PCBs' threat to public health and the environment is still elusive. The discovery of the mechanism for Ah Receptor mediated toxicities in the mid 1970s diverted attention to the more prevalent ortho rich PCBs lasting until recently. Our results confirm the fact that the toxicities of the most stable congeners of PCBs, especially with rigid structures and racemic properties, are still the open problem which has to be solved.

Conclusions

1. The river Elbe is contaminated first of all by Delor 103, whereas the river Morava by Delor 106. Total contamination of the river Elbe as compared with river Morava is approx. 5 times higher.
2. Concentrations of non-ortho and mono-ortho-chlorinated PCBs in *Dreissena polymorpha* are considerably lower when compared with the content in rivers, while the 2,2'-di-ortho-chlorinated PCBs are practically the same. Dominating accumulation of tri-ortho chlorinated biphenyls as well as the pronounced accumulation of 2,6-di-ortho- and tetra-ortho-chlorinated PCBs has been found. This is most probably caused by high thermodynamic stability and rigid structure of mentioned compounds (dihedral angle, aromatic rings perpendicular to each other, internal barriers of rotation).
3. Fish have relatively low metabolism ability for high chlorine content of PCB congeners. Biotransformation appears to be limited to congeners with tri- and tetra-ortho chlorinated positions. Lesser chlorinated PCBs are more easily biotransformed. It is

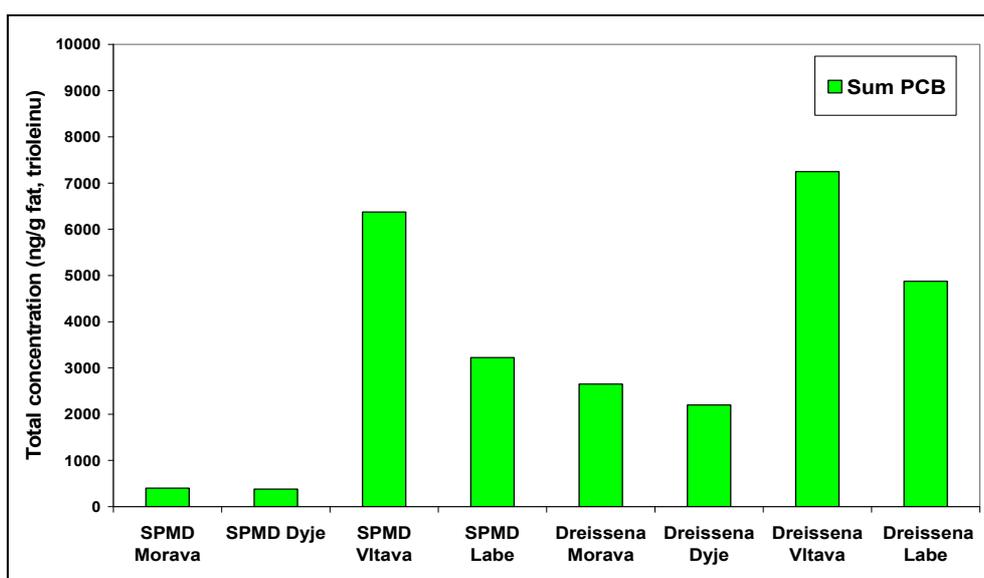
generally accepted that bioconcentration of PCBs in aquatic organisms correlates with the degree of chlorination, the stereochemistry and also with lipophilicity.

- Up to now only the TEFs values are used for the determination of PCBs contamination for toxicity limits determination taken into account the low chlorinated PCBs, where the three- and tetra-ortho chlorinated PCBs are missing.
- The presence of chiral compounds in the environment is therefore of emerging significance, because many biological processes can be enantioselective and many of these compounds are released into environment as racemic mixtures. The existence of optical isomers between congeners of polychlorinated biphenyls has a bearing on the toxicity and metabolic interactions of these compounds. Thus, toxicities of the most stable congeners of PCBs, especially with rigid structures and racemic properties, are still the open problem which has to be solved.

Comparison of selected of POPs at selected profiles

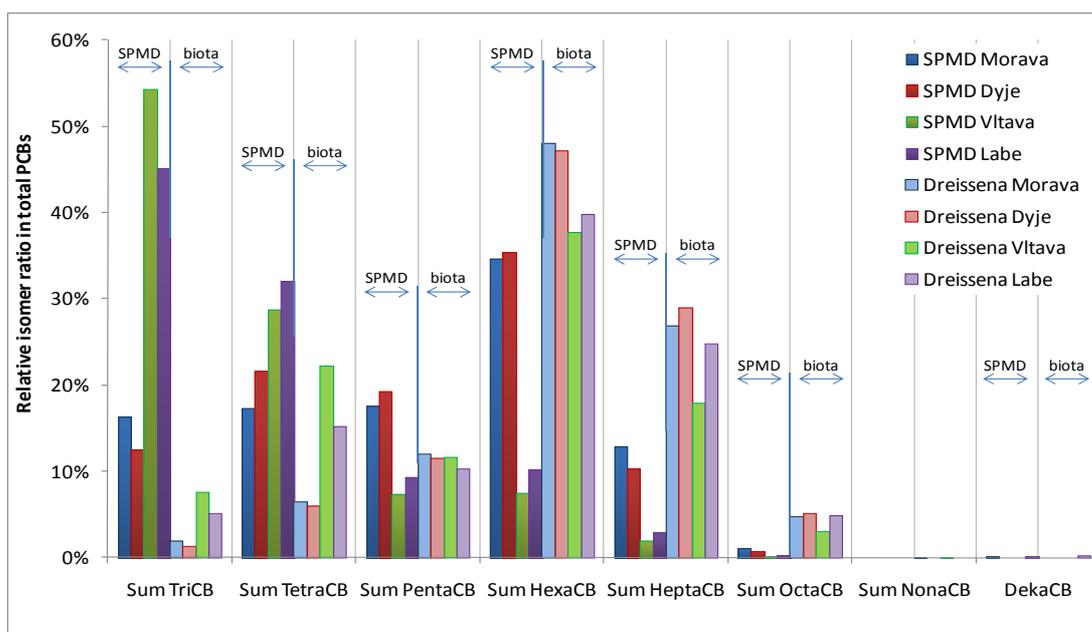
This work was realised within framework of pilot studies, realised in 2001-2002 and it is part of the study, discussion above (PCBs from the point of view of the ortho- side chlorinated congeners). Comparison was realised within PAHs, PCBs, PBDEs and PCDD/Fs. Main aim of the study is revealing differences between biotic and abiotic sampling systems, with main impact of revealing bioaccumulation from the point of view of level chlorination. Exposure of SPMDs was performed for 30 days, together with *Dreissena polymorpha*, in selected profiles of Bohemian and Czech rivers. Concentration are expressed in comparable unit with basement to SPMD and *Dreissena* respectively. Results were based on the same amount lipid, i.g. 1 g allowing their comparison.

It is generally stated, that intake of POPs from water by biotic organisms is connected with metabolism and depuration.

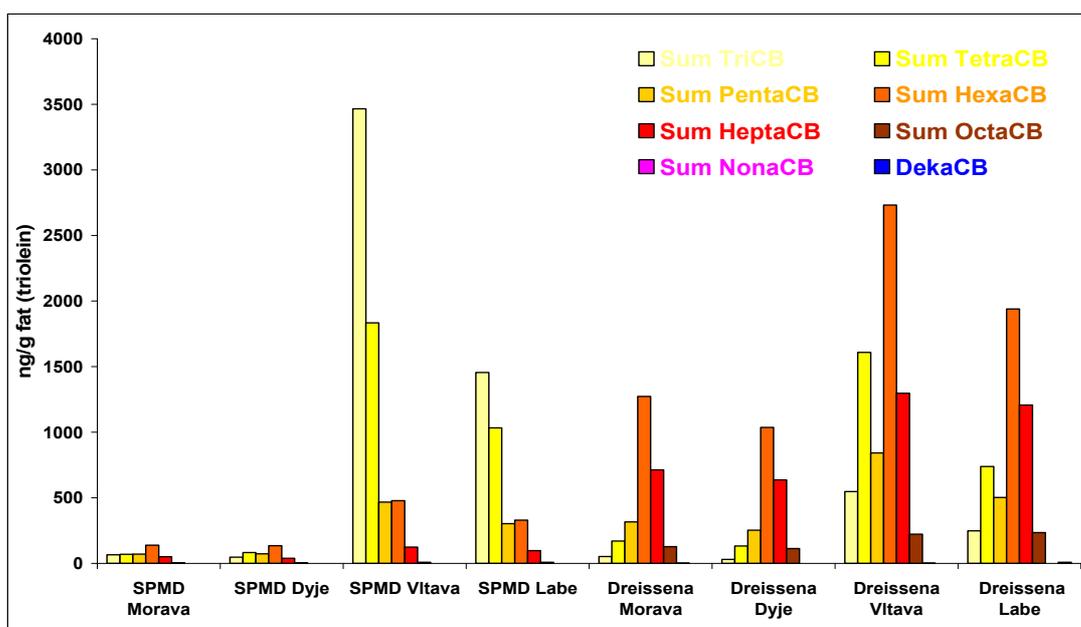


Graph 15 Biotic/Abiotic comparison: total concentration of PCBs

As to PCBs, there are apparent higher concentrations in biota than in SPMDs, but not equally: Moravian about 5-6 times, Bohemian are nearly equal (about 1,1-1,5 times). Higher contamination exhibits Bohemia rivers (Elbe, Vltava) than Moravia ones (Odra, Dyje) – approximately 5 times. From homologue profiles (Graph 16), there are apparent lower concentrations of lower-chlorinated PCBs, like tri-, tetra-CB in SPMDs, whereas penta- to hepta- was higher in *Dreissena polymorpha*. It can be accounted to negligible depuration of low-chlorinated biphenyls or accumulation of contaminants from food chain and non-soluble fraction.



Graph 16 Biotic/Abiotic comparison: relative ratio of PCBs

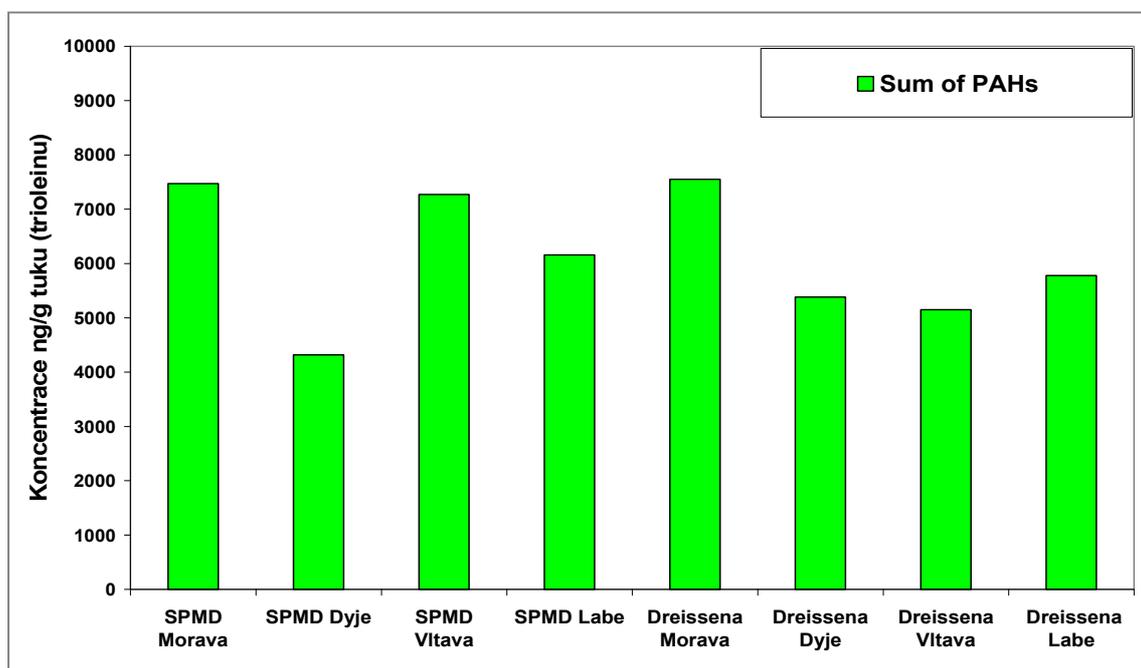


Graph 17 Biotic/Abiotic comparison: PCBs isomer profiles

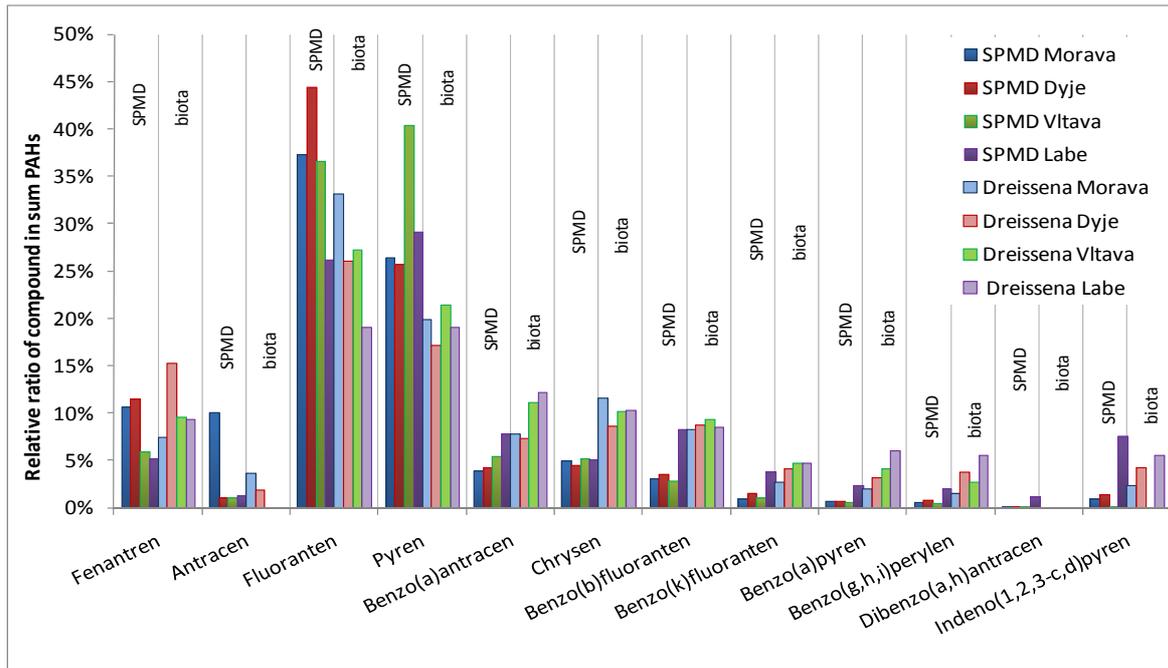
Relative homologue profiles are similar for *Dreissena polymorpha*; however SPMDs exhibit different

profiles for Bohemia and Moravia rivers. This fact is accounted to ability sequestering contaminants without any further transformation; thus using the SPMDs is a more suitable way for identification of contamination sources than biota. On the other hand, identification of PCBs in biota reveals intake of PCBs from both water (soluble) and non-soluble fraction. From the graph, in SPMDs Tri- to Tetra-CB congeners prevail in Bohemia (more polluted) rivers. In spite of these findings Hexa- and Hepta-CB are dominant in *Dreissena polymorpha* samples, while Tri-CB was found by factor about 5 comparing to SPMDs.

As for PAHs, the comparison between *Dreissena polymorpha* and SPMDs has similar results to PCBs. However, PAHs are readily more biodegradable than chlorinated compounds. It results into only slightly elevated concentration of PAHs. Even in some cases, there is a faster depuration than uptake, resulting in the lower accumulation in *Dreissena polymorpha* than in SPMDs (Elbe and Vltava River). It is noteworthy that in spite of different contamination of composition from the point of view of individual compounds, there is no surprising different metabolism by biota. As in the case of low chlorinated compounds, we can observe similar behaviour at the case of PAHs as well: from the ratio of individual PAHs compounds it is apparent that phenanthrene, anthracene, fluoranthene and pyrene dominate in SPMDs, due to their readily metabolism in biota. On the contrary, benzo(a)anthracene to indeno(1,2,3-cd)pyrene are dominating in the biota, due to less degradability. In the SPMDs, there is apparent accumulation without metabolism.

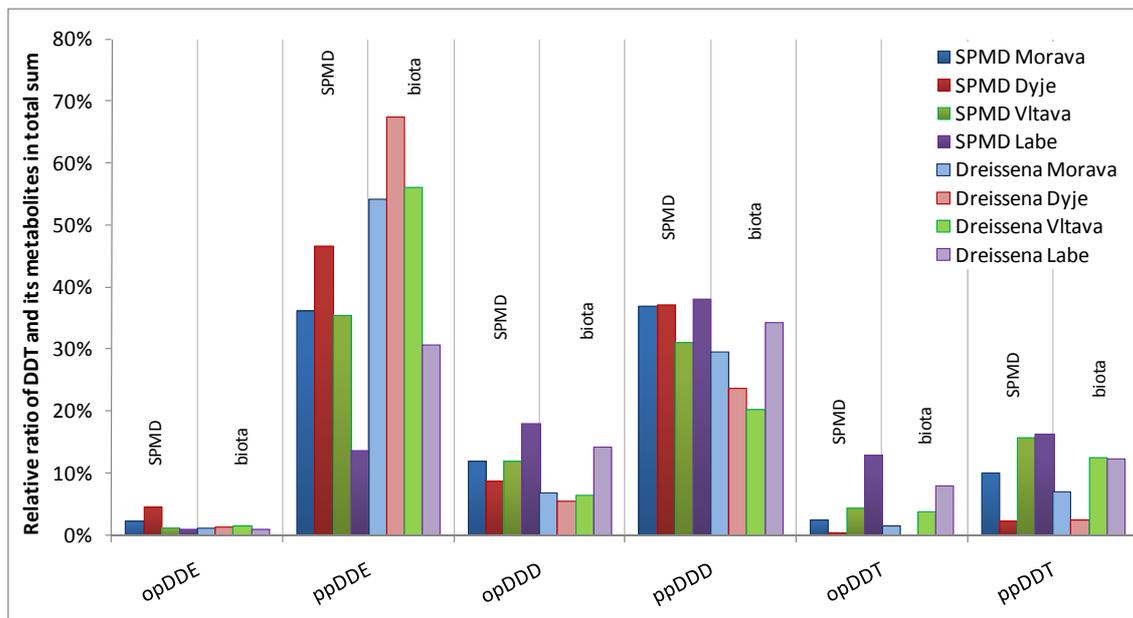


Graph 18 Biotic/ Abiotic comparison: total concentration of PAHs



Graph 19 Biotic/ Abiotic comparison: relative ratio of individual compounds in sum of PAHs

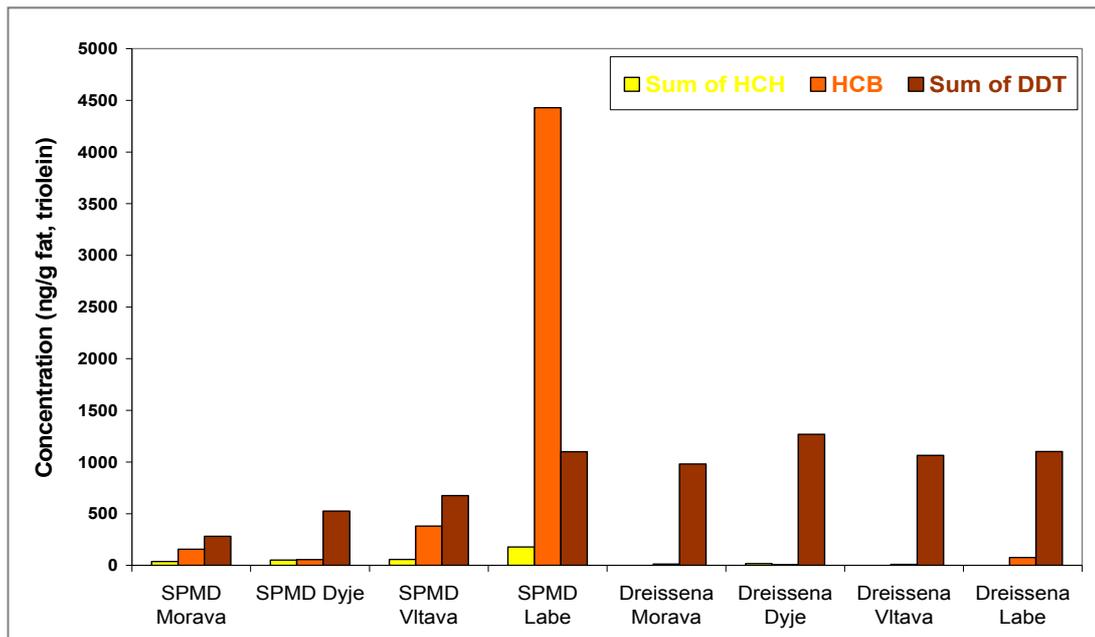
Distinctive results are apparent from DDTs and their metabolites (see Graph 20 and Graph 21). At first, it is generally known that both DDEs and DDDs are breakdown products of DDTs. DDTs do not occur naturally in the environment, DDEs and DDDs strictly follows the appearance of DDTs. From the relative ratio, it is apparent that DDTs is also slightly degraded by *Dreissena polymorpha*, mainly to DDE, where that product dominates in biota over that in SPMDs.



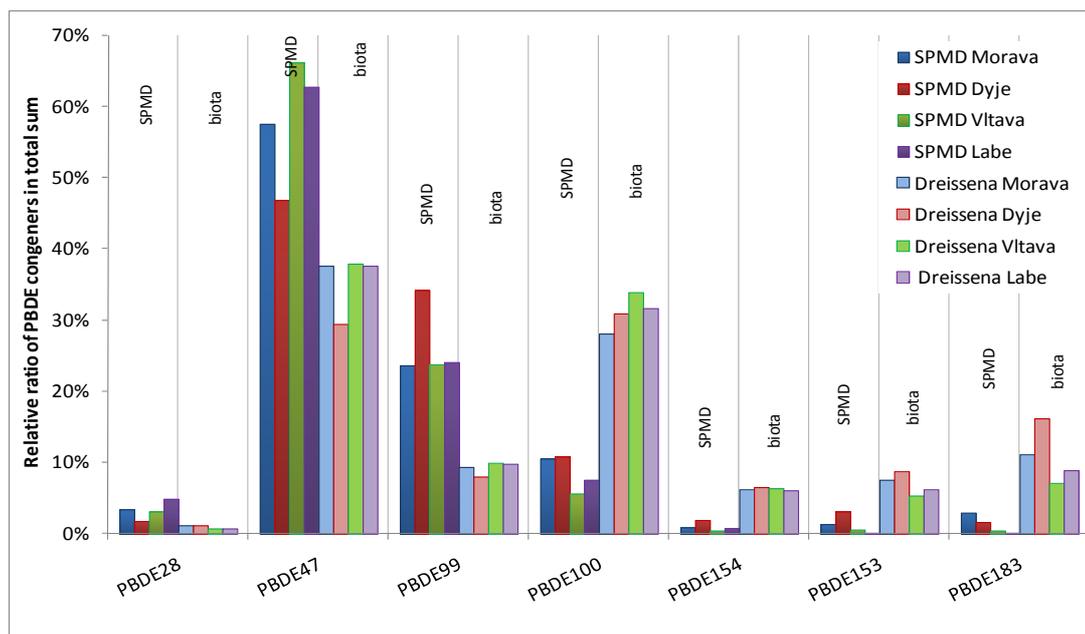
Graph 20 Biotic/ Abiotic comparison: relative ratio of DDTs and their metabolites in total sum

On the contrary, DDDs are dominating in SPMDs. Provided that SPMDs do not make a metabolisation, *Dreissena polymorpha*'s metabolisates more readily to DDEs. Considering similar behaviour of any biotic organism that opens discussion about ratio DDD/DDE, causing various

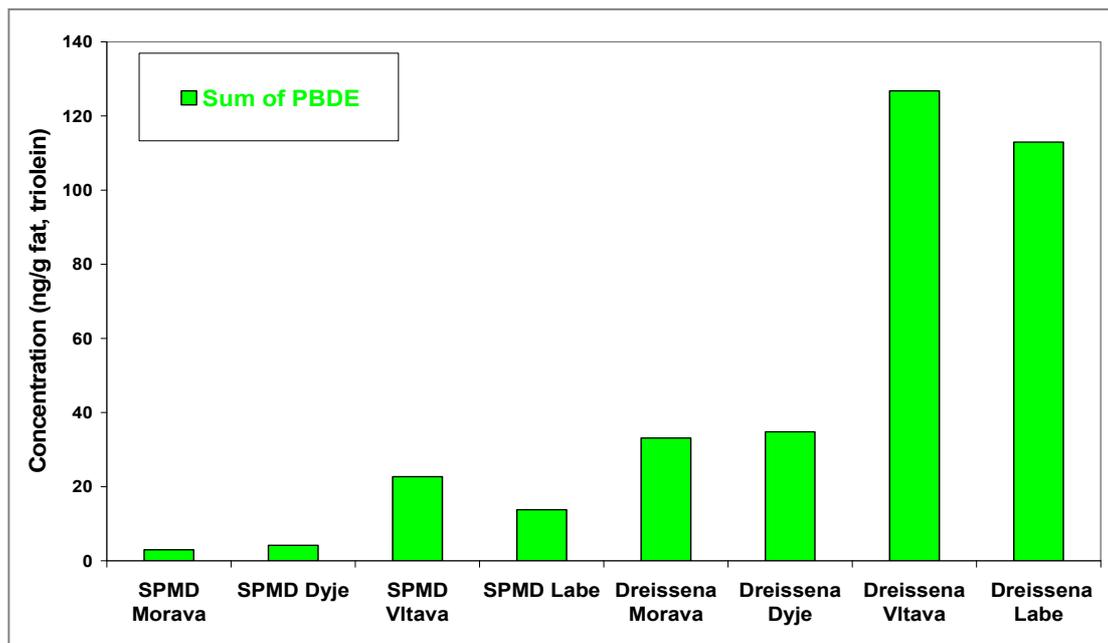
health effects should be opened. However, deep insight to this issue exceeds scope of this work.



Graph 21 Biotic/Abiotic comparison: concentration of HCHs, HCB and DDTs



Graph 22 Biotic/Abiotic comparison: relative congener ratio of PBDE



Graph 23 Biotic/Abiotic comparison: concentration of PBDE

The HCH and HCB, HCHs are partly metabolised, whereas HCBs is not, probably due to rigid molecular structure and stability. This results in comparable results in SPMDs and biota cases. Apparently, from the graphs above, the intake of DDTs that is consequently metabolised to DDEs and DDDs (see above) in biota is not only from dissolved phase.

Serious intake is thus from food chain. That underlines that all rivers are historically contaminated by DDTs that is stored in solid compartments, including sediment. Despite to this fact, Elbe River dominates due to presence around the recent producer - Spolana Neratovice.

Properties of PCBs similar to PBDEs were recently postulated (see Graph 22 and Graph 23). From the point of degradability view, it seems to be acceptable. It is apparent that Bohemia and Moravia rivers are differently contaminated. Elbe and Vltava seems to be sing of effluents, most probably from WWTP. However, major intake of PBDEs into biota most probably originates from sediment and food chain. This intake seems to be in all rivers, but for Moravian is less remarkable than for Bohemian ones.

The mostly dominating congener is PBDE47, that is readily degradable than PBDEs and all higher congeners. A special phenomenon is apparent for congener PBDE100 that is remarkably accumulated in biota from both food chain and dissolved phase, with no metabolisation. If background contamination can be avoided (from analysis of biota before exposure as a blank, having negligible PBDE100 contamination), metabolic formation PBDE100 from PBDE47 cannot be also excluded.

Conclusions

1. Different accumulating mechanisms in biota are participating on the POPs uptake: combination processes run in parallel, mainly bioaccumulation (both dissolved and solid phase), biomagnification (from the food chain) and metabolisation.
2. Only bioconcentration process directs POPs exposure at SPMDs, without metabolisation.
3. The *Dreissena polymorpha* is suitable for evaluation of possible health risk, whereas SPMDs is more suitable for sources identification.
4. For routine monitoring, where both risk evaluation and source identification is the main goal, both biota and abiota is necessary to be used.

Special application for POPs assessment: WWTP

In this work, special application for assessment of POPs in Waste Water Treatment Plant Ostrava is described. There were two goals: (i) to verify application of SPMDs in dirty application, with strong conditions (biofouling and concentration), (ii) to verify toxicological approach for evaluation of POPs, and (iii) to make identification of priority contaminants and their levels, contributing to major pollution, as the long-term response.

Assessment of contamination of quality of sludge produced by various cleaning technologies is becoming very challenging at present time. Main reason is usual the contamination by POPs, arising from various sources. One of the most important task and present challenge for assessment is the long-term monitoring of POPs in all input streams. Due to functional attributes of SPMDs, their applications seem to be interesting alternative for the long-term monitoring, resulting in timely-weighted average contaminants. As complementary to chemical parameters, toxicity tests seem to be valuable response to overall assessment.

Until this application, no similar applications of SPMDs were found worldwide. Within this application, there were following parameters assessed: PAHs, PCBs (all detected), and PCDD/Fs (17 WHO I-TEF 1998 parameters). Based on good experience of recent toxicity studies, there were also following tests realised: inhibition of light production of bioluminescent bacterium *Vibrio fischeri*, acute toxicity test with cladophora *Daphnia pulex*, rainbow trout *Oncorhynchus mykiss* and the test of inhibition of algal growth of *Scenedesmus subspicatus*. Within this study, it was the first application of *V_{tox}* parameter for SPMDs, as defined above. For a comparison, conventional grab sampling close to integral sampling was realised. Basic experimental overview is given in following table.

Sampling	Sample Kind	Monitored parameters
Grab	Water	PAHs (12 of 16 US EPA) Toxicity tests
Grab	Sewage sludge	PAU (12 of 16 US EPA) Toxicity tests
SPMDs (integral)	Water	PAHs (12 of 16 US EPA) PCDD/F (I-TEF WHO, 1998) Toxicity tests

Table 8 Basic experimental overview

Standard (grab) sampling was performed according to valid Czech (European/ISO) sampling standards for waters and solid materials. The sampling was performed prior SPMD deployment and samples were analysed by validated (accredited) analytical methods. SPMDs sampling was performed according to recommended good SPMDs practise, as described above.

As mentioned previously, this application was realised on wastewater treatment plant (WWTP) in Ostrava city. This WWTP is used for cleaning of wastewater from three different sources: sewage water, sewage water with industrial wastewater and wastewater directly from a coking plant. Capacity of treatment plant is approx. 184300 m³/day. Common technological behaviour and improvement is focused to quality improvement of produced sewage sludge in accordance with the requirements of Council Directive No. 86/278/EEC for sewage treatment plants.

Main focus was put to industrial water inlets (coking plants Šverma and Svoboda). Overall technological scheme is given in Figure 11.

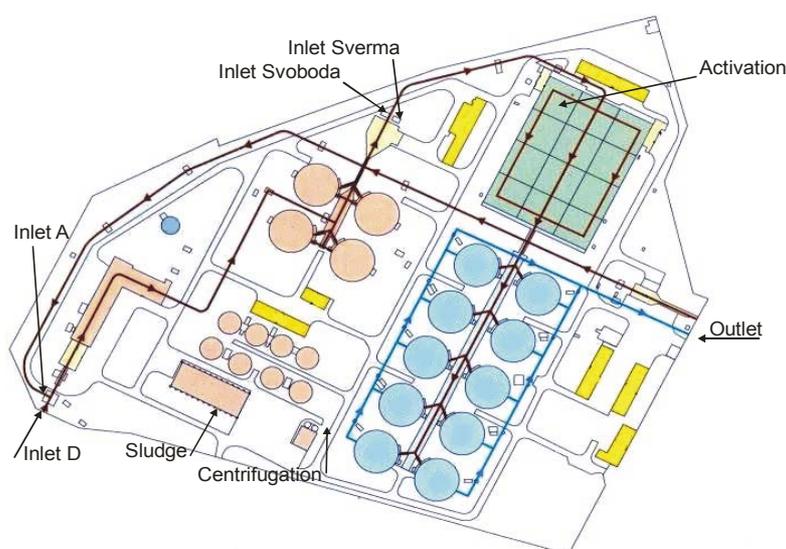
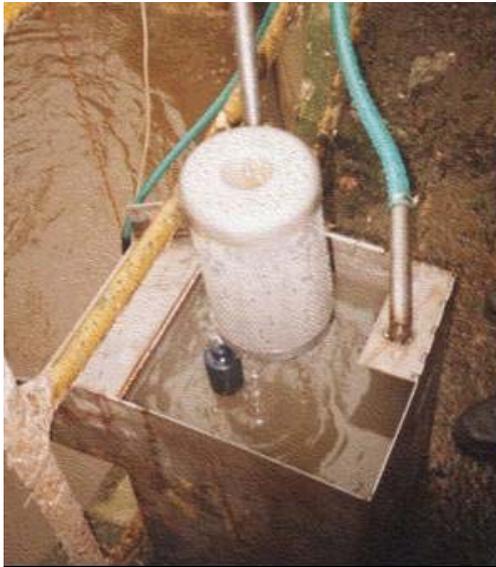


Figure 11 Basic scheme of sewage treatment plant.

Due to strong flow, it was necessary for SPMDs application to prepare special stainless-steel

vessels for diverting input streams, as shown in the picture below. Otherwise the strong stream could cause damaging SPMDs, despite its placing into protective shroud. This application, however, used no PRCs, due to lack of experience to PRCs approach, even for WWTP.



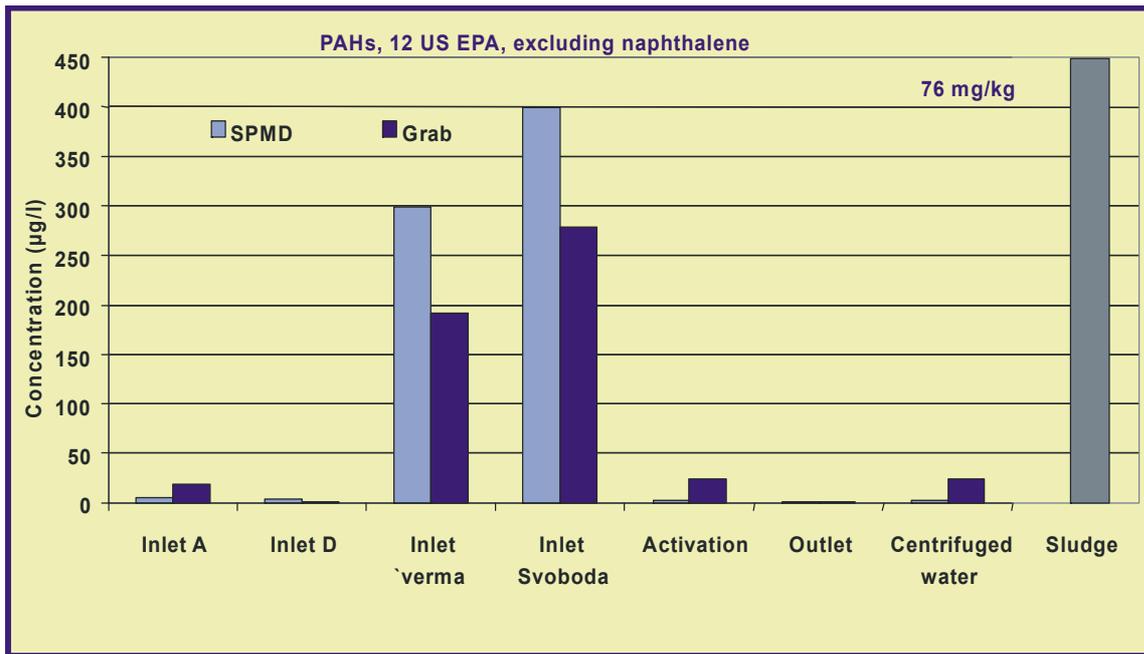
Picture 1 Sampling arrangement (inlet D – wastewater recipient); before exposure



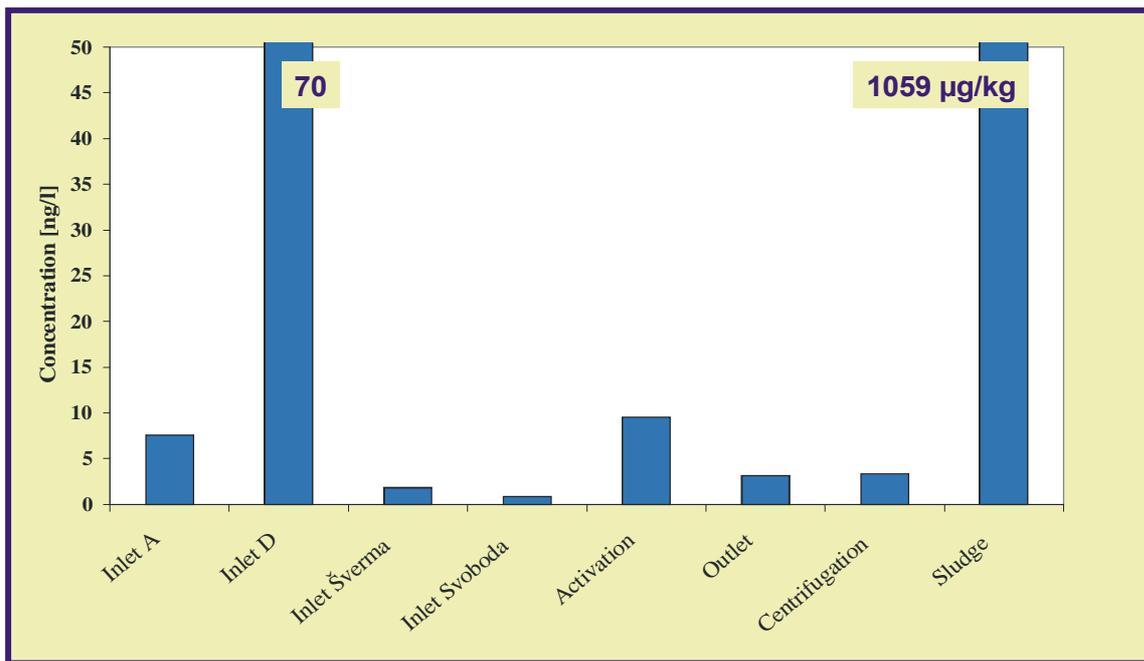
Picture 2 Sampling arrangement (inlet D – wastewater recipient); after exposure

Results from SPMDs exposure are presented in Graph 24 to Graph 28 for chemical parameters; results from particular toxicity tests are in Graph 29.

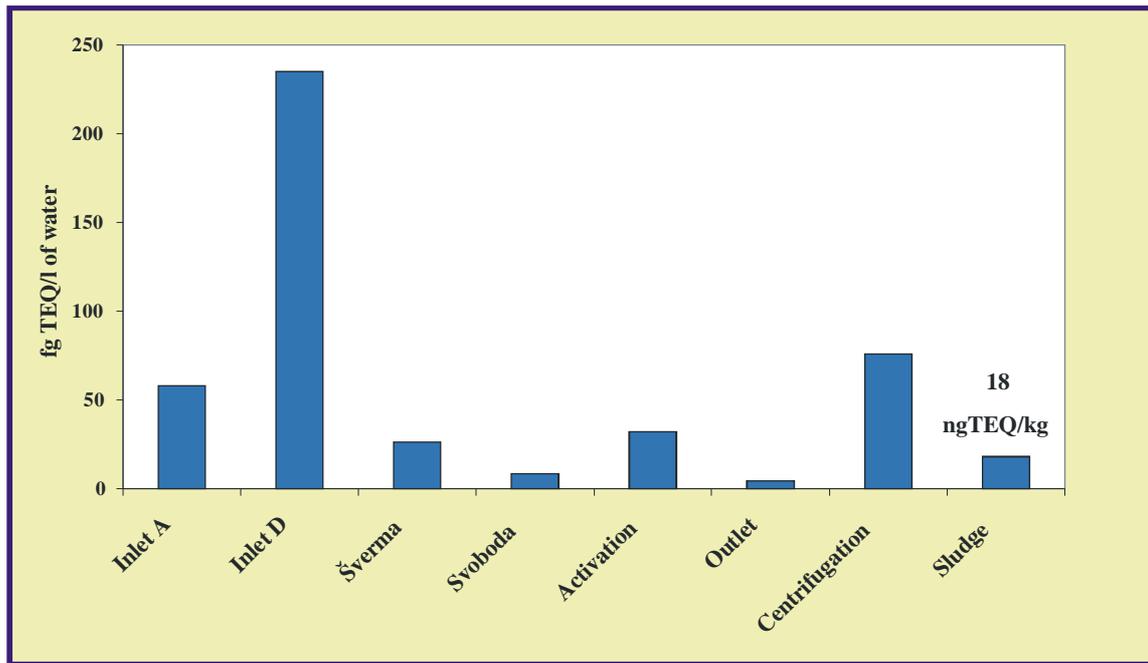
From comparison of measured PAHs (see Graph 24) in all sampling places the same compound profile of both sampling methods (grab and SPMDs). It is apparent (for the particular industrial contamination). Quantitative levels are different at 3 places which are probably caused by various reasons: (i) profile Inlet A: timely variable concentration of PAHs, as a result of an disadvantage of grab sampling, (ii) water from activation tanks and water from centrifugation process are strongly influenced by presence of colloids in water. PAHs results also show applicability of SPMDs for highly contaminated samples, like those from coking plants (Šverma or Svoboda).



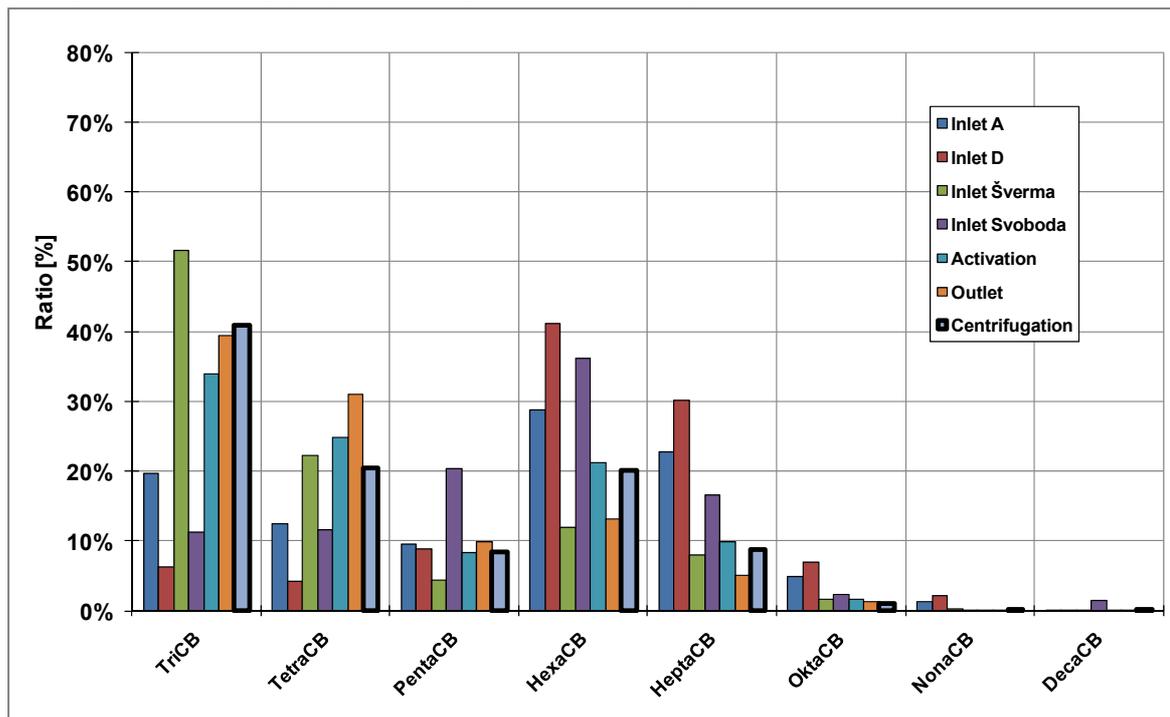
Graph 24 Comparison of grab and passive sampling for PAHs



Graph 25 Concentration profile of PCBs in particular streams and sludge



Graph 26 Contamination profile of PCDD/Fs in particular streams and sludge

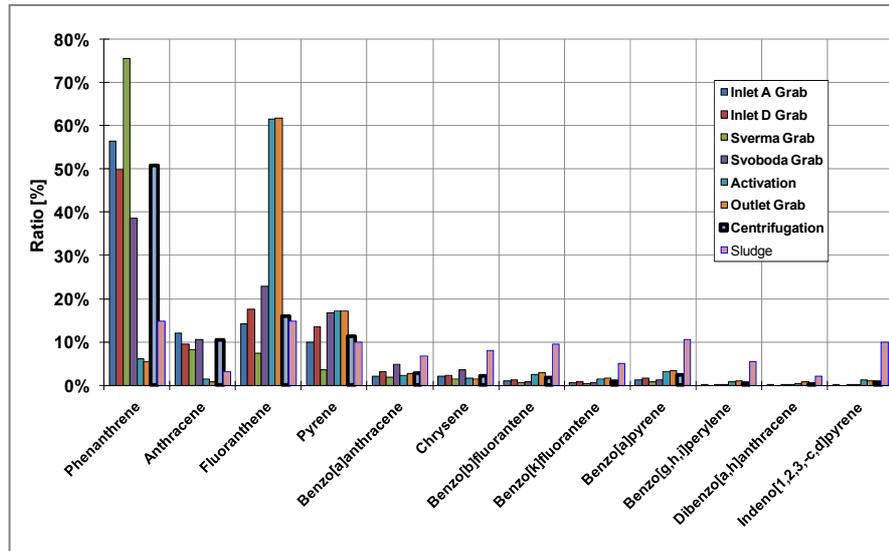


Graph 27 Profile of PCBs isomers; distribution of PCBs on solid (sludge) and liquid phase

Values measured by SPMDs exhibit high value of results with combination qualitative/quantitative profile monitoring, and toxicity testing as well. All this is the result of long-term continual, integral sampling, as shown in Graph 27 and Graph 28 to trace overall PCBs and PAHs profile.

There are dominant hexa-isomers in the graph of PCBs isomers, distinctive for technical mixture Delor 106 or analogous mixture. This profile is distinctive both for sludge and recipient D, that is

from the PCBs intake dominant. On the other hand, profiles for industrial waters (Svoboda, Sverma) are different; despite it is the same kind of source (coking plant).



Graph 28 Profile of PAHs; distribution to solid (sludge) and liquid phase

As for PCDD/F, the complexity of PCDD/Fs is apparent from chromatograms (see Figure 12), showing spectrum of accumulated compounds in SPMDs, in comparison to isotopic labelled compounds added to samples during sample analysis (GC-MS/MS analysis, tandem mass spectrometry, Thermo Finnigan). In these chromatograms, there are apparent various congeners, found in collected samples, worth attention for further quantification and analysis of their toxic relevance, especially for PCBs, due to given facts above.

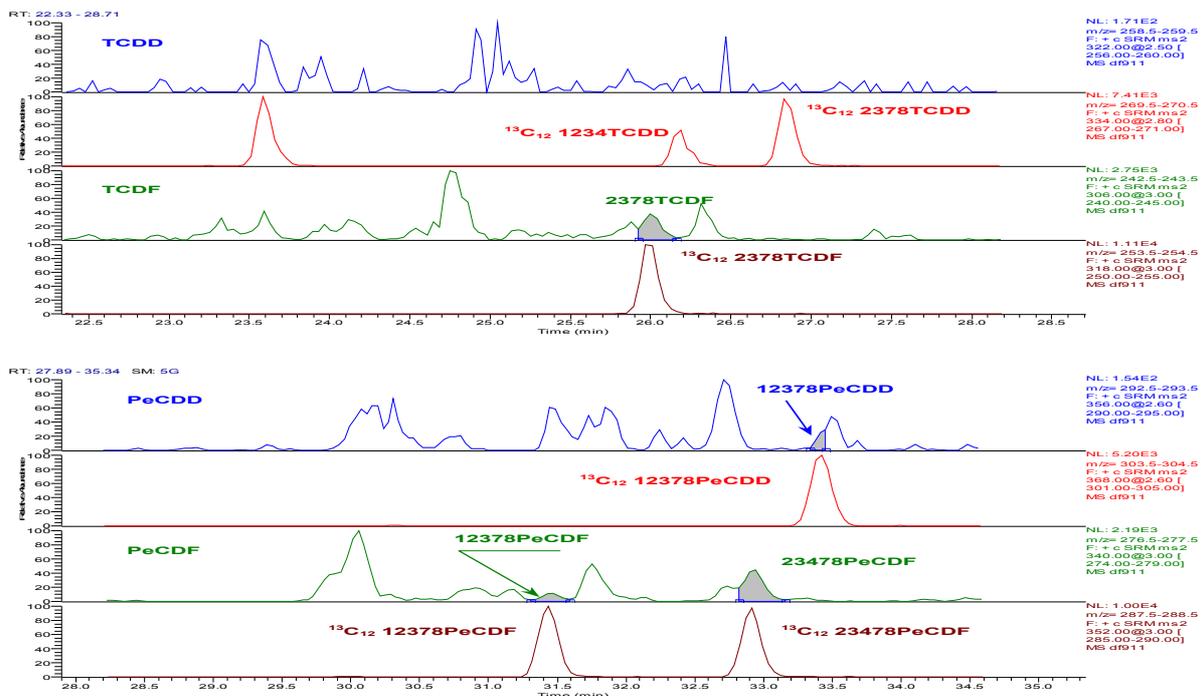
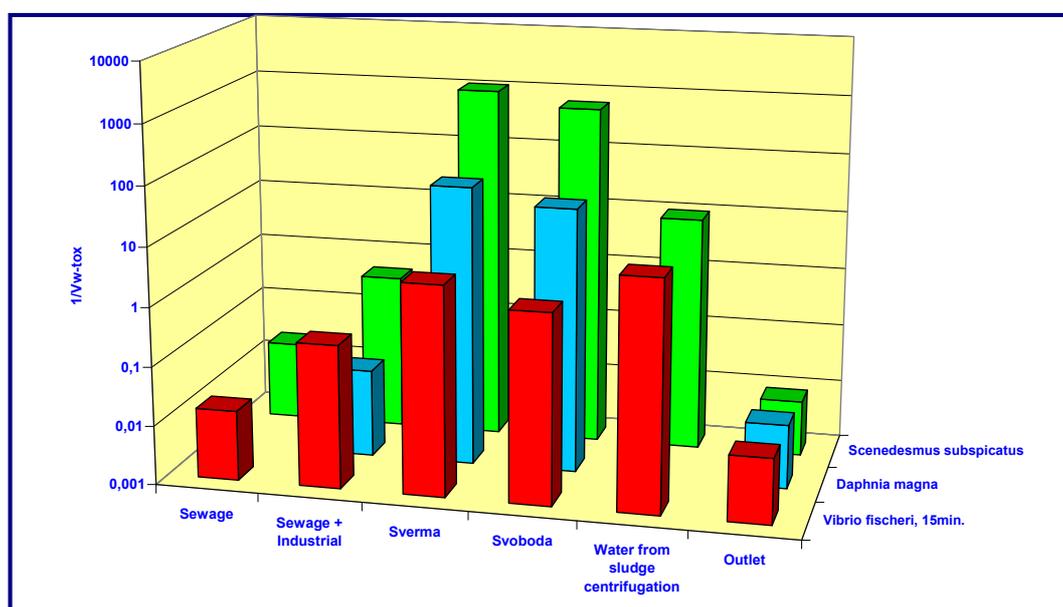


Figure 12 Chromatograms of selected PCDD/Fs (Inlet D)

Within framework of this study, both chemical and ecotoxicological relevance was studied. Graph 29 shows V_{tox} values from these inlets, expressing the ecotoxicological responses on various places. Very important response of V_{tox} is centrifuged water after second step of cleaning and V_{tox} of recipient Cerny Creek (Outlet) downstream of WWTP, causing direct consequent contamination of Odra River. Despite the high level on chemical contamination, Cerny Creek means the lowest value of leaving contamination of WWTP. This also corresponds with the lowest values of V_{tox} that demonstrate a decrease of toxic potential in inlets during the course of cleaning and a good level of toxicity in the recipient comparative to an overall “toxicity” of surface water. As for organism comparison, all from tested ones have given similar response that means selective response of those selected and considered as standard ones for waste assessment.



Graph 29 Toxicity evaluation of WWTP streams

The concentration exceeding the limit for sludge according to 86/278/EEC is apparent from the total PCBs and PAHs concentration. Concentration of PCDD/Fs in sludge fulfils the limit. Total concentration of chemical parameters well correlates to performed toxicity tests, shown in Graph 29. There are evident various sources of contamination (from relative congener profiles of PCBs, Graph 27), even where these originate from one kind of industrial plant. As for PAHs, there are various dominant compounds, distinctive for coking plants, playing major sources of contamination. So, e.g., Graph 28 shows dominance of phenanthrene, fluoranthene and pyrene. There is also the distribution shown of particular PAHs between liquid and solid phase. The most important fact is that all POPs, including PAHs contributes to solid phase. This fact underlines the necessity to accept limits for those POPs in sludge, especially due to its consequent use in agriculture.

Toxicological responses on all monitoring profiles are demonstrated in the Graph 29. From relative comparison, the less toxic is the output stream, flowing into Ostravice River. The highest

toxicity is exhibited in industrial sources, flowing from coking plants – Sverma and Svoboda. From the point of view of contamination of produced sludge and its contamination by organic contaminants, like PAHs, the highest priority must be focused on quality of industrial waters prior the cleaning on municipal waste water treatment plant. It provides the necessary judgment about further technologies to be involved for industrially contaminated streams.

Conclusions

Following conclusions can be derived from this pilot study:

1. The SPMDs seems to be a suitable tool for qualitative and quantitative monitoring in key profiles of sewage treatment plant.
2. Toxicity testing plays important role in overall quality assessment of both inlet/outlet water and produced sludge due to limited set of known chemical parameters to be accessed.
3. This complementary approach should be based on tests performed in advance (pilot study) for particular treatment technology and kind of treated waters.
4. The SPMDs technique exhibits features of high capacity tool for industrial streams to be accessed (e.g. high concentration PAHs, PCBs, etc...).
5. For direct estimation of POPs contamination of produced sludge, grab sampling is recommended with combination commonly available analytical techniques, due to unpredictable distribution of POPs between liquid and solid phase,
6. It results from PAHs, that they are apparent in all sampling profile of compounds, both from integral and grab taken samples. Despite good agreement of results from integral to grab sampling, there are differences in quantitative profiles, caused by different distribution of POPs to solid and liquid phase. For water and activation, the difference is probable caused by presence of colloids. For collector A, difference is probably given by variable concentration, resulting from grab sampling.
7. All measured parameters show, that major problem is focused to sludge, represented by solid phase at cleaning process. It underlines the necessity to accept special limits for assessed POPs, especially if used in agriculture.
8. With respect to exceeding the limits, it is expected adoption of control limits soon. Otherwise the usage of sludge would bring contamination to soil, and consequently to food chain. At the same time, monitoring of the technological process itself seems to be valuable due to serious contribution of contaminants from input streams. The intermediates seriously contribute to sludge quality as well.
9. For further applications, the use of PRC for on-site calibration will lead to improvement of produced results.

Assessment of POPs in Rivers in the Czech Republic

Presented results reflect parts of large base of complex monitoring data set obtained within the framework of biomonitoring study, realised since 1999, under the the guaranty of the Czech Hydrometeorological Institute and Ministry of Environment. Due to complexity of monitored compounds to be monitored and reaction to WFD implementation into Czech practice, decision was made to support since 2001 biomonitoring of organisms by passive sampling techniques. This decision has initiated the use of SPMDs (for non-polar compounds) and DGTs (for metals), latter of POCIS (for pharmaceuticals and pesticides), within a series of pilot studies, realised in 2001 – 2002, on selected profiles of Czech Republic, motivated by the first set of exposures in Czech Republic started in 1997., A series of tests for full validation and accreditation under EN ISO 17025 standard, for SPMDs, DGTs and POCIS was realised followed with the project “*Research and validation of new integral sampling methods of persistent organic compounds, heavy metals with toxicity tests for monitoring applications*”, under 203/04/1457, by Grant Agency CR. Since 2003, SPMDs, DGTs together with biotic organisms were, applied within the largest biomonitoring study in Europe (any may be in World) with involvement of passive sampling devices, that continues up present day, with extension to polar compounds (pharmaceutical, pesticides), monitored by POCIS.

Exposure of passive samplers and biotic organisms have been realized in parallel, generally since May to June (for passive samplers) and since May to July for biota⁶. At early stage, all results were primarily taken from total concentrations, based on passive samplers and published Rs, utilising the average measured temperature within exposure time. Since 2004, results were transferred into ambient concentrations using PRCs approach. For *Dreissena polymorpha*, results were taken on lipid base, whereas results from exposed membranes were used on the SPMDs base, especially for the case of comparison (see application “Comparison of SPMDs and biotic sampling systems” above).

List of sampling profiles is in the Table 9, their allocation in the map in Figure 13.

No.	River – Profile Name	Abbreviation	Profile Code
1	Odra – Bohumín	Odr-Boh (1163)	1163
2	Opava – Děhylov	Opa-Děh (1145)	1145
3	Morava – Lanžhot	Mor-Lan (0401)	0401
4	Dyje – Pohansko	Dyj-Poh (0402)	0402
5	Jihlava – Ivančice	Jih-Iva (1187)	1187
6	Svratka – Židlochovice	Svr-Žid (1180)	1180
7	Labe – Debrné	Lab-Deb (1002)	1002
8	Labe – Valy	Lab-Val (0101)	0101
9	Labe – Lysá	Lab-Lys (0102)	0102
10	Labe – Obříství	Lab-Obř (0103)	0103
11	Labe – Hřensko	Lab-Hře (0104)	0104
12	Vltava – Zelčín	Vlt-Zel (0105)	0105

⁶ In early phase, there were some exposures realised twice a year.. However, due to economic limitations, once a year exposure was definitely adopted, from May to July.

No.	River – Profile Name	Abbreviation	Profile Code
13	Ohře – Louny	Ohř-Lou (4006)	4006
14	Sázava – Nespeky	Sáz-Nes (3324)	3324
15	Berounka – Srbsko	Ber-Srb (1089)	1089
16	Lužnice – Bechyně	Luž-Bec (4004)	4004
17	Otava – Písek	Ota-Pís (4005)	4005
18	Bílina – Ústí	Bíl-Úst (1123)	1123
19	Lužická Nisa - Hrádek nad Nisou	Luž-Hrá (1130)	1130
20	Jizera – Předměřice	Jiz-Pře (4003)	4003
21	Bečva – Troubky	Beč-Tro (3670)	3670

Table 9 List of monitoring profiles (CHMI, biomonitoring)

It is noteworthy, that since 2003 the assessment of POPs in 15 Bohemia and Moravia Rivers by PS and biota was initiated with 19 profiles; since 2007, there were new 2 profiles added.

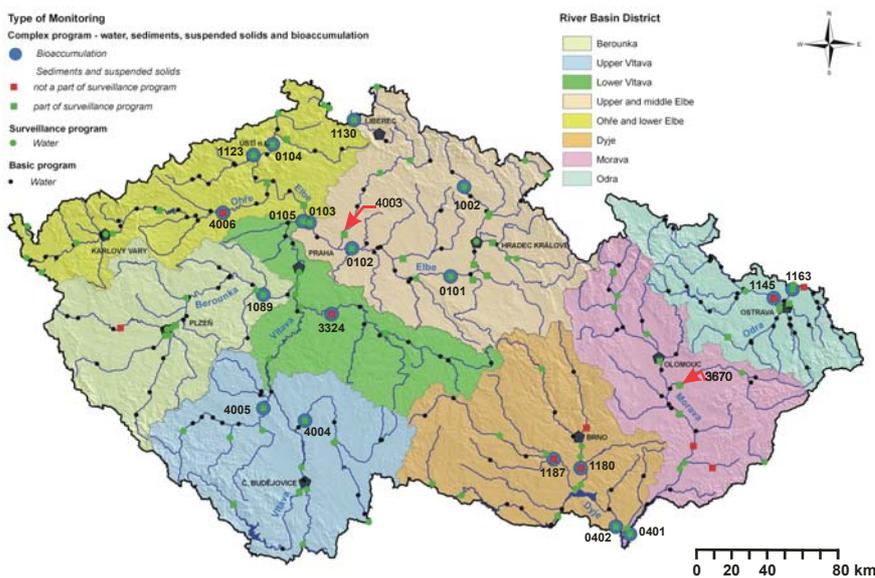


Figure 13 Map of sampling places
Two new profiles for year 2007 are marked by red arrow

Configuration for sampling was realised as depicted above. Protective shrouds were fitted recently to floats used in biomonitoring studies. Schematic arrangement is given on the Figure 14.

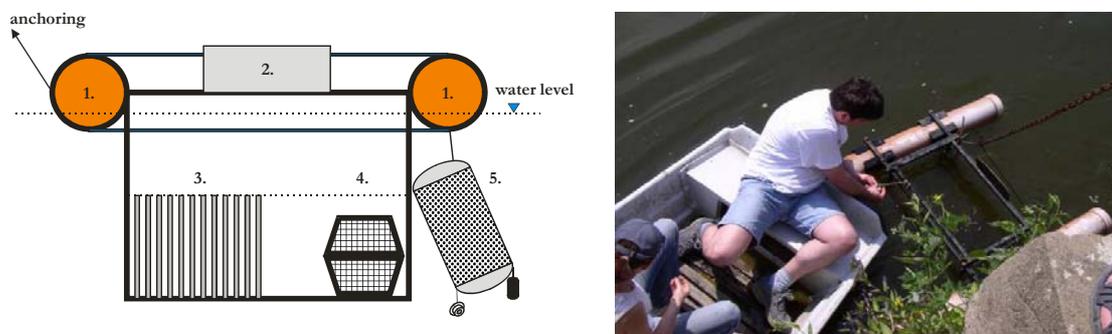


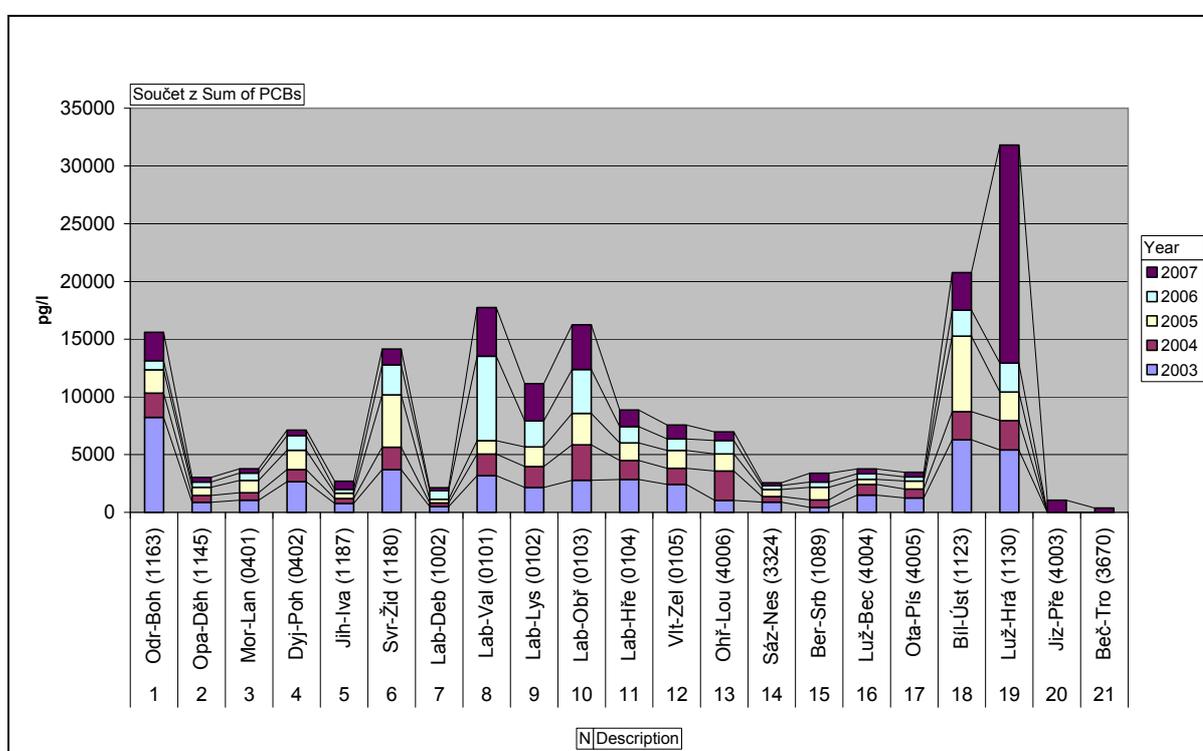
Figure 14 Schematic arrangement of sampling by biota and passive sampling techniques
Legend: 1. floats, 2. solid frame construction with sign, 3. plates for algae growth, 4. cage for Dreissena polymorpha, 5. protective shroud with SPMDs, POCIS, DGTs and thermo data-logger. Right-hand picture: one of many site installations (Sázava – Nespeky, 2006)

Overall results – descriptive and exploratory part

Profiles for each group of parameters are presented on Graph 30 to Graph 72. Due to non-normal distribution, outliers, descriptive data are mentioned as both average and median; the median is only robust parameter to outlying data. The period 2003-2007 is discussed.

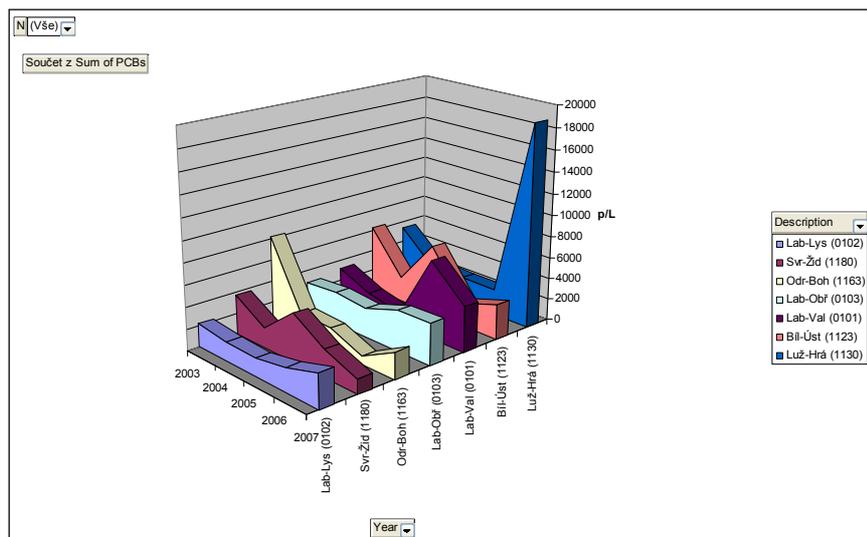
PCBs

For PCBs, the highest concentrations were found in Bílina River in Ústí n/L. ($C_{\text{average/median}} = 4155 / 3263 \text{ pg/l}$), Elbe-Valy ($C_{\text{average/median}} = 3549 / 3172 \text{ pg/l}$), Elbe-Obříství ($C_{\text{average/median}} = 3249 / 3064 \text{ pg/l}$), Lužická Nisa ($C_{\text{average/median}} = 6363 / 2540 \text{ pg/l}$), Odra-Bohumín ($C_{\text{average/median}} = 3121 / 2112 \text{ pg/l}$) and Svatka-Židlochovice ($C_{\text{average/median}} = 2828 / 2611 \text{ pg/l}$). The lowest contamination was found in Elbe-Debrné ($C_{\text{average/median}} = 426 / 330 \text{ pg/l}$), that can be considered as a background value for monitor, Jihlava-Ivančice ($C_{\text{average/median}} = 539 / 454 \text{ pg/l}$), Berounka-Srbsko ($C_{\text{average/median}} = 678 / 638 \text{ pg/l}$) and Opava-Děhylov ($C_{\text{average/median}} = 605 / 596 \text{ pg/l}$).



Graph 30 Czech Rivers: total concentration profiles of PCBs

The middle low concentrations were also found in Jizera-Předměřice ($C_{\text{average/median}} = 1048 \text{ pg/l}$) and Bečva-Troubky ($C_{\text{average/median}} = 379 \text{ pg/l}$), but further monitoring can reveal a temporal trend. Total trend of contamination of PCBs from 2003 to 2007 can be (in majority places) considered as alternating (Svatka-Židlochovice, Odra-Bohumín, Labe-Valy), and uprising (Labe-Lysá, Labe-Obříství). Remarkably uprising is in Lužická Nisa-Hrádek. Forthcoming concentrations, considered as a sum along all profiles, can be found as uprising till the year 2007, see Graph 30. If the trend is to be confirmed in 2008, acting management for detailed identification of contamination sources shall be realised.

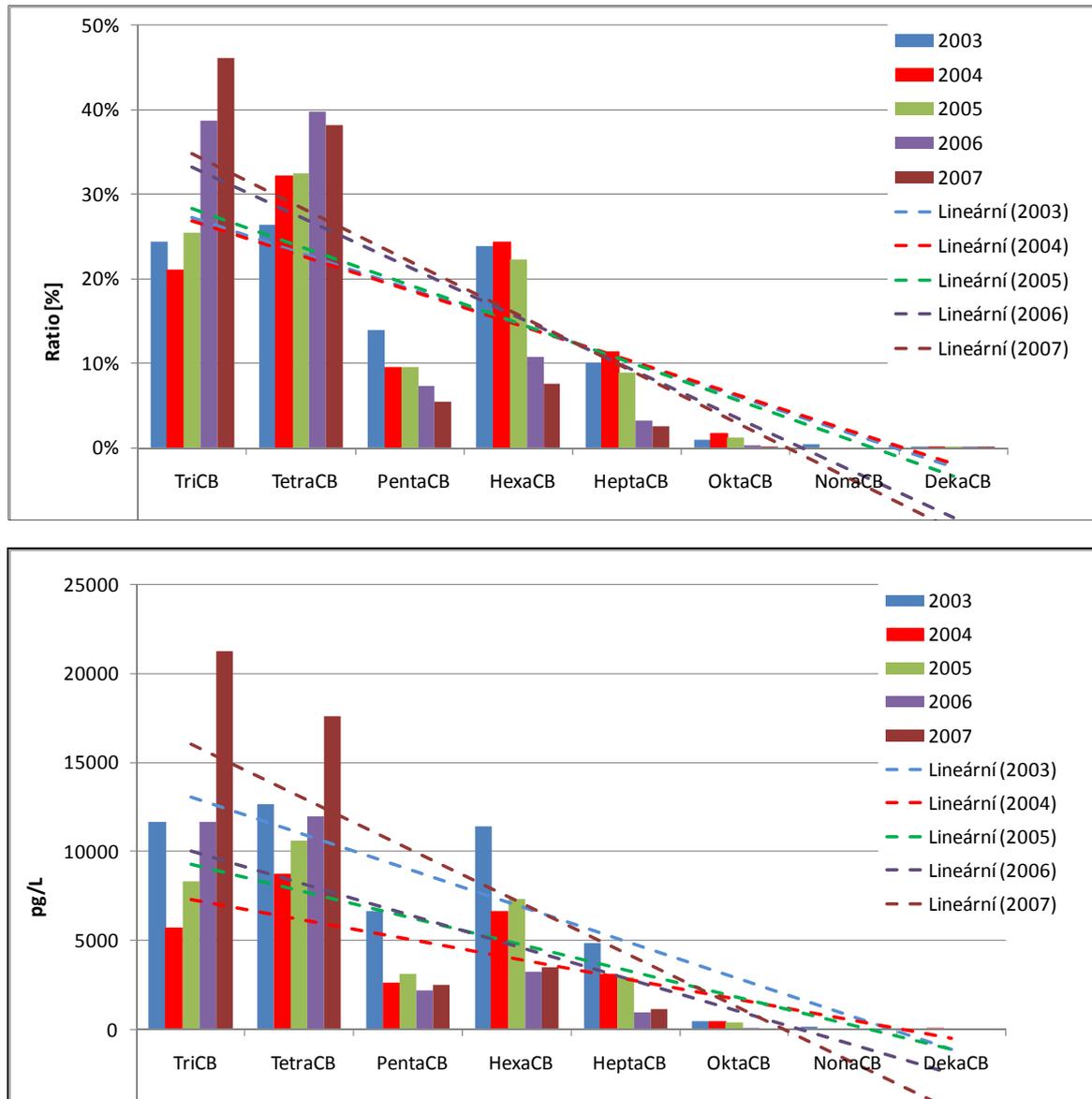


Graph 31 Trend of PCBs contamination in selected profiles (2003-2007)

As for probable sources, the elevated contamination by PCBs most probably comes from industrial sources, despite banned production of PCBs in 1980s in former Czechoslovakia. Since beginning of production, over 21kt were manufactured in Chemko Strážské and over 11kt was used inside Czechoslovakia^{77, 242-244}. Due to their thermal stability, resistance to chemicals, high permittivity, flame-retardant properties, etc., they were widely used as cooling fluids, dielectrics in transformers, plasticizers in paints and varnishes, hydraulic fluids and heat transfer agents. Technical mixtures, imported to Czech Republic were produced as Delor 103, dominating di-, tri-, and tetra-homologues (used as dielectric fluids for capacitors), whereas Delor 106 was dominantly represented by penta-, hexa-, and hepta- CB (used for paint factory). Due to this massive usage any elevating contaminations are not surprising.

With respect to Delor 106 and Delor 103 recently used in technical mixtures, there is an apparent changing of ratio of isomeric patterns a year. Roughly, based on summary concentration of PCBs isomers and its development from 2003 to 2007, there are Tri- and Tetra-CB isomers uprising (3-16%), whereas, Hexa-CB is decreasing proportionally (1-15%). Exceptional values of the 2003 year reflect the consequence of validation of methods and the application of an approach derived only from Rs, without PRCs. Making provision for this seems to be a breaking point in Penta-CB; Octa- to nona-CB can be considered as neutral in term of magnitude of measured values. As to spatial distribution of PCBs with respect to PCBs isomeric pattern, results are presented in Graph 32. The Tri- to Deca-CB are in the order from the left to the right. Moravian and Bohemian rivers are apparently different in the term of isomer patterns. In Odra-Bohumín, there is apparent decrease up to year 2005 with dominating Tri-Tetra isomers, proportional to Hexa- and Hepta-CB, increasing up to 2007. Total composition can be found in Graph 32 - 33.

Detailed isomer profiles of PCBs are in Graph 34 to Graph 52, for each monitoring profile.



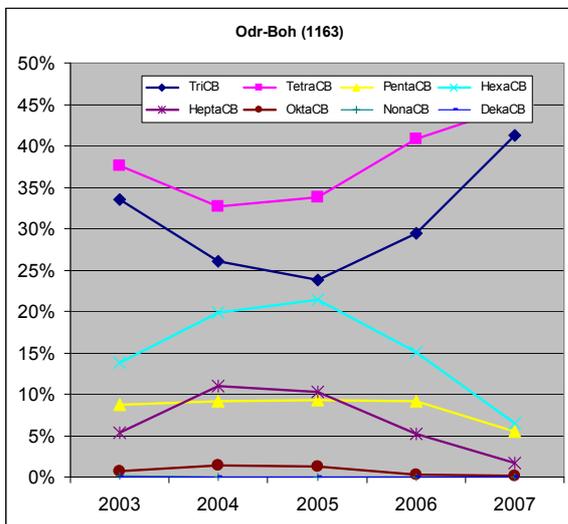
Graph 32- 33 Rough development of relative and absolute PCB isomers (from 2003 to 2007)
Tri-TetraCB – elevation, PentaCB - breaking point, Hexa-HeptaCB – decrease

Contamination of Elbe-Valy most probably originates from Pardubice-Semtín, with dominant content of Delor 103⁷⁷. However, composition of PCBs varies up to Elbe-Obříství, behind Spolana Neratovice. It means that Spolana Neratovice can also contribute to PCBs contamination by Delor 106, despite recently found dominant contamination by OCPs and PCDD/Fs^{16, 77, 295}. For potential source identification in Elbe River, there were homologue profiles used along the whole stream, presented in Graph 40 to Graph 45; there is the detailed profile along the whole river up to Vltava profile. From the contamination point of view, there is apparent diluting effect by Vltava.

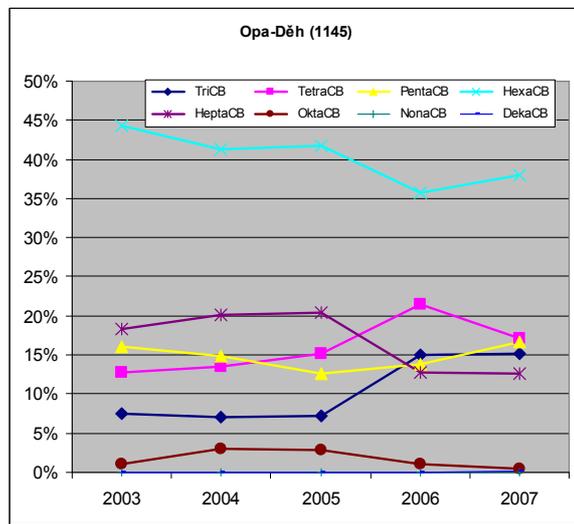
The contamination of Bílina-Ústí n/L. most probably originates from Spolchemie chemical plant, where the contamination of PCDD/Fs and HCBs was also found. Another source is WWTP. There is some speculative contamination arising from hazardous waste incineration plant. However, this has not been convincingly documented so far; conclusions are in good agreement to recent

studies¹⁶.

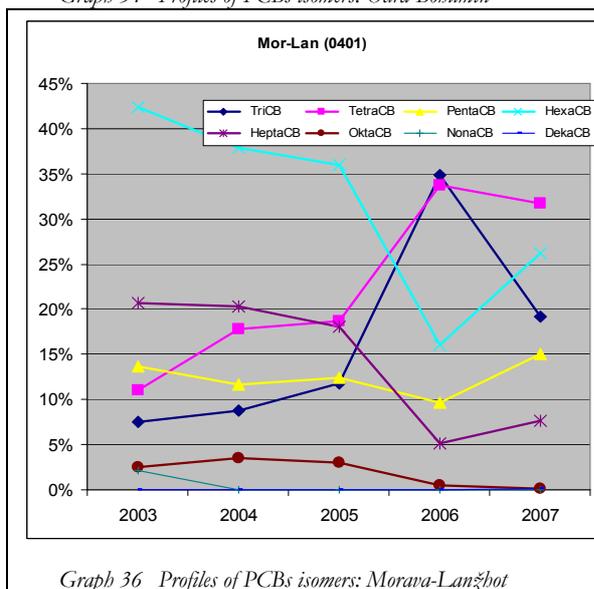
Contamination of PCBs found in Odra-Bohumín most probably originates from various sources of Ostrava Region^{133, 296, 297}, recently industrially contaminated area, with various effluents from: waste water treatment plant, effluents from coke-plant (see application Special application for POPs assessment: WWTP) and steel-work industries, areas with capacitors fluids, etc.... From Graph 31, there is an apparent increase of low-chlorinated biphenyls, namely Tri-CB and Tetra-CB; Penta-CB does not change, Hexa-CB and Hepta-CB are decreasing. In can be attributed increased amount of total PCB, mainly in 2007 with low chlorinated PCB metallurgical industry, using residual amounts of different components hydraulic fluids ^{77, 248, 249, 298}, with dominant Tri- to Tetra-CB. Effluent can mostly be observed from WWTP, partly cleaning industrial sources, and local industrial treatment plants to be monitored in near future, in focus recent findings, mainly realized in Poland^{133, 296, 299, 300}.



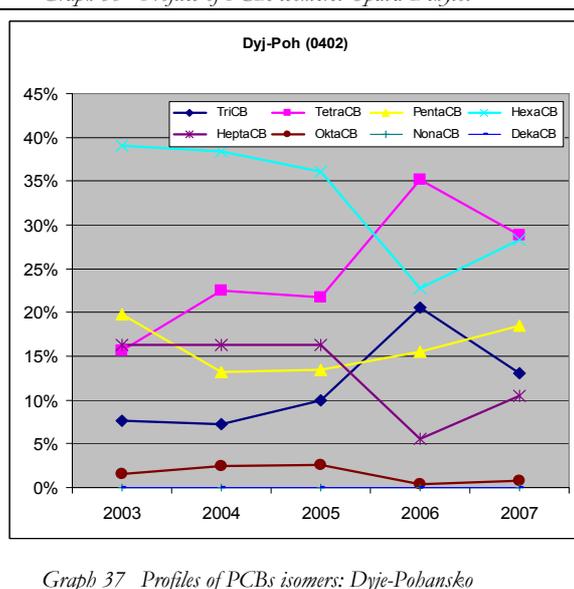
Graph 34 Profiles of PCBs isomers: Odra-Bohumín



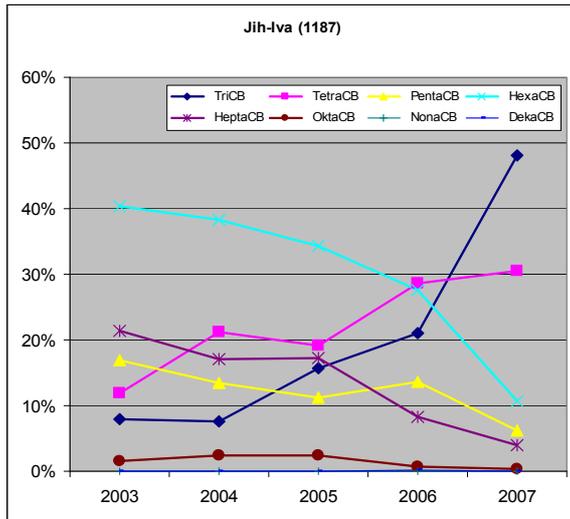
Graph 35 Profiles of PCBs isomers: Opava-Děhýlov



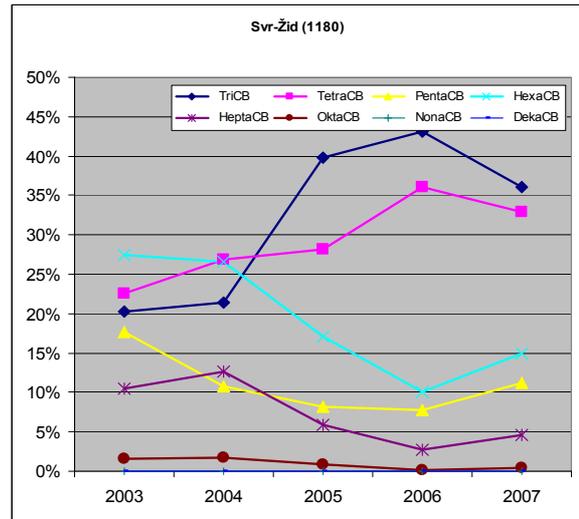
Graph 36 Profiles of PCBs isomers: Morava-Lanžhot



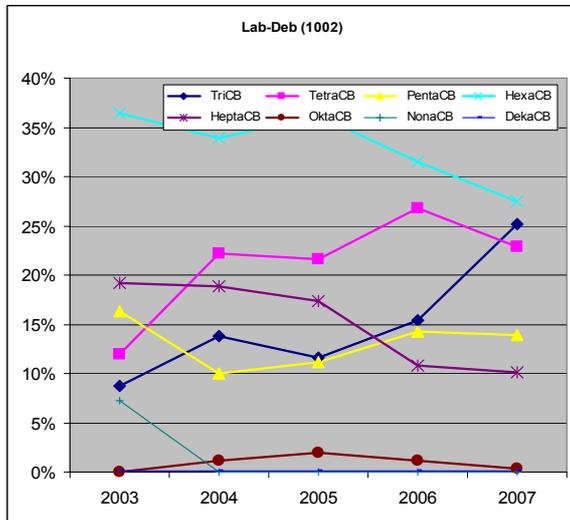
Graph 37 Profiles of PCBs isomers: Dyje-Pohansko



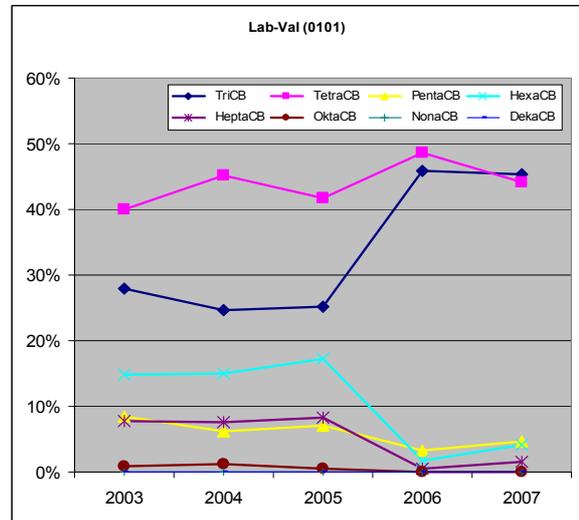
Graph 38 Profiles of PCBs isomers: Jihlava-Ivančice



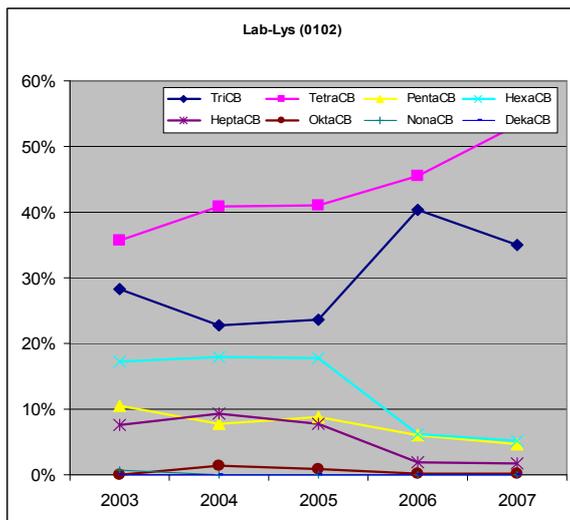
Graph 39 Profiles of PCBs isomers: Svatka-Židlochovice



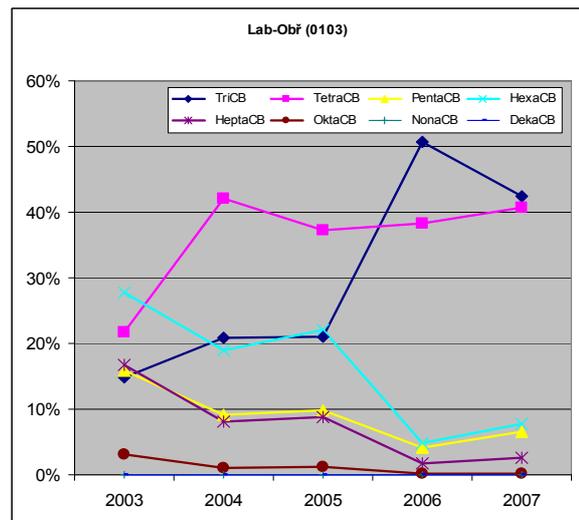
Graph 40 Profiles of PCBs isomers: Labe-Debrné



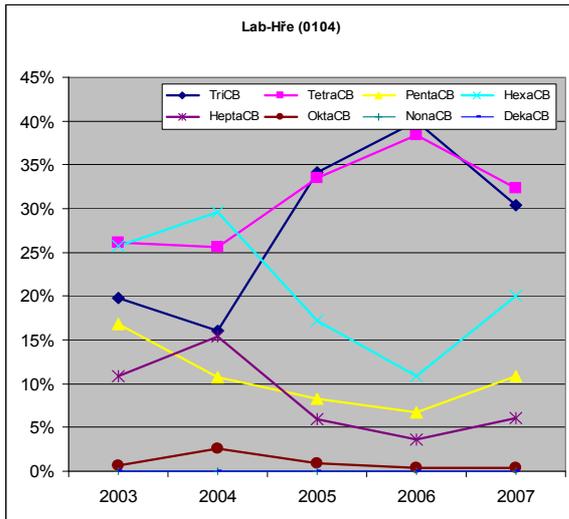
Graph 41 Profiles of PCBs isomers: Labe-Valy



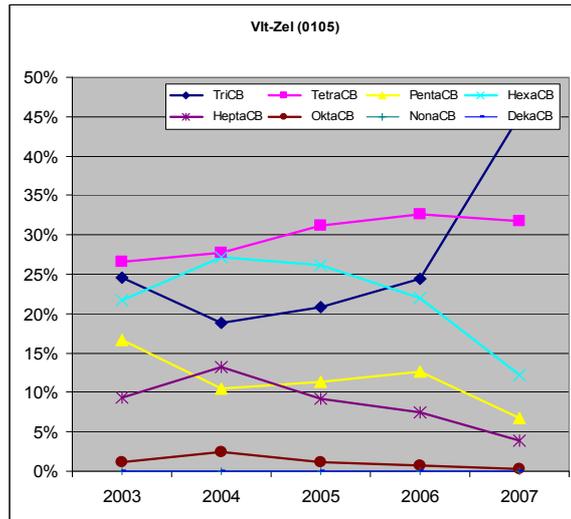
Graph 42 Profiles of PCBs isomers: Labe-Lysá



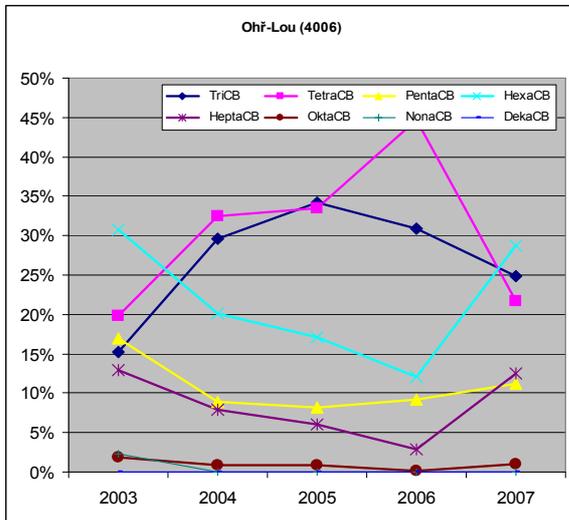
Graph 43 Profiles of PCBs isomers: Labe-Obřezství



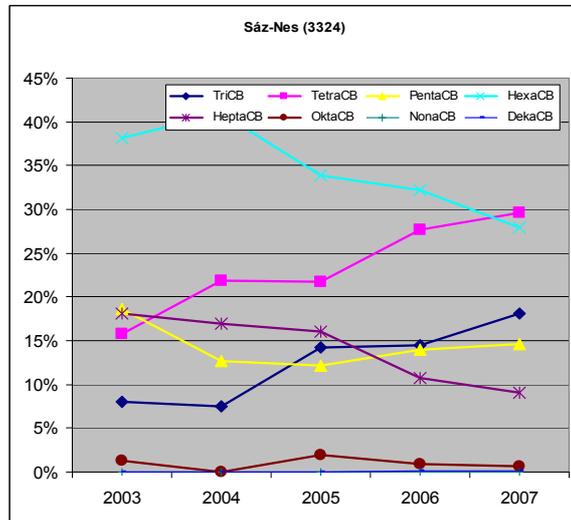
Graph 44 Profiles of PCBs isomers: Labe-Hřensko



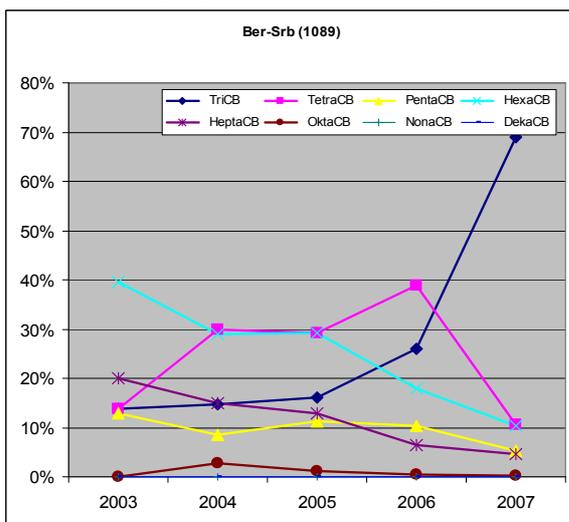
Graph 45 Profiles of PCBs isomers: Vltava-Zelčín



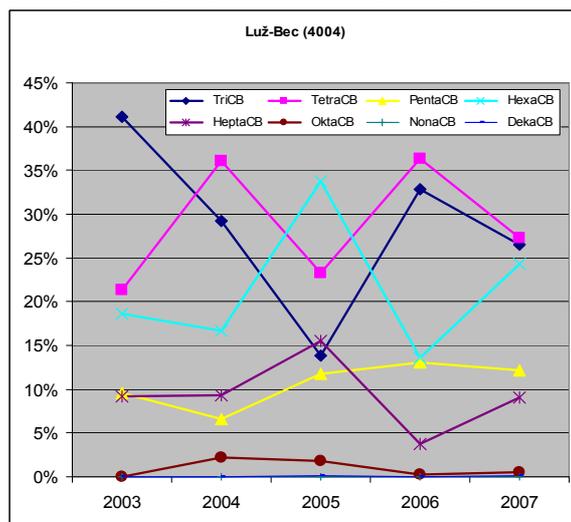
Graph 46 Profiles of PCBs isomers: Ohř-Louny



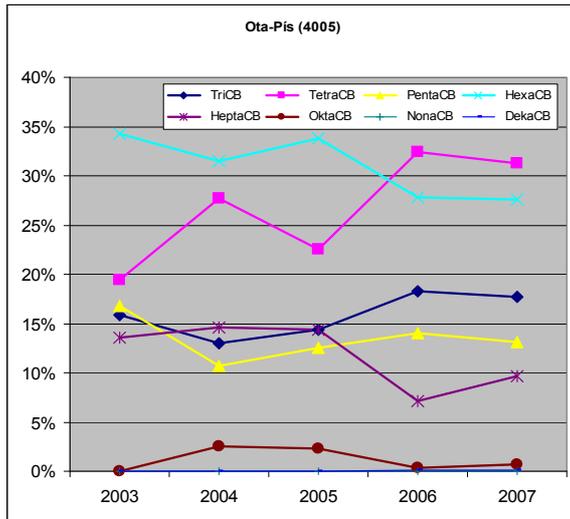
Graph 47 Profiles of PCBs isomers: Sázava-Nespeky



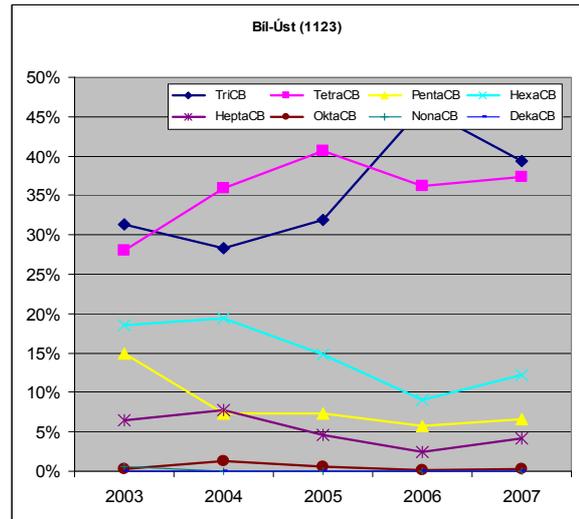
Graph 48 Profiles of PCBs isomers: Berounka-Srbsko



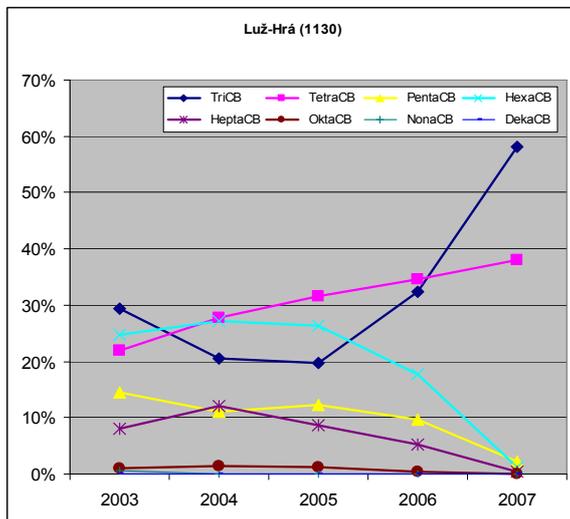
Graph 49 Profiles of PCBs isomers: Lužnice-Bechyně



Graph 50 Profiles of PCBs isomers: Otava-Písek



Graph 51 Profiles of PCBs isomers: Bílina-Ústí nad Labem



Graph 52 Profiles of PCBs isomers: Lužice-Hrádek n. Nisou

Graphs 30-48 PCBs isomer profiles on individual sampling sites

Legend:

Axis: Y – relative ratio, Y – year

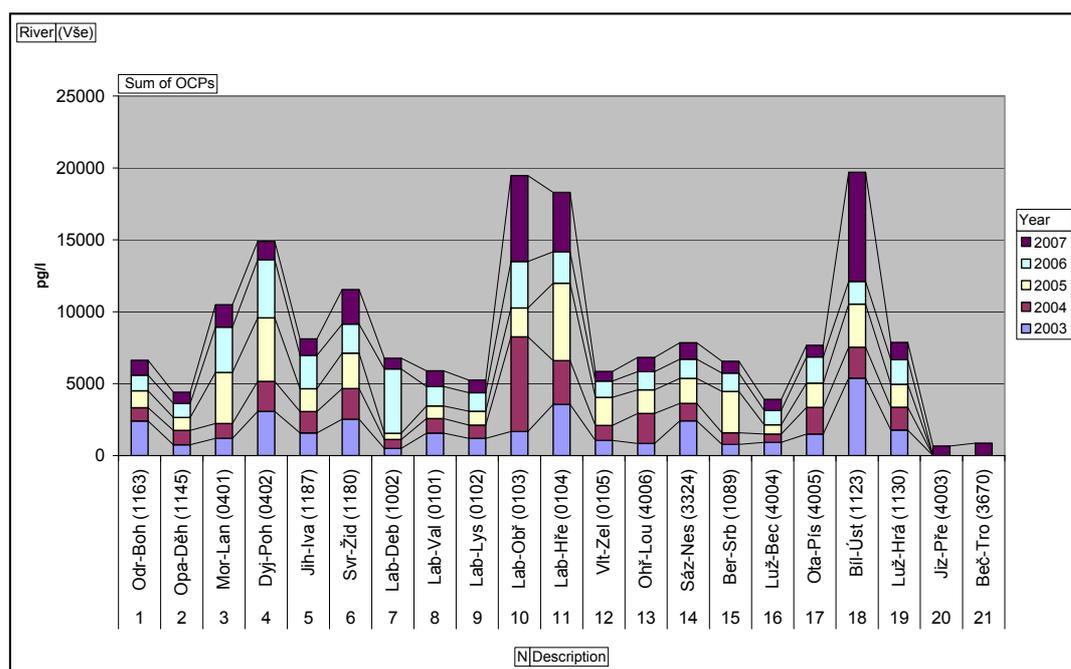
Profiles: excluded profiles, exhibiting no trends and involved into monitoring system in 2007 (Jiz-Pře (4003) and Beč-Tro (3670))

OCPs

Overall contamination by OCPs in all rivers with temporal trends is demonstrated by Graph 53. Major contamination⁷ is identified in following profiles: Bílina-Ústí ($C_{\text{average/median}} = 3939 / 2991$ pg/l), Elbe-Obríství ($C_{\text{average/median}} = 3892 / 3231$ pg/l), Elbe-Hřensko ($C_{\text{average/median}} = 3659 / 3557$ pg/l). On the contrary, the lowest concentration (about three times) was found in Jizera-Předměřice ($C_{\text{average/median}} = 663$ pg/l), Lužnice-Bechyně ($C_{\text{average/median}} = 781 / 770$ pg/l), Bečva-Troubky ($C_{\text{average/median}} = 859$ pg/l) and Opava-Děhylov ($C_{\text{average/median}} = 880 / 908$ pg/l). Based on total sum of OCPs, the contamination exhibits slightly decreasing trend (omitting episodic exceptions in year 2005). From the profile graph, the most apparent of OCPs, is caused in majority by DDTs and

⁷ Data from years 2003-2007

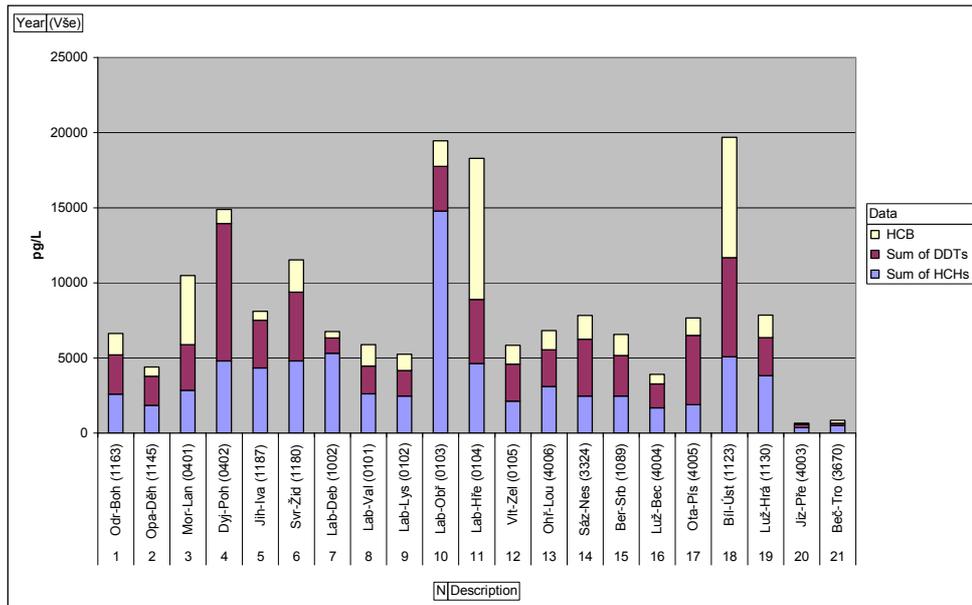
HCHs, from previous use⁷⁷, similarly like for the case of PCBs - see cumulative Graph 54, below.



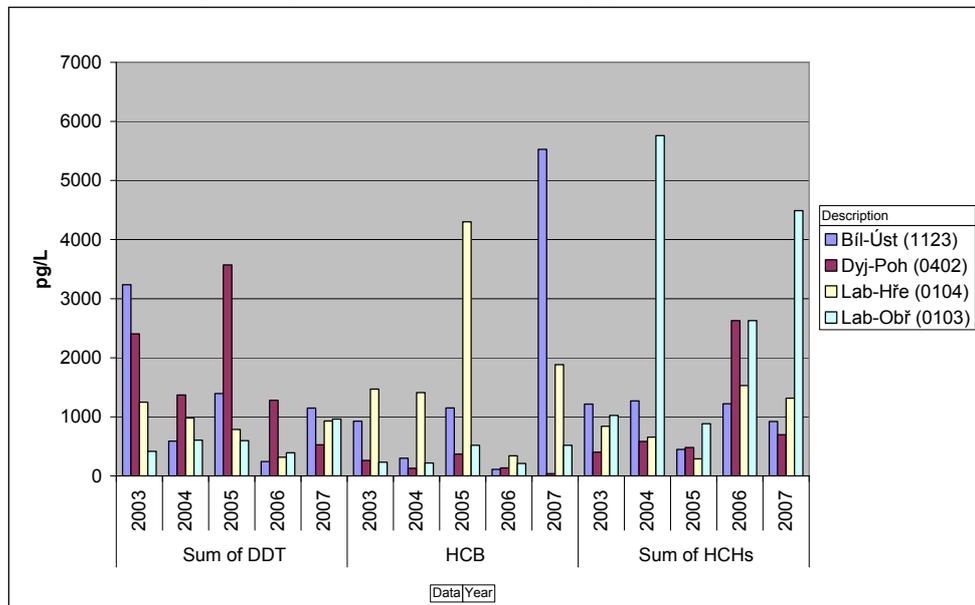
Graph 53 Czech Rivers: concentration profile of OCPs

As to the total concentration OCPs, the Bohemian rivers seem to be more contaminated than the Moravian ones, with major sources in Elbe and Břilina rivers, caused by recent production of selected OCPs. Temporal trend of the most contaminated profiles are in Graph 55. Variations among years are apparent from the profiles as well as sampling places, making difficulties in formulation of serious conclusions about exact causalities. As results from published data²⁵¹, there is a relative good correspondence with burdens of soil and sediments, found in the order of hundreds ng/g^{9, 12, 14}, or at biotic organisms^{77, 248}, despite SPMDs exhibits only dissolved phase, without metabolic mass exchange. Some exceptions have been shown in contamination at the level of thousands ng/g. The relative distribution of individual OCPs reflected historical profile. The total OCPs trend levels in the river sediments and water decreases at the sites with continuous sources several years after floods. If alternation of OCPs contamination appears, it requires further attention to their sources, tributaries and sub-tributaries, mainly for following rivers: Elbe, Břilina and Dyje.

As for DDTs and its metabolites, the use of DDT increased enormously worldwide after World War II, primarily because of its effectiveness against the mosquito that spreads malaria and lice that carry typhus. In Czech Republic, problems with contamination by extensive use of DDT began to appear in the late 1940s. There were two main production plants: Spolana (Neratovice) and Spolchemie (Ústí nad Labem)⁷⁷.



Graph 54 Czech Rivers: cumulative concentration profiles HCB, DDTs and HCHs



Graph 55 Czech Rivers: temporal trends of DDTs, HCB and HCHs in selected profiles

Since 1947 it is known that many species of insects have developed resistance to DDT^{301, 302}; despite that DDTs has been broadly used. DDT is not readily a rapidly metabolized by organisms; apart from it is stored in the fatty tissues, that is consequence of log K_{ow}, having strong accumulation potential in SPMDs as well^{301, 302}. The biological half-life of DDT is about eight years; that is, it takes about eight years for an animal to metabolize half of the amount it assimilates⁸⁶. After 1974, the use of DDT was no longer permitted in Czech Republic; definite prohibition of its use was after 1984, however not respected by Russian Army. From recent data^{1, 77}, over 7 kt of DDTs was used, dominantly in agriculture, not in industry. Due to long-term use, no surprise was that residuals were found in some environmental compartments, mainly in sediments, due to strong accumulation

usual from producing plants, in various mixtures. According to published inventory data, there is a good correspondence to contamination found in sediments^{81, 251, 303}. However, higher contamination in profile Debrné than in Valy has not been reported, considering Synthesia-Pardubice not as a remarkable contamination source. From total HCH profiles, most contaminated Moravian rivers are Dyje, Jihlava, Svratka, whereas Bohemian, dominating in total OCPs contamination, is Elbe and Bílina.

The HCB was produced in Spolana-Neratovice and used as wood preservatives, as a fungicide for treating seeds and as intermediate in organic syntheses. Technical grade HCB contains up to 2 % impurities (1.8 % pentachlorobenzene and 0.2 % 1,2,4,5-tetrachlorobenzene), including higher chlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls⁷⁷. Additionally, HCB may be formed as an unwanted by-product in the syntheses of other chemicals, from high-temperature sources and from chlor-alkali³⁰⁴⁻³⁰⁸. Its occurrence is apparent From the Graph 54 in all profiles, as well as in background profile Debrné. Slightly uprising contamination was found behind Spolana in Obráství profile, whereas the highest contamination exhibits the Hřensko profile, that was also identified recently in respect to recent flooding episode in 2002^{309, 310}. Apparently, there is no contribution found from the Vltava-Zelčín profile, the uprising contamination arises from contamination behind this profile. A detailed identification was not realised. Comparing the published data referred to flooding episode from various compartments³⁰⁹⁻³¹¹ and based on this work, we can confirm Synthesia a one source of HCB, however not the only one. This controversy can be explained by (i) methodological errors in analysis (an insufficient separation from PCB, apparently found in Synthesias lagunes and forming serious contamination by PCBs, see above), and/or by (ii) strong boundary HCBs only in sediment, deeper layers, with only limited release into phase sequestered by SPMDs, and/or, finally by (iii) definite eradication of HCB by flooding episode in 2002. The controversial results of HCBs also exist in inventory^{248, 249} apart to inventory⁷⁷, where concentrations found in sediment behind Synthesia (Elbe-Valy) were at the same orders of magnitude as in other profiles. Anyway, detailed look inside seems to be important for further observations. On the other hand, occurrence in Elbe and Bílina well corresponds to data from the inventories^{77, 248, 249}, found in sediments in tens of ng/g.

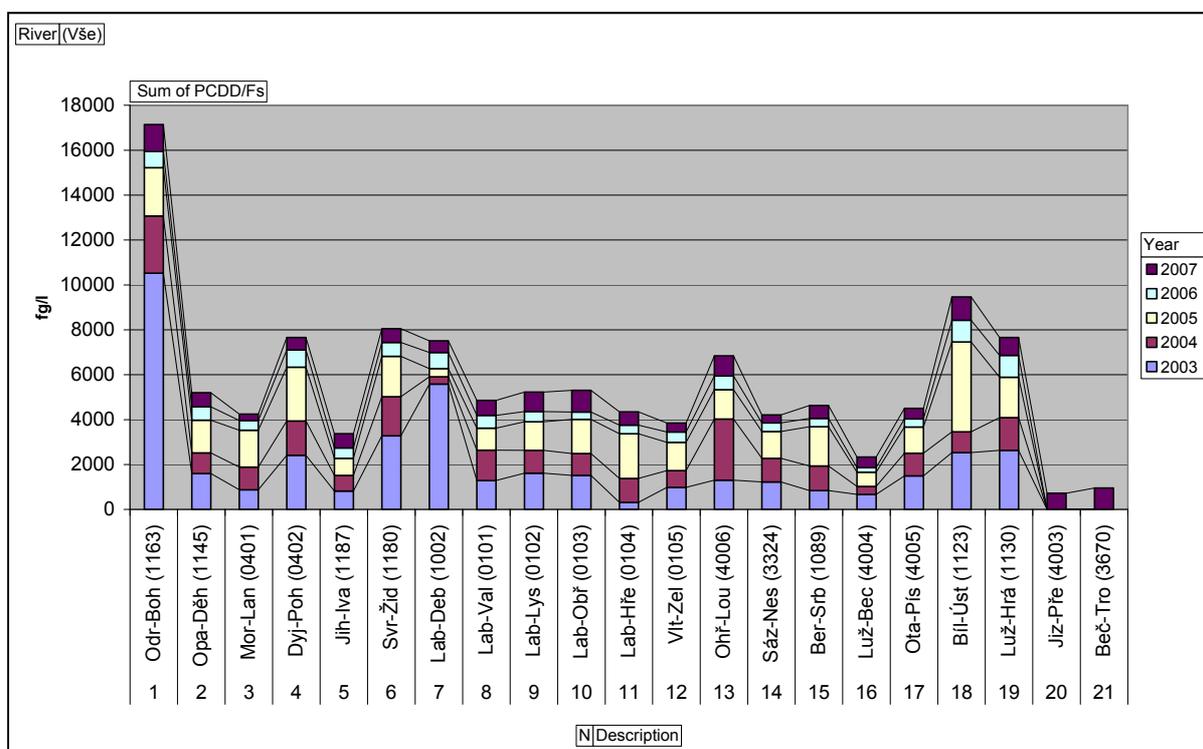
Contamination of HCBs yields the lowest contribution (6-24%, average 21%) to total OCPs, with exceptions of Elbe-Hřensko (51%), Bílina-Ústí n. Labem (41%) and Morava-Lanžhot (44%). The HCHs and DDTs contribute nearly with the same composition (with 15-62%, average 42% and (25-79%, average 36%), respectively. Detailed overview of composition is depicted in the Graph 56.

As a main contamination sources of OCPs in various compositions for Elbe and Bílina rivers, there were Spolana Neratovice identified and Spolchemie Ústí nad Labem due to historical production. The Synthesia Pardubice, undoubtedly is an apparent source of POPs, as documented by inventory^{77, 248, 249} in various lagoons. But effluents of POPs did not show a serious intake of dissolved phase into Elbe River, as results from Elbe-Valy profile. Other profiles were considered as

affected by historical use of OCPs. Detailed identification of OCPs sources would require further concern of all tributaries, by means of methodology described above, mainly because of controversial results.

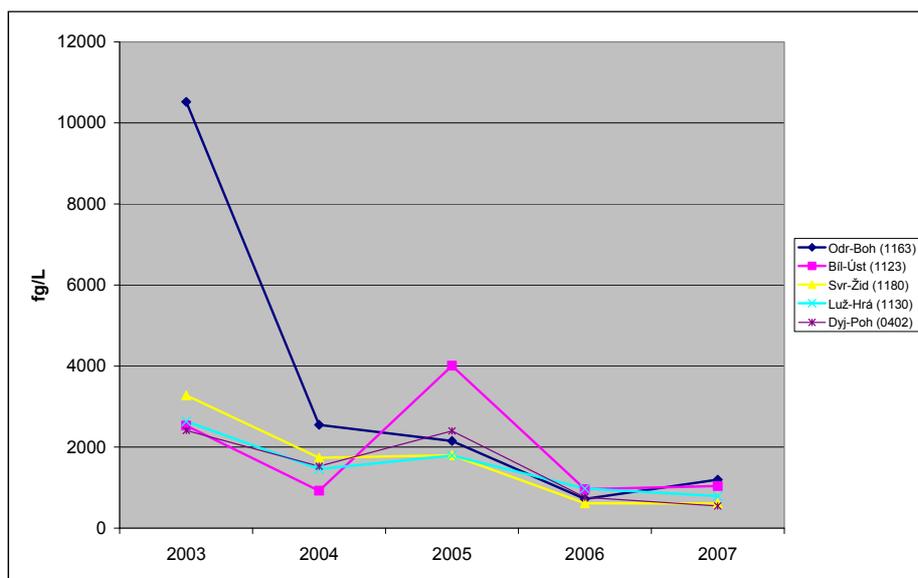
PCDD/Fs

Overall contamination by PCDD/Fs is summarized in the Graph 57. Among all sampling profiles, the most remarkable profiles are Odra-Bohumín ($C_{\text{average/median}} = 3428 / 2152 \text{ fg/l}$), Bílina-Ústí n. Labem ($C_{\text{average/median}} = 1892 / 1040 \text{ fg/l}$), Svratka-Židlochovice ($C_{\text{average/median}} = 1610 / 1739 \text{ fg/l}$) and Lužická Nisa-Hrádek ($C_{\text{average/median}} = 1531 / 1457 \text{ fg/l}$). In Odra, the found contamination has relevance to various industrial sources, like steelwork companies and WWTP effluents as well as landfills of Chemical plant Ostrava-Hrušov²⁴⁹. The other profiles seem to have other industrial nature, as derived from isomeric profiles. No surprise is the contamination in Bílina-Ústí (as corresponding to OCPs contamination, see above), or Lužická Nisa, behind effluent of Municipal Incineration Plant Liberec.



Graph 57 Czech Rivers: concentration profile of PCDD/Fs

There is a lack of data for a comparison, because no other systematic monitoring campaign was realised recently focused on PCDD/F content. On the other hand, as seen from the list of measured isomers by SPMDs, majority was below the LOD (number of observations with LOD within the range 84-100%), meaning that contamination of PCDD/Fs falls into the range of low environmental contamination, even after accumulation by SPMDs (only sequestering the dissolved phase).

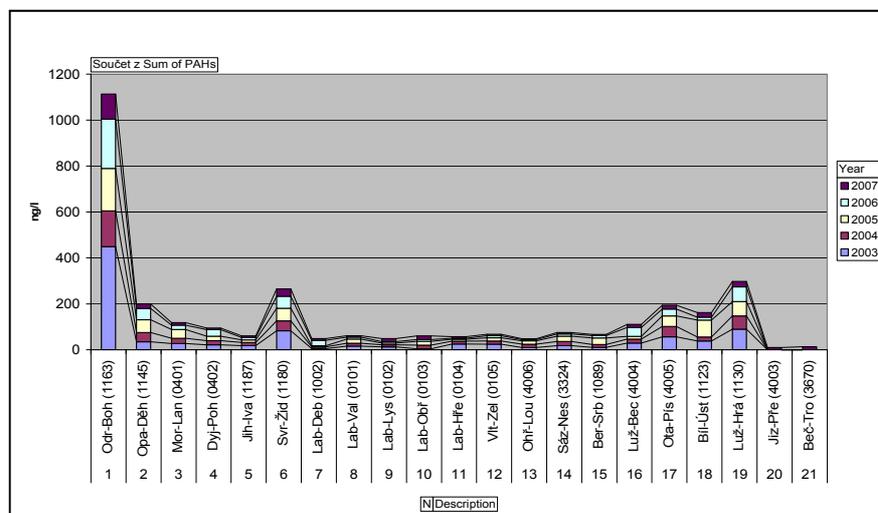


Graph 58 Czech Rivers: temporal trend of sum PCDD/Fs in selected profiles

There are apparent exclusions for isomers measured above LODs, mainly TCDD, TCDF, PeCDF (number of observations with LOD within the range of 0-16%). From the temporal trends point view, as evaluated from the most contaminated profiles, the overall trend is decreasing (see Graph 58).

PAHs

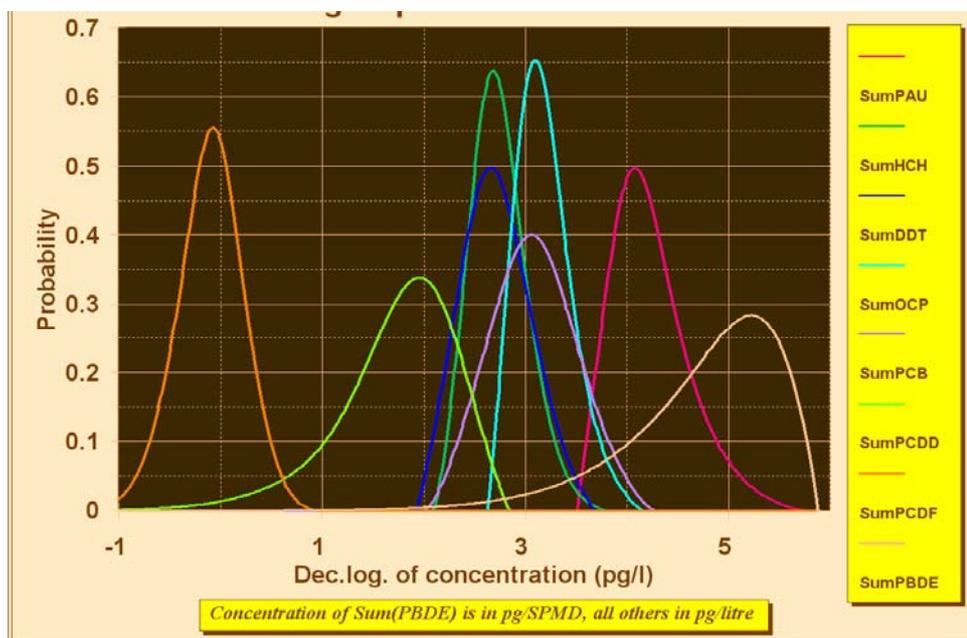
It results from the Graph 59, that there is an apparent dominant contamination by PAHs in Odra-Bohumín profile ($C_{\text{average/median}} = 223 / 185 \text{ ng/l}$). This profile reflects the contamination from present and historical industrial sources, mainly coke plant, not appeared elsewhere in Czech Republic. The other slightly dominating profiles are Lužická Nisa ($C_{\text{average/median}} = 59 / 63 \text{ ng/l}$) and Svatka – Židlochovice ($C_{\text{average/median}} = 53 \text{ ng/l}$). On the contrary, the cleanest areas were found in following rivers: Jizera, Bečva, Ohře, Elbe, Jihlava, Berounka, and Sázava, that exhibit $C_{\text{average/median}}$ from 10 to 15 ÷ ng/l.



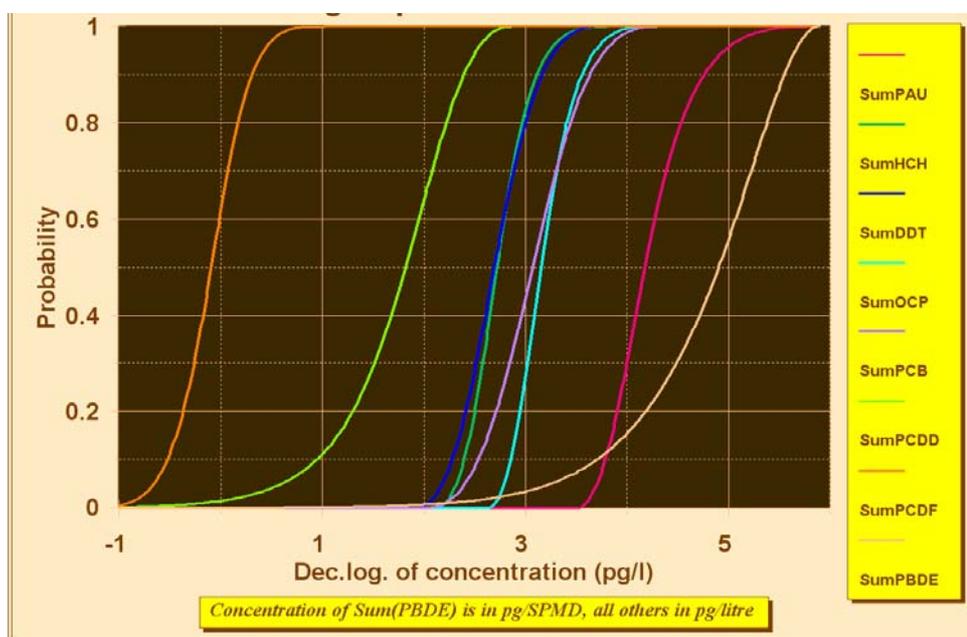
Graph 59 Czech Rivers: concentration profile of PAHs

Statistical and gnostic analysis

The total concentration levels of groups of parameters are presented in Graph 60 and Graph 61 by probability distribution and density functions.



Graph 60 DFs of selected POPs

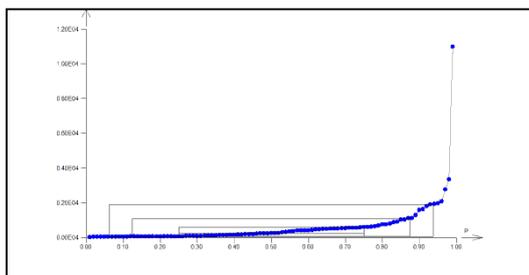


Graph 61 CFs of selected POPs

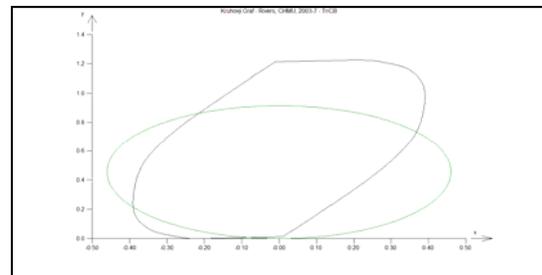
Ranges and values of the concentrations are apparent from these graphs, represented by DF originated in both pilot and monitoring data sets (years 2001-2007). Concentrations of group of pollutants differ by order of magnitude. Distributions differ not only by mean levels, but also by the variabilities, reflected in the shape of DF. Apparently, distributions are not normal (Gaussian), domains are finite, densities are asymmetric, mainly for PBDEs, PAHs, PCBs. Special attention is to

be put to PBDEs. Due to high variability of data, robust (gnostic) approach was used in data evaluation. The shape of PBDE's DF can be roughly found as similar to PCDDs, reflecting their similar bioaccumulation property with respect to SPMDs, as well as similarity of molecular structures.

Statistical EDA has revealed not-normal distribution, densities asymmetric, parameters like skewness and kurtosis has revealed necessity data transformation. The Box-Cox transformation was performed on non-normal data, prior multivariate data analysis. Then, PCA was performed, to explain the variance–covariance structure of a complex multivariate data set through a few linear combinations of the original variables (in this case, isomers PCB, selected congeners PCDD/F, Σ PAHs, Σ HCHs, Σ DDT's (consisting of DDT's, + metabolites DDDs and DDEs), selected PBDEs, and toxicological parameters). Principal component analysis was performed as a pattern recognition method to compare the bioconcentration of mentioned parameters in SPMDs.

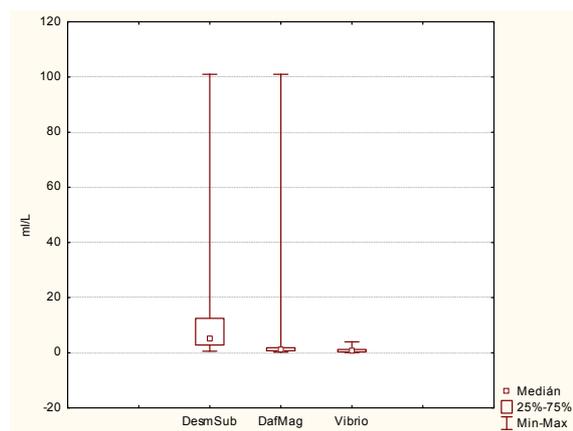


Graph 62 *Quantile-box plot*
It indicates some outliers at high values and asymmetric, skewed distribution

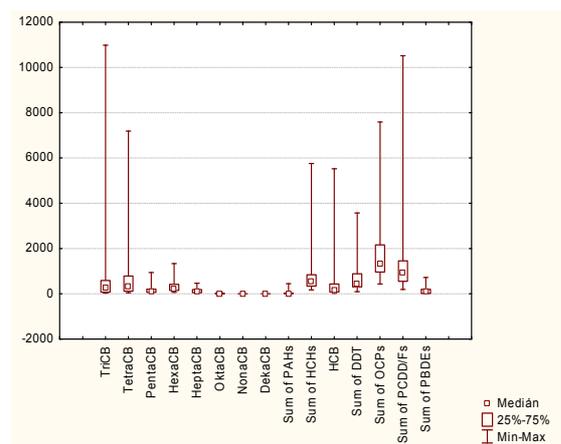


Graph 63 *Circle plot*
It indicates the difference from normal distribution

Selection of parameters for multivariate analysis was based on the grouping of parameters and variation, as is apparent from box and whiskers plots for both chemical and bioassays. Those, having negligible variance were omitted (e.g. some PCDD/Fs congeners).



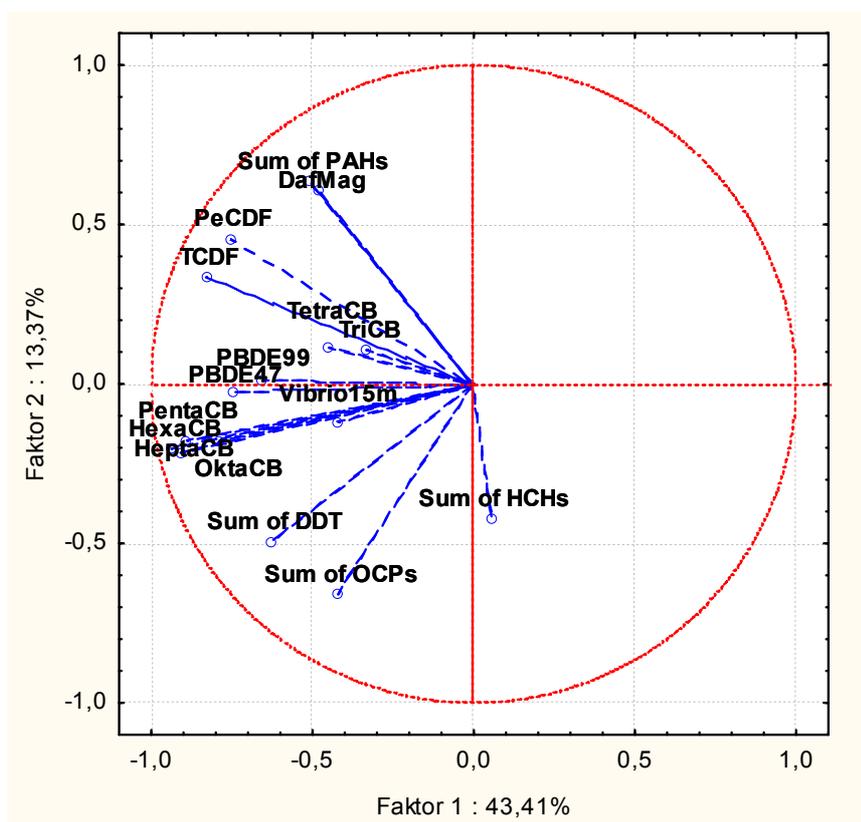
Graph 64 *Box and whisker plots (bioassays)*



Graph 65 *Box and whisker plots (chemicals)*
PCBs, OCPs, PBDEs (pg/L), PAHs (ng/L), PCDD/F (fg/L)

To obtain information about the relationships among the variables, the principal component

analysis was applied to reveal the eventual clustering of the samples among sampling periods,. Two components, whose total variance gives 58.4%, are seen in the graph below. Similarities or interactions among POPs (active variable), including toxicity (supplemental variable) parameters can be well seen from PCA Graph 66, along with all observations. There are major clustering Σ PCBs – Σ Tri- Σ TetraCB, Σ PBDEs, Σ PAHs, together with Σ TCDF and Σ PeCDF. The second cluster is formed by higher chlorinated Σ PCBs (Σ Penta- Σ OctaCB), Σ DDT's (and their metabolites) and Σ HCHs.

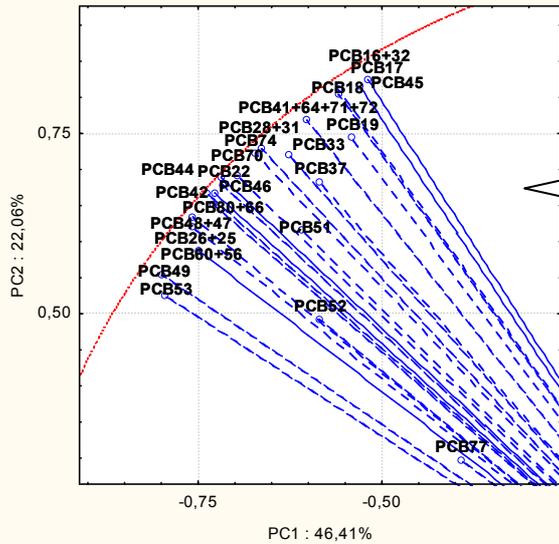


Graph 66 Czech Rivers: plot components weights of POPs groups

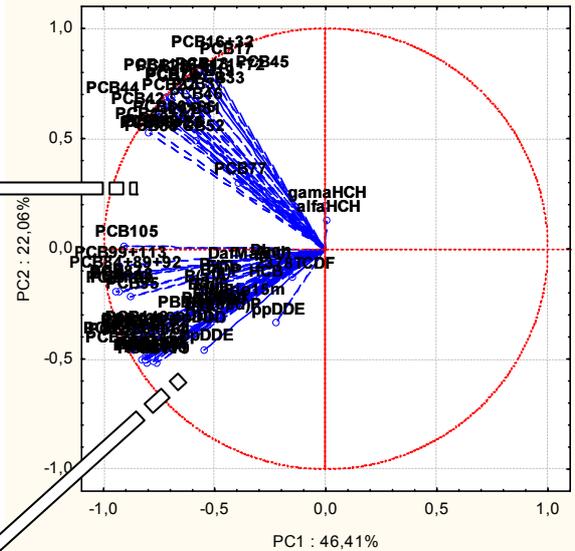
A detailed insight to similarities of individual groups of POPs can be obtained in the graphs below, where parameters and their correlations are demonstrated.

Factor	Eigenvalues	% of total variance	Cumulative Eigenvalues	Cumulative %
<i>Eigenvalues for individual compounds</i>				
1	34,80925	46,41234	34,80925	46,4123
2	16,54261	22,05682	51,35187	68,4692
3	8,01511	10,68682	59,36698	79,1560
4	2,36176	3,14901	61,72874	82,3050
<i>Eigenvalues for groups of POPs</i>				
1	6,945759	43,41099	6,94576	43,4110
2	2,138467	13,36542	9,08423	56,7764
3	1,962420	12,26513	11,04665	69,0415
4	1,409568	8,80980	12,45621	77,8513

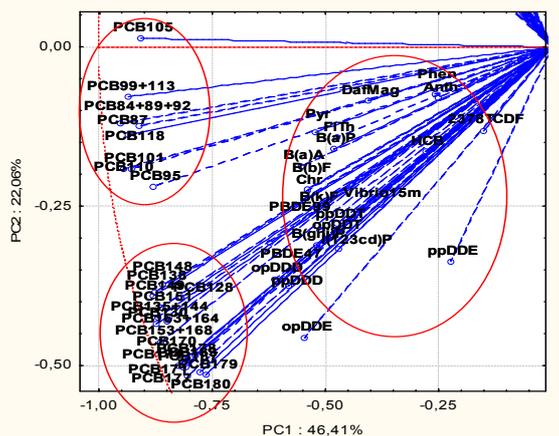
Table 10 Czech Rivers: eigenvalues for individual compounds and their groups
Yellow field are relevant factors to be used for analysis



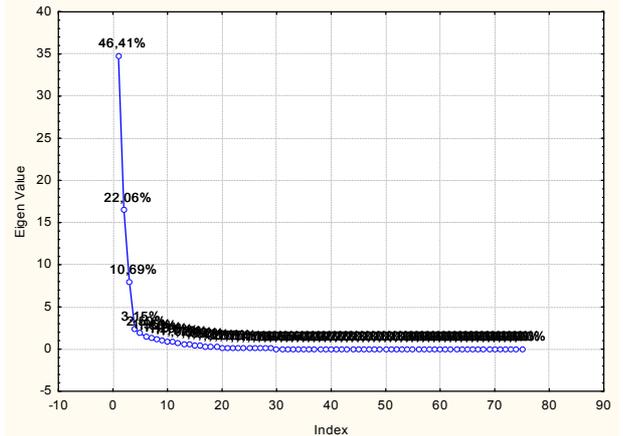
Graph 67 Czech Rivers: plot component weights (detail of PCBs)
 The cluster of individual congeners, mostly affiliated to group of TriCB and TetraCB isomers



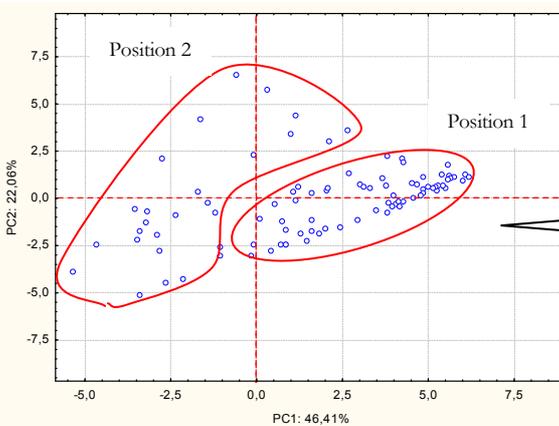
Graph 68 Czech Rivers: plot component weights of individual compounds
 Selection of LODs: <10% LODs, denoted as X=LOD (Statistica™)



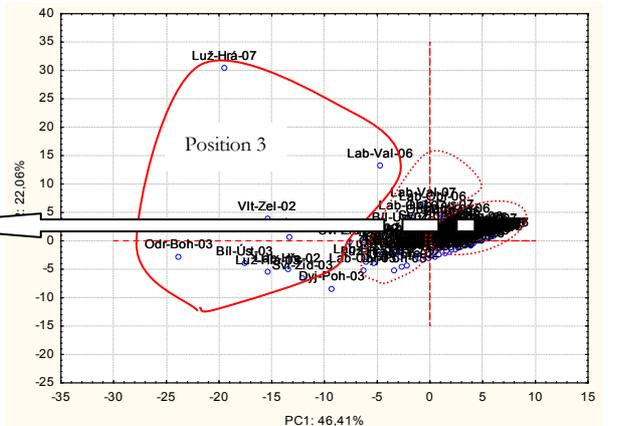
Graph 69 Czech Rivers: plot component weights (detail of PCBs, OCPs, PBDE, PCDF and toxicity)



Graph 70 Czech Rivers: scree plot
 PC1-PC4 can be used for analysis (Statistica™)

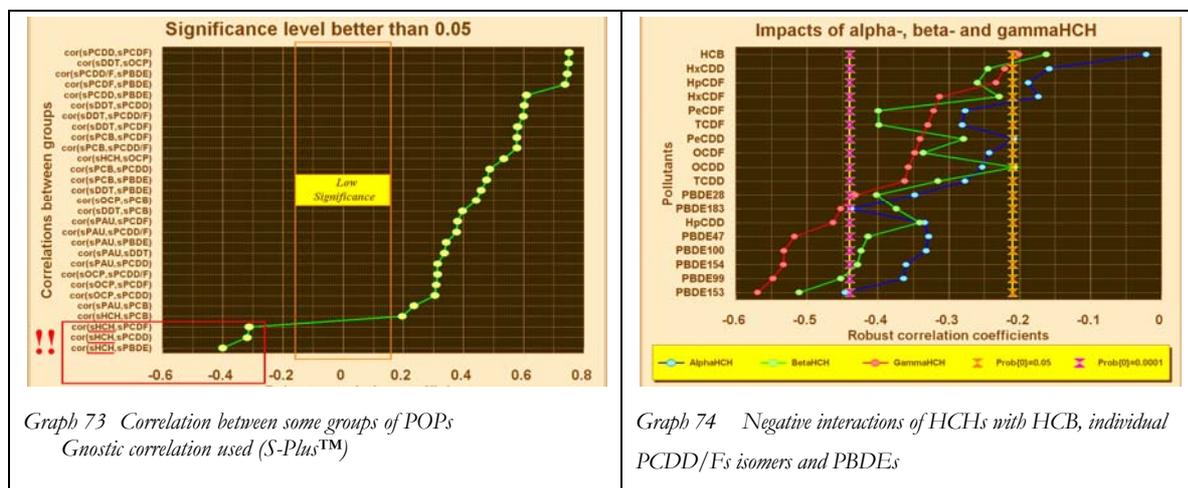


Graph 71 Czech Rivers: plot component score (detail of clusters)



Graph 72 Czech Rivers: plot component score (total composition of clusters)

Despite the better results in PCA model based on individual compounds than for their groups, the latter model was used for evaluation of quality correlation coefficients. At first step, the PCA was used as an exploratory method based on non-robust, implicitly set-up Pearson correlation; results are shown in Graph 67 - Graph 72. This approach was followed by robust multivariate methodologies. Robustly estimated correlation (gnostic) coefficients^{59, 114, 160} were tested for statistical significance; results are given on Graph 73 and Graph 74.



From those Many significant correlation coefficients are thus observed and confirmed. There are not only positive correlations, represented by \sum DDTs, \sum PCDD/Fs, \sum PCBs, \sum PAHs. There also exist negative interactions, represented mainly by γ -HCH. The main correlations were found with \sum PBDE. Relations of groups reflect relations between individual compounds/congeners. Individual correlations are described below. These correlations most probably do not reveal relationship in the sense of common reaction mechanisms. It reveals most probably basic facts that while HCHs decrease in general, PBDE contamination from year to year rise due to accumulation in environment. These results would require special attention in management and regulation.

Conclusions

- SPMDs has proven to be a suitable tool for both quantitative as well qualitative screening for monitoring of POPs in rivers. Its applicability extends the possibility of source identification of chemicals in combination with toxicity tests using the *Daphnia magna*, *Desmodemus subspicatus*, and *Vibrio fischeri*.
- The realised observations show, that further detailed identification of sources are needed for application of water management with the aim of source identification and abatement. Within this study, the main contamination sources were identified in following profiles: Bílina-Ústí nad Labem, Elbe-Valy, Elbe-Hřensko, Elbe-Obříství, Lužická Nisa, Odra-Bohumín, and Svratka-Židlochovice.
- Contamination sources exhibit temporal and spatial variations or trends, mainly by PCBs. Those were identified by timely-weighted accumulation by SPMDs.

- It has been found that PCB contamination, toxicity and its behaviour in environment can be dependent on its structure. Further research in this field is needed.
- Distributions of data differ not only by mean levels but also by their forms. Distributions are non-Gaussian (“normal”). Domains are finite, densities asymmetric.
- It was shown that is welcomed if used combination of methods, especially if data do not fits normal distribution and there are in small data sets. In such cases for correlation, the best results are expected if used robust correlation, ig. Spearman, Kendall or gnostic, instead classical Pearson’s.
- There is an apparent difference in the scree plot, when the first level of grouping (i.g. as isomers for PCBs, sum of DDTs, HCHs) is used instead individual compounds, the loss of cumulative variance can be in the magnitude of 20% and more, if all factors are used for analysis. For that reason, at the case of POPs, it is very advisable to pass through very detailed exploratory analysis, leading to very detailed insight into both groups as well as individual compounds.

Identification of endocrine disrupting chemicals by SPMDs

Endocrine disrupting chemicals (EDCs) represent a variety of chemical classes, such as persistent organic compounds like PCBs, PAHs, OCPs, PBDEs, hormones, and other industrially formed compounds, like dioxins. Those compounds can cause many adverse effects in aquatic environment³¹²⁻³¹⁶ and alter the hormonal and homeostatic systems that enable the organism to communicate and respond to its environment. Nowadays, basic scientific research shows that the mechanisms are much broader than originally recognized, i.g. through nuclear hormone receptors. Thus, endocrine disruptors act via nuclear receptors, nonnuclear steroid hormone receptors, nonsteroid receptors, orphan receptors, enzymatic pathways involved in steroid biosynthesis and/or metabolism, and numerous other mechanisms that converge upon endocrine and reproductive systems^{317, 318}

In the case of complex pollution, there is limited information from chemical parameters, thus, evaluation of toxicological response in the relation to chemical pollutions can help to fill gaps in total EDCs assessment. If standard SPMDs are used, than both calibration data with uptake rates (Rs) or newly performance reference compounds (PRCs) can be used for evaluation of ambient concentration, reflecting timely average concentration (TWA) of dissolved contaminants in water. The semipermeable membrane devices (SPMDs) of standard arrangement were used (see above) for identification of non-polar organics: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs), polybrominated difenyl ethers (PBDEs), triclosane (TCS) and methyltriclosane (MeTCS) within the framework of assessment of endocrine disrupting chemicals (EDCs). The bioassays in parallel in vitro responses to reveal cytotoxicity (Cyt 1/IC50), antiestrogenicity (AES 1/IC50), antiandrogenicity (AAND 1/IC50) and AhR-mediated activity (Bio-TEQ) was used to chemical parameters.

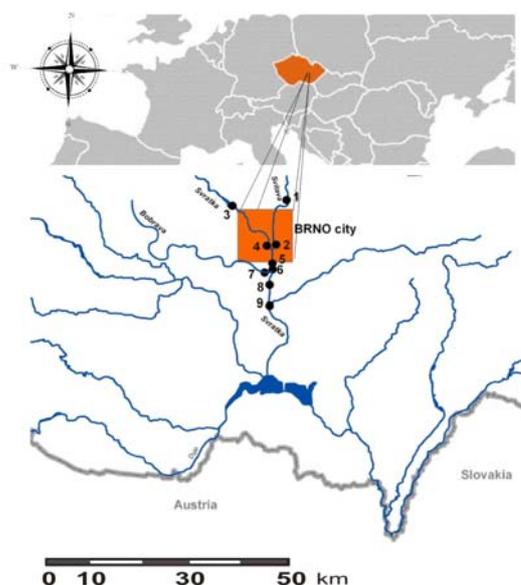


Figure 15 Sampling places

The study was focused on the impact of Brno agglomeration and municipal waste water treatment plant effluent on upper (lower) streams of Svitava and Svratka rivers.

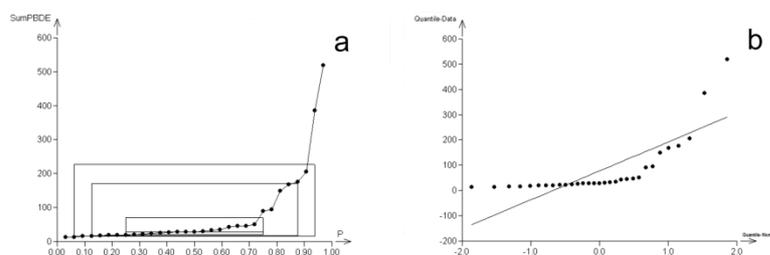
Sampling places are given on the Figure 15. They were selected to reveal contamination caused by Brno agglomeration: Svratka upstream (site 3), Svitava upstream (site 1),

profiles before junction (sites 4 - Svratka, and 2 - Svitava), profiles at the Central WWTP Brno, 500000 equivalent inhabitants, Modřice (site 5 - influent and site 6 - effluent), downstream profiles after junction (site 7 - Bobrava, site 8 - Svratka - Rajhradice), and comparable profile reflecting output from small WWTP, 500

equivalent inhabitants (site 9 - Svratka - Židlochovice).

For exposure, two SPMDs for chemical analysis and one for toxicity were used. A $^{12}\text{C}_{13}$ labelled PCBs (PCB 3, 8, 37 and 54) were used as PRCs, together with deuterated PAHs (acenaphthene, fluorene, phenanthrene, and chrysene). Standard sampling arrangement was used (see above). Passive samplers have been exposed in water for 28-30 days, in spring and autumn in 2007 and 2008. Chemical parameters were measured by GC-MS/MS system by isotopic dilution³¹⁹. Four reporter gene bioassays were used to measure receptor-mediated activities³²⁰.

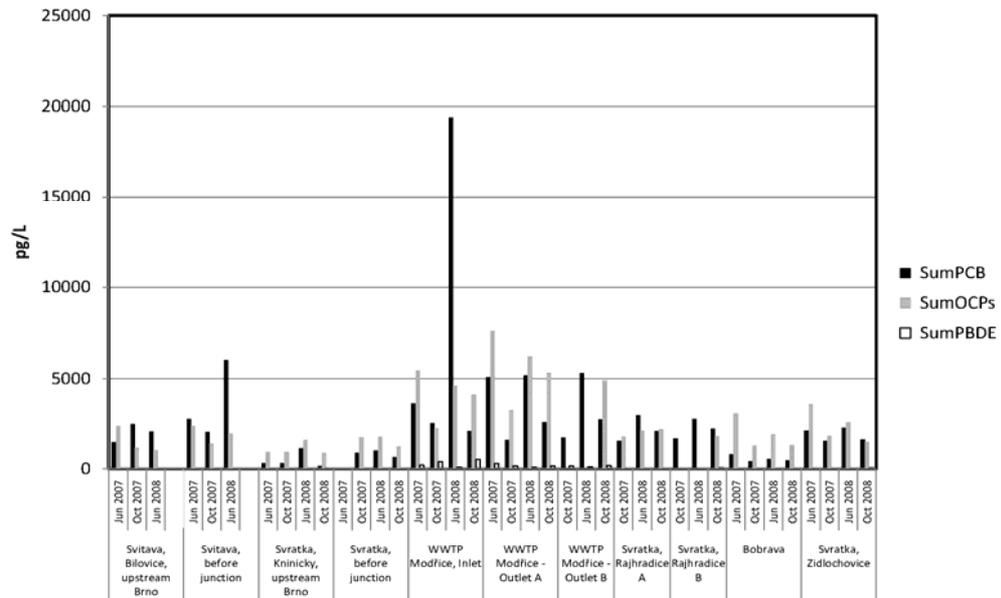
- (1) *Diagnostic plots in the EDA* was used for selected example ΣPBDE . The quantile-box plot (Graph 75a) indicates some outliers at high values and asymmetric, skewed distribution. The normal probability plot (Graph 75b) checking a normal distribution does not exhibit close agreement of the sample points with a straight line. A combined sample skewness and kurtosis test leads to the test statistic is $21.13 > \chi^2(0.95, 2) = 5.992$ and therefore normality of data distribution was rejected.



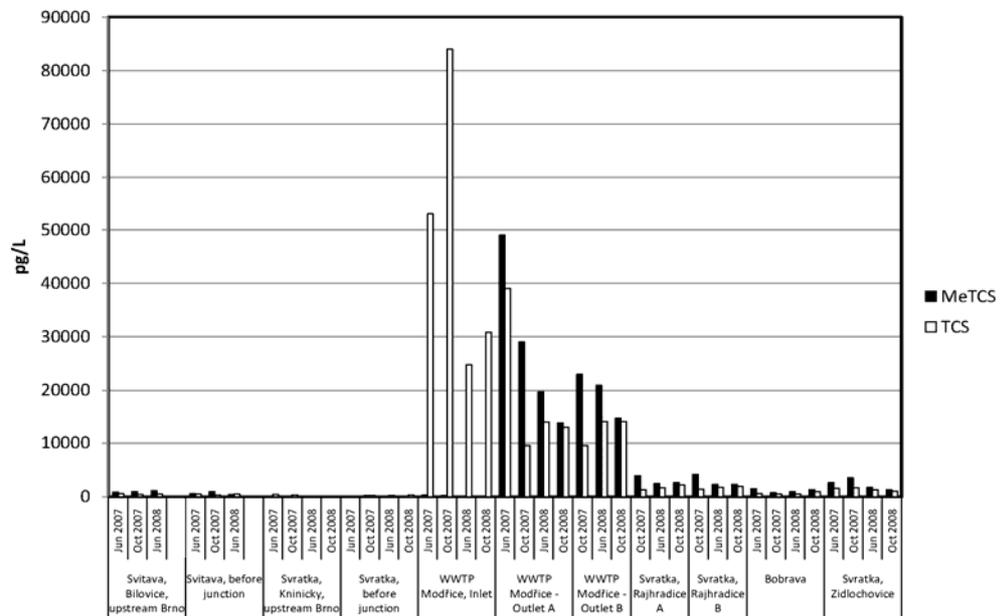
Graph 75 EDCs in Brno: Diagnostic plots of EDA

- (2) All pollutant levels exhibit temporal and seasonal variations. From the graph patterns, it is apparent that POPs like PCBs, OCPs and PBDEs except of TCS or MeTCS were found at all

sampling places. As expected, major source of contamination by TCS and MeTCS originates from WWTP effluent – see Graph 77. However, for POPs in both Svitava and Svatka rivers, there were found concentrations of PCBs, OCPs, and PBDEs, due to their historical use as a result of human anthropogenic activity Graph 76. As for temporal trends, samples from WWTP in summer (June) exhibit higher contamination than in autumn (October). It can be accounted to lower (about 10%) water flow in summer than in autumn.

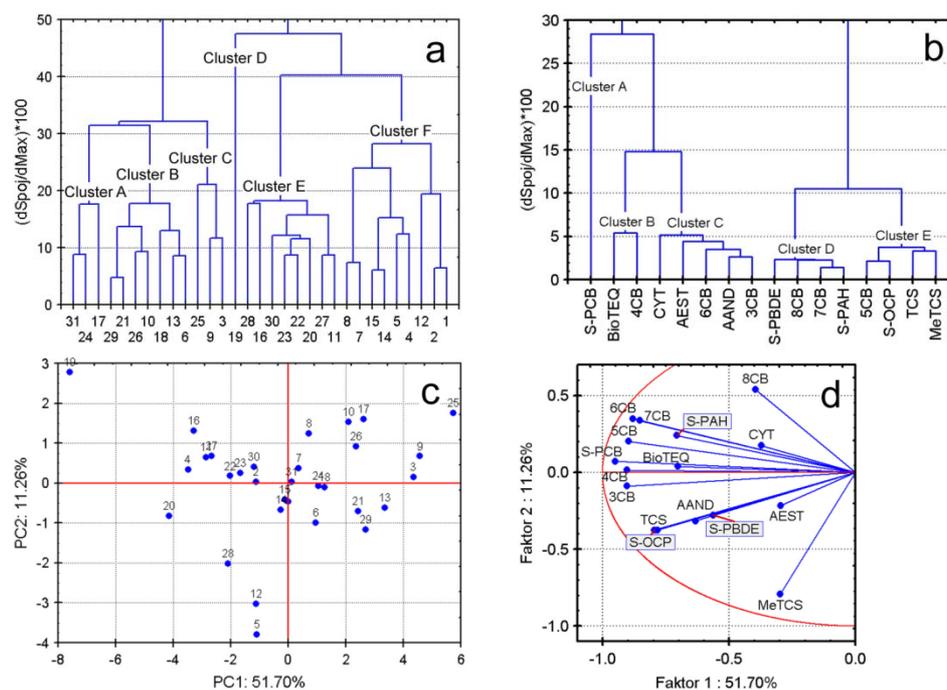


Graph 76 EDCs in Brno: concentration of PCBs, OCPs, and PBDEs



Graph 77 EDCs in Brno: concentration of MeTCS and TCS

- (3) Cluster analysis CLU: The dendrogram of water samples resulting from CLU (Euclidean distance and Ward's agglomerative method) of Box-Cox normalized data set is represented.



Graph 78 EDCs in Brno: dendrograms and PCA of sampling sites and parameters

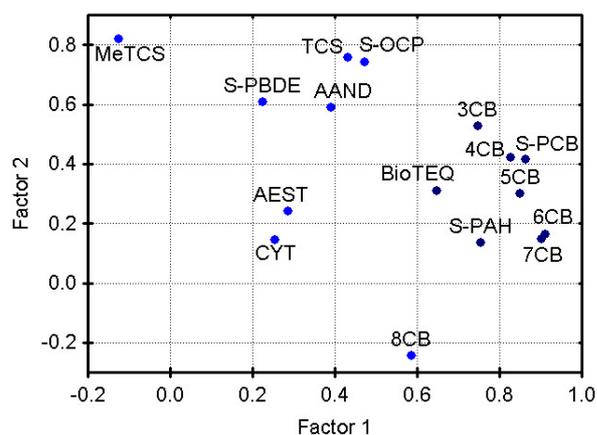
Dendrograms of clusters formed of objects and variables found by the cluster analysis of Box-Cox normalized data using the Ward method. It shows that the sampling points could be grouped into four main clusters A through D. Dendrogram constructed with the use of the weighted mean method shows also samples monitored in 2007 and 2008 in four main streams I (Svitava), II (Svratka), III (Bobrava) and IV (Modřice). Cluster A concerning samples taken mostly in 2008 strongly differs from all other clusters B, C and D. The Cluster D contains samples from 2007 only, that reveals similarities among all Svitava profiles, characterised by POPs occurrence. On the contrary, Svratka downstreams exhibits similarity to Modřice outlet/inlet, from the POPs point of view, that reveals no special significance on POPs arising from WWTP effluent. It underlines that POPs occurrence is not strongly affected by WWTP and they occur in both upstream and downstream of assessed rivers. Certain similarity of Svratka downstream profiles (Rajhradice and Židlochovice) is found in 2008 (represented by Cluster C), together with WWTP inlet/outlet, and with Svitava profile (before junction). Finally, Cluster B can be attributed to Bobrava profiles in both years, Svratka upstream, Svratka before junction, and finally, Svitava upstreams. Cluster A represents an outlying sample No. 19 only, constituted by water sampled in 2008.

Dendrograms of variables bring quite an agreement between both approaches, the weighted mean method and Ward method. Five constructed clusters contain exactly the same variables when both cluster methods are applied, the weighted mean method and Ward method. Cluster A contains one outlying variable \sum PCB. Cluster B being close to cluster C contains BioTEQ and

4CB while cluster C contains CYT, AEST, 6CB, AAND and 3CB. Another two clusters D and E seem to be similar but cluster D contains Σ PBDE, 8CB, 7CB and Σ PAH while cluster E contains 5CB, Σ OCP, TCS and MeTCS. This outlying sample is attributed to MeTCS occurrence that can be accounted to biodegradation product of TCS due to biological process of WWTP. As for toxicity responses, cytotoxicity was higher for samples from locations downstream of Brno city. Treatment processes in the WWTP decreased the cytotoxicity of the waste waters. SPMD samples elicited significant AhR-mediated activity. The antiestrogenic activity appeared at most sampling sites.

Note: for identification of observations, see source data on CD.

- (4) *Principal components analysis PCA:* The PCA was applied to the Box-Cox normalized data set (16 variables) to compare the composition patterns between the analysed water samples and to identify the variables that influence each one. The first two principal components PC1 vs. PC2 explained about 62.96% of the total variance. The analysis of the data shows that: (a) PC1 which explains the 51.70% of the total variance, is mainly influenced by the normalized concentration of compounds gathered in clusters A-D (b) PC2 (11.26% of the total variance) is influenced by the normalized concentration of MeTCS. The content of all four clusters is similar as it is found with the PCA. Main cluster is formed by PCBs and Tri- to 7CB isomers, together with PAHs, and with BioTEQ response. As for detailed expression, TEQ values are mostly defined by low-chlorinated isomers 3CB and 4CB that dominantly form sum of PCBs. The source can be attributed to Delor 103 or analog (Aroclor A1016), used in former Czechoslovakia. There was more than 11000 tons of Delors produced and applied in the former Czechoslovakia within the period of 1959 to 1984⁷⁷. This is the main reason why some parts of the Czech Republic are still contaminated with PCBs. This contamination of the fields and rivers by Delors is the result of 25 years PCBs production and their use in the Czech Republic. Delor 103 contains mainly the mono-ortho and 2,2'-di-ortho-chlorinated biphenyls. Dominating accumulation of tri-ortho chlorinated biphenyls as well as the pronounced accumulation of 2,6-di-ortho- and tetra-ortho-chlorinated PCBs has been found. There are different opinions for understanding the reasons of these accumulation phenomena. Very high thermodynamic stability and rigid structure of compounds mentioned above might be the possible and serious reason for accumulation in the biota, as well as in SPMDs. This attribution can explain correlation of low chlorinated biphenyls with AhR-mediated activity. On the contrary, 8CB forms other group and does not exhibit close correlation with other isomers. As for cytotoxicity and antiestrogenicity, both parameters exhibit close correlation, without close correlation to any observed POPs chemicals, in SPMDs. As for OCPs, close correlation to TCS was found, less close to occurrence PBDEs and antiandrogenicity.
- (5) *Factor analysis FA:* The rotation Varimax of the axes defined by PCs (FA) explains again the 63% of the variability of the Box-Cox normalized data but it partially modifies the weight of the



Graph 79 EDC in Brno: Factor analysis, Varimax rotation

normalized variables in three varifactors obtained Graph 79. It can be seen that: (a) VF1 is mainly influenced by the polychlorinated biphenyls (3CB, 4CB, 5CB, 6CB, 7CB, Σ PAHs, Bio-TEQ and Σ PCB), (b) VF2 is influenced by MeTCS and by the sum of organochlorinated pesticides (OCPs). Both methods, PCA and FA, lead to same clusters, i.e. 3 clusters and two outlying variables MeTCS and 8CB.

Conclusions

- The following order of levels of monitored chemicals were found: PAHs > TCS > MeTCS > PCBs > OCPs > PBDEs.
- From the point of view biotests, there is apparent AhR-mediated activity (dixon-like activity), that corresponds to low chlorinated PCBs.
- Occurrence of antiestrogenic, as well as antiandrogenic activity assessed by in vitro tests on all profiles under WWTP, is in good agreement with TCS, OCPs and PBDE occurrence, as confirmed by used statistical methodologies. This fact shall lead to management of WWTP or further technological changes, to be generalized for similar water treatment plants designed without special handling of EDCs.
- Occurrence of OCPs and PBDEs is closely connected with their historical and present use, respectively. Occurrence of MeTCS is considered as episodic, outlying, and related to TCS. Apparent correspondence of MeTCS to TCS content was found, revealing direct mechanism of MeTCS formation by biological waste water treatment.
- The most rigorous estimate of location is represented by the re-transformed mean \bar{x}_R after Box-Cox transformation of original data. This estimate can be taken as the best for each element studied, if statistical approach is used for data evaluation.

Aspects of analysis of multivariate data

Following topics describe some of aspects of realised data analysis, supporting the requirement of robustness of analytical approaches. Among the most important, correlation and regression methods were applied. Besides common statistical approaches, gnostic approach was also used. Conclusions from the applications are especially relevant for POPs and their levels, as found above using SPMDs. It is noteworthy that data transformation (e.g. Box Cox), despite it rigorousness, tends data to interdependence, as was found in practical applications within this thesis.

Due to some consequences, that are relevant for small data samples³²¹, mainly: (i) statistical tests

have relatively low power, (ii) probability of selection of most appropriate parametric distribution will be low, (iii) use of empirical distribution may be problematic, (iv) parameter estimates will have high statistical error; approximation of the sampling distribution may be relatively poor, the use both non-robust statistical correlation (Pearson), robust statistical correlation (Spearman, Kendal) and robust gnostical seems to be practical, for further PCA application.

Correlation coefficients of data

From available correlations, *non-parametric* methods were used. An overview of used methods is given in the Table 11.

ID	Approach description	Method source
SP	Common statistics	Pearson
SK	Robust statistics	Kendall
SS	Robust statistics	Spearman
GN	Mathematical gnostics	Kovanic

Table 11 Czech Rivers: overview of used approaches and methods of correlation

Using these methods, following correlation coefficients were estimated for given groups of POPs and selected toxicity tests.

Method	Correlation	Value	Pr(0)	Method	Correlation	Value	Pr{0}
SP	(P2,P3)	0.734	0.000	SS	(P5,P6)	0.772	0.001
SP	(P1,P2)	0.654	0.002	SS	(P2,P5)	0.711	0.003
SP	(P2,P5)	0.624	0.004	SS	(P1,P2)	0.526	0.026
SK	(P5,P6)	0.602	0.000	GN	(P5,P6)	0.763	0.001
SK	(P2,P5)	0.556	0.001	GN	(P2,P5)	0.739	0.003
SK	(P1,P2)	0.404	0.016	GN	(P1,P2)	0.486	0.026
SP	(T3,T2)	0.418	0.075	SS	(T3,T2)	0.559	0.018
SK	(T3,T2)	0.368	0.027	GN	(T3,T2)	0.524	0.022

Table 12 Czech Rivers: correlation coefficients for POPs groups

P1 - PCDD/Fs, T1 - *Daphnia magna*, P2 – PCBs, T2 - *Vibrio Fischeri*, P3 – PAHs, T3 - *Desmodemus subspicatus*, P4 – HCHs, P5 – HCB, P6 – DDTs (*S-Plus*TM)

It is apparent from the survey, that all correlations inside the groups are positive. There is only one correlation insufficiently significant, that of the *Desmodemus subspicatus* and *Vibrio Fischeri*. As for toxic effect of pollutants, following table gives significant correlations with $Pr\{0\} < 0,05$.

Correlation	Correlation coefficient			
	SP	SK	SS	GN
(P3,T3)	0.657	0.439	0.617	0.643

Correlation	Correlation coefficient			
	SP	SK	SS	GN
(P6,T1)	0.255	0.462	0.574	0.501
(P3,T1)	0.481	0.392	0.556	0.424
(P5,T1)	0.345	0.368	0.493	0.384

Table 13 Czech Rivers: correlation coefficients among chemicals and toxicological responses

P1 - PCDD/Fs, T1 - *Daphnia magna*, P2 – PCBs, T2 - *Vibrio Fischeri*, P3 – PAHs, T3 - *Desmodemus subspicatus*, P4 – HCHs, P5 – HCB, P6 – DDTs (*S-Plus™*)

As seen from the results, the toxicities of PCDD/Fs, PCBs as well as HCHs are insignificant. There are significant correlations of PAHs, DDTs and HCB, all positive.

Method	SP	SK	SS	GN
SP	0	0.136	0.147	0.161
SK	0.136	0	0.084	0.087
SS	0.147	0.084	0	0.047
GN	0.161	0.087	0.047	0

Table 14 Czech Rivers: comparison of various correlation coefficients and methods

The importance of used correlation approaches is apparent from the results above. There is quantitative expression of mean square difference (D) between vectors of correlation coefficients. From this overview and results compiled above, the smallest difference between vectors is between Spearman's and gnostic correlations that documents the best fit between those methods. Both methods are the most suitable for further evaluation of relationship of data. However, Kendall's method seems to be acceptable too, but an important feature should be taken in account: gnostic correlation coefficient is estimated by the gnostic distribution functions, which enable usage of censored data. Classical Pearson's correlation, despite its wide use, is less robust and could be used only exceptionally for the purpose of POPs data with their specific trends, outlying and censored data, as shown by slightly controversial results above (marked as yellow background).

From types of commonly used parametric (Pearson) and nonparametric correlation coefficients (Spearman, Kendall and gnostic), the choice of the most robust has following *practical* aspects:

- Spearman (SS), assuming that the variables under consideration were measured on at least an ordinal scale. Spearman's R can be thought of as the regular Pearson product moment correlation coefficient, that is, in terms of proportion of variability accounted for, except that Spearman R is computed from ranks.
- Gnostic (GN) correlations are non-linear with respect to data due to the curvature of data spaces. Gnostic estimate of the correlation coefficient of the data can be obtained by the formula similar to the statistical one^{59, 230}, but using the theoretically proven relation to data probability estimated by distribution functions.

Regression models

Among all methods applied to robust estimation of the parameters of linear multidimensional regression models, models based on the gnostic theory and alternatively on the statistical methodology were applied. The methods that were chosen are below, in the Table 15. All functions were applied by S-Plus™ 6.2 package, with the package of gnostic programs. Main goal of this task was verification of regression models of chemical effects on toxicity, namely of PAHs and PCBs on *Vibrio fischeri*, based on preliminary results of exploratory and correlation analysis referred to above.

Method ID	Function Method	Coeff. Det. R	STD	M(w)	M(Ef. PCB)	M(Ef. PAH)
0	wt.OLS	0.624	0.0212	1.000	+0.2676	-0.0239
1	wt.gnostic	0.986	0.0044	0.586	+0.0119	-0.0105
2	wt.andrews	0.684	0.0182	0.925	+0.0236	-0.0209
3	wt.bisquare	0.684	0.0182	0.925	+0.0236	-0.0209
4	wt.cauchy	0.697	0.0176	0.895	+0.0228	-0.0202
5	wt.fair	0.979	0.0063	0.405	+0.0075	-0.0077
6	wt.OLS	0.647	0.0203	0.988	+0.0257	-0.0209
7	wt.gnostic	0.683	0.0188	0.960	+0.0247	-0.0216
8	wt.andrews	0.704	0.0173	0.880	+0.0223	-0.0199
9	wt.bisquare	1.000	0.0137	2387.5	+21.605	-13.604
10	wt.cauchy	0.624	0.0212	1.000	+0.0268	-0.0239
11	wt.fair	0.660	0.0194	0.953	+0.0248	-0.2209

Table 15 Czech Rivers: overview of applied methods for regression analysis and their results

All methods were realised as Weighted Least Squares. The best results was reached by the method No. 1 (gnostic) with $R=0,986$ and $STD = 0,0044$, together with No. 5 (“fair”) with $R = 0,979$ and $STD = 0,0063$. Another appropriate method was No. 8 (“Andrews”) with $R = 0,704$ and $STD = 0,0063$. In all results, there is apparent opposite effect of PCBs against PAHs. This fact is explainable by grouping effect and loosing information distinctive for both PCBs and PAHs. However, regression methodology plays important complementary methodology, especially when a robust method is used. The use of the most robust methods is advisable, even if data are of low scale and/or normality criteria are not met.

Evaluation of data from biomonitoring by GA

As a result from some important UN actions (e.g. Stockholm Convention for POPs), several studies for monitoring of POP in various environmental compartments were realised, except to those presented above by means SPMDs. It is worth saying that routine monitoring data had often a typical feature: small data set for which the statistical approach was difficult to be applied, so that gnostic analysis (GA) played an important role for proper interpretation of measured data. Demonstrations presented as examples stem from monitoring selected POPs and heavy metals in

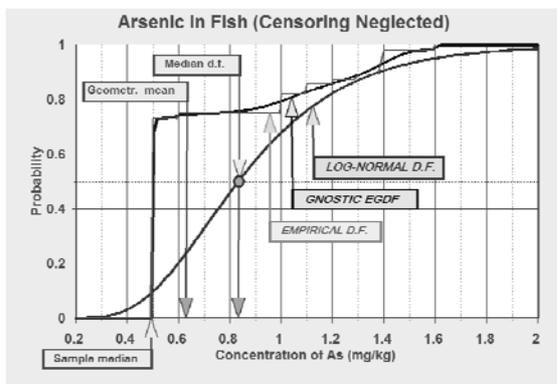
Czech rivers in biotic organisms. There are following criteria considered:

- (i) basic^{112, 113} (valid for statistical and gnostic methods)
 - distribution functions (d.f. or DF): empirical (EDF),
 - homogeneity test of a data sample,
 - bounds of a data sample,
 - heteroscedasticity,
- (ii) extended⁵⁹ (valid mainly for gnostic methods):
 - estimating local (ELDF), and estimating global (EGDF),
 - weights of data,
 - cross-section data filtering,
 - data censoring,
 - robustness of used distribution functions,
 - interval analysis of a data sample.

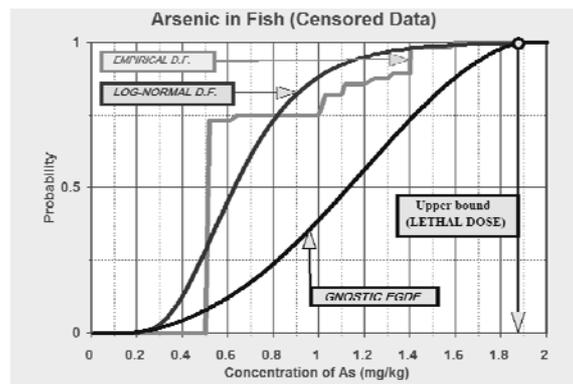
It is important to say that this list of tasks is to be understood as an example of gnostic approach to data evaluation, not as a complete review of results. For simplicity and better understanding, results of some analyses are presented in the following graphs. Data were analysed from the following data matrix:

- 56 observations, from which 41 are below the limit of detection (LOD),
- 4 organisms used as sampling methods,
- 3 selected parameters (arsenic, mercury, DDD as metabolite of DDT's).

There are apparent differences among statistical and gnostic d.f. in Graph 80. If the evaluation of contamination were provided by discrete statistics only (sample's *median*, *mean*, *geometric mean*, *median of the log-normal d.f.*), the data would be misinterpreted. Gnostic d.f. yields more information from data and a better understanding of experimental phenomena. Information that 41 data were measured below the limit of detection has not been used in the case of Graph 80.



Graph 80 Comparison of DFs (statistical log-normal and gnostic ELDF)



Graph 81 Comparison of DFs and data censoring

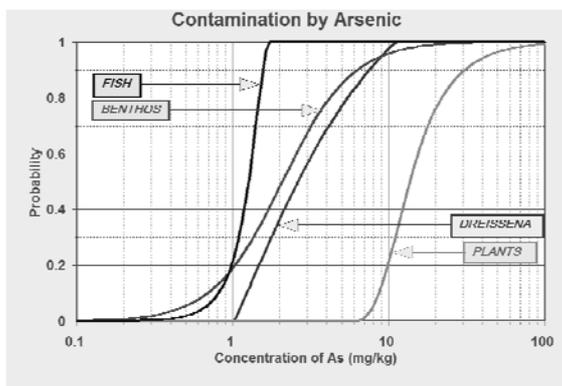
As mentioned above, gnostic d.f. can make use of the information that some data were censored (in this case it relates to data below the sensitivity threshold). Even more, gnostic d.f. EGDF has a valuable feature of estimating the upper bound of data support. For a biotic organism, this can be understood as a limit value, which the organism cannot survive. This can be a new challenge for newly designed assessment studies.

In general, a comparison of sampling methods plays an important role for understanding various ways of intakes of various contaminants in water ecosystems. Such comparison must be provided in following circumstances: (i) sampling and analysis is provided by the same source of uncertainties, (ii) sampling method is the source of the most relevant uncertainty of generated data (analytical uncertainty plays a negligible role in all the complex causes). Both mentioned conditions have been met for further evaluations.

To identify features of sampling methods, we can: (i) compare results of one POP for various sampling methods, (ii) compare results of one sampling method for various POPs with the aim to define which sampling method should be used in future evaluation of monitoring a given POP.

The application of gnostic d.f. to compare four biotic organisms is shown in Graph 82. Since 1999, there were 4 organisms used, to evaluate the accumulation of POPs:

Benthos, fish, *Dreissena polymorpha*, and plants. When d.f. are used for interpretation, we can see: (i) how individual organisms are sensitive to particular pollutants, (ii) how reliably can be their upper bound of accumulation of POPs estimated.



Graph 82 Comparison of four sampling methods

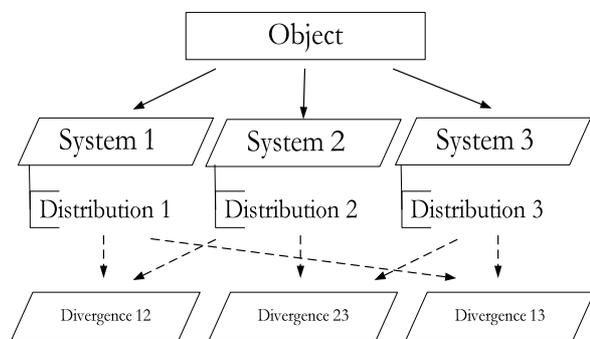


Figure 16 Verification of conclusions from gnostic studies

It can be seen in the graph that two organisms (fish and *Dreissena polymorpha*) enable the maximum dose (which can be interpreted as the lethal dose) to be estimated as the upper bound. The lower bound of the data support can also be of interest. It can be determined by means of the gnostic EGDF, too. Similar conclusions can be drawn from cases of other contaminants, like p,p- DDD, as the metabolite of DDT's (for the Elbe river). Benthos confirmed the differences between other parameters, and thus differences between sampling systems. On the contrary, there is a similarity for fish with *Dreissena polymorpha*, which is in agreement with theoretical assumptions.

As for general procedure, the basic data flow in gnostic analysis of uncertain data is shown in the

Figure 16. This scheme describes steps of quantitative evaluation of results resulting in the **divergence values** measured as local differences between distribution functions. Minimum divergence exhibit *Dreissena polymorpha* and fish. The maximal divergence is assigned to Benthos and fish. The divergences are summarized in Table 16.

Contaminant	Minimum Divergence		Maximum Divergence	
	Organism	Value	Organism	Value
p,p-DDD	<i>Dreissena polymorpha</i> Fish	0.033	Benthos Fish	0.111
Arsenic	<i>Dreissena polymorpha</i> Benthos	0.029	Benthos Fish	0.231
Mercury	<i>Dreissena polymorpha</i> Benthos	0.045	Benthos Fish	0.286

Table 16 Divergences of biotic organisms on various parameters

Conclusions

- Among various correlation methods, positive and mostly significant correlation was found for following parameters: (i) HCB, DDTs, (ii) HCB, PCBs, and (iii) PCDD/Fs, PCBs. That correlation generally corresponds to both physical properties and common occurrence of those groups.
- When using correlation among groups, special attention must be paid to avoid loss of information, due to various effects, as denoted above. To lower those effects, data censoring is recommended to be accounted for, to make use of all data for evaluation. Using only non-censored data leads to distortion of relationship and misinterpretation of groups.
- In the evaluation of toxic effects, there is an apparent correlation among PCB (directed by HexaCB), OCPs (DDT and metabolites and HCB) with *Vibrio fischeri*, and *Daphnia magna* with PAHs and PCBs (directed with PentaCB). All correlations are positive.
- As from used correlation, the best method was reached by Spearman and gnostic correlation, as evaluated by relations between vectors. Those correlations are recommended for cases of data with data featured with both outliers and hidden local trends.

Development of quality criteria for SPMDs

As previously declared, particular attention must be focused to (QA/QC), reflected into sample chain of custody, with close inherence to Sampling Plan. To make SPMDs acceptable as standard sampling methods recognised by environmental bodies, some critical criteria must be met. Despite that SPMD approach is widely used by environmental investigators, we have established a way of

declaring general rules for acceptance by regulatory and resource management agencies (this is derived from recognition by Czech Accreditation Institute). Those criteria are based on QC and QA parameters, the overview of which is presented in the Table 17. Additionally, those criteria can be used for declaration of the conditions formulating assumptions for comparability of results. It proves that SPMDs may soon be used as a method for data in litigation.

Parameter, requirement	Way of acceptance (how to meet criteria, given parameters)
Accuracy of sampling, linkage to published calibration data	Publications with experimental Rs (calibration data); PRC approach
Extensive QA/QC (blanks)	Any (<i>economic</i>) combination of blanks (fabrication, transport, field, laboratory)
Reproducibility	Multiple deployment of SPMDs See application and demonstration below.
Repeatability	Round-robin test (for various environmental compartments) ⁸
Qualification for proper handling	Personal certification Experience (e.g. pilot studies, research, etc.)
Chain of custody, Sampling plan	Standard operational procedures (SOPs) General GLP principles (<i>reporting</i>)
Applicability/selectivity for chemicals	Value of log Kow Used analytical methods
Limit of detection	Uptake rates (Rs) availability Performance of used analytical method Time of exposure SPMDs Kind of matrix (sampled environment)
Maximum capacity	SPMDs design Uptake rates (Rs) Time of exposure Deployment conditions (temperature)
Sampling efficiency	Using PRCs approach
Standardisation, comparability among measurements	Using standard tools ONLY (<i>declared manufacturing QA/QC</i>) SOPs (described procedures) General GLPs (practice in laboratory)

Table 17 Way of acceptability for used SPMDs for litigation

Uncertainty estimation

As one of important part for validation and acceptance is the identification of uncertainty, as a condition of testing the laboratories in accordance with EN ISO/IEC 17025 standard. The procedure for uncertainties description has various steps (Ref): (i) specification of measurement range, (ii) identification of uncertainties, (iii) quantification of identified uncertainties, and finally (iv)

⁸ IPSIC (International Passive Sampling Interlaboratory Comparison); currently running IPSIC 2010 at the case of issue of this thesis

evaluation of combined and extended uncertainty. It is noteworthy, that overall SPMDs procedure, incorporating analytical methods is too complex for exact uncertainty computation, so that uncertainty estimation had to be used.

As a basement for uncertainty quantification, the identification of uncertainty sources was provided. To overcome the complexity, the effort was focused on those, which are considered as the most important; those critical uncertainties are given in Figure 17, in the form of Fish-bone (Ishikaw chart).

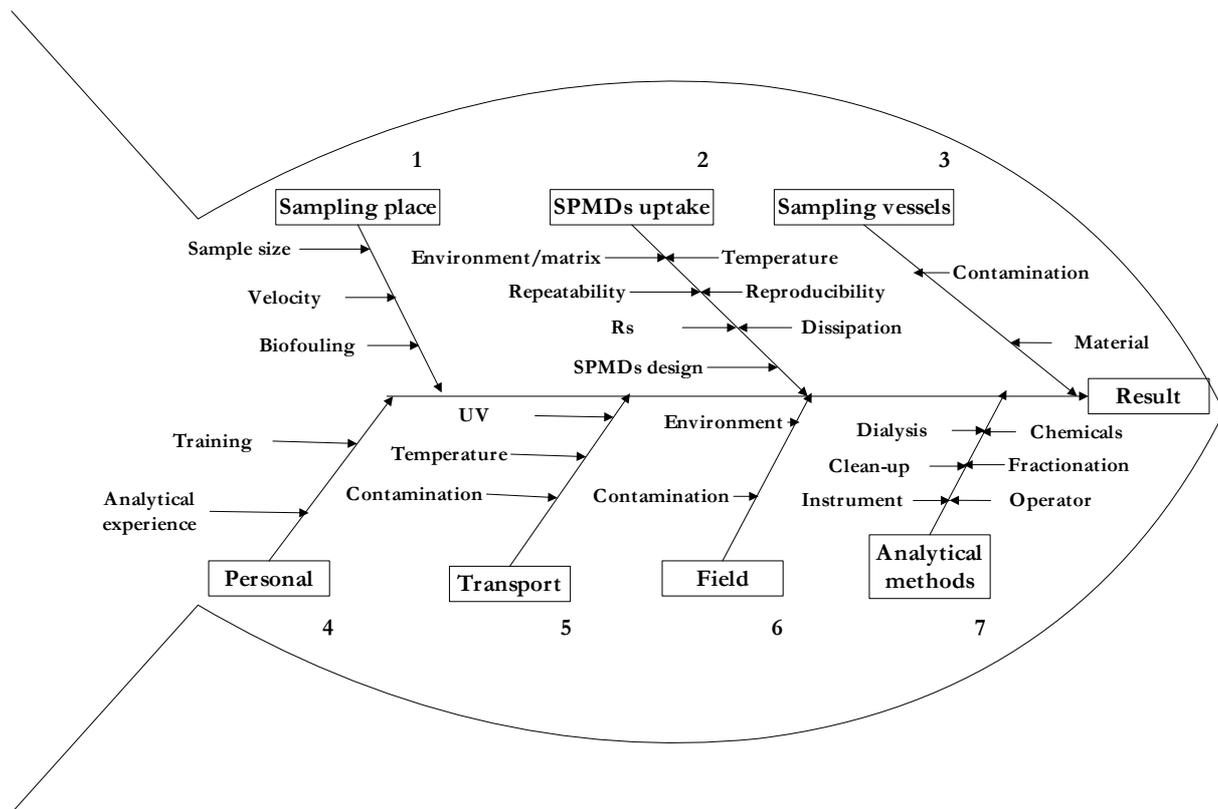
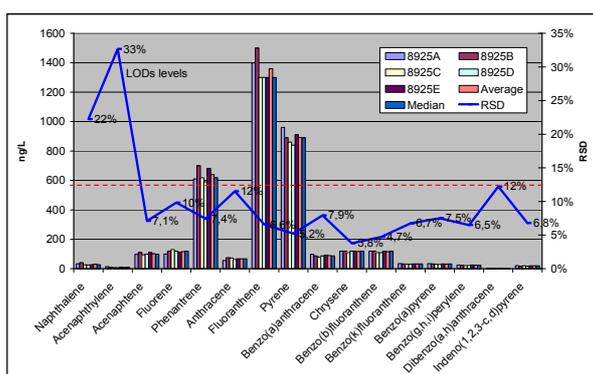
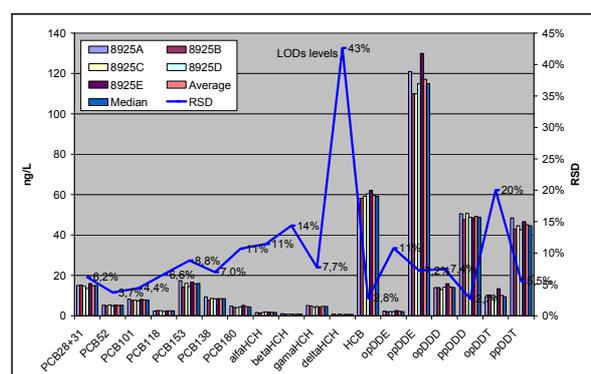


Figure 17 Identification of uncertainties for SPMDs (Fish-bone)

The most difficult task is the quantification of commonly used methods, e.g. by (i) “down-top” procedure which includes all identified uncertainty sources, which are well described and quantified, “down-top”, which is based on performance of used methods. However, neither of those procedures is clearly defined for SPMDs; this requires a detailed insight (future research work is desirable). Even more, due to lack of information about some parameters, like *reproducibility* (from intercalibration study), , there is practically impossible nowadays to describe overall uncertainty in the most rigid way. However, results of this thesis have contributed to one important part – *repeatability* in various water ecosystems as described above, in following chapter. Overall results from the comparison of results exposed in replicate deployment are summarized into following Graph 83 - Graph 88. It was shown (see below Evaluation of repeatability) (from RSD evaluation, RSD among SPMDs exposed



Graph 87 QA/QC: parallel results of individual PAHs, surface water, N=5



Graph 88 QA/QC: parallel results of individual PCBs and OCPs, surface water, N=5
PCBs - within indicator congeners

Detailed results can be found among source data, as supplied in digital form only to this thesis. To evaluate reproducibility, uncertainty of testing methods must be mentioned. As from validation protocols, uncertainty was set up within 20-30%. From all data, there is apparent RSD within 5-15%, involving both sampling and analytical uncertainty. For exceptional cases, where values are approaching LODs, RSD is about within 20-40%.

Glacial water - toxicity

At the Poděbrady sampling site, two parallel SPMDs for toxicity testing were deployed. These membranes were used for proving repeatability of chosen bioassays for this study. There are summarized data of these parallel bioassays in table 8. Dose-response curve of algal bioassays was not typical sigmoid curve, so its 95% confidence intervals of EC50 are wide. This event is in toxicity testing not unusual and does not mean that the experiment should be repeated. This is to see in comparing similar EC50 values of both parallel membranes. Values of V_{tox} of parallel membranes fit well. Improvement should be done in test with crustacean *Daphnia magna*. As this organism is relatively big, it needs bigger volume of tested media together with higher consumption of SPMD aliquot. The volume of SPMD sample is always limited (especially in a case of samples with low toxicity, where no extensive dilution can be realized), so a development of miniaturized bioassay would be beneficial.

	Poděbrady		
	Parallel Membrane A	Parallel Membrane B	Standard Deviation
<i>Desmodesmus subspicatus</i>			
EC50, ml.l⁻¹	25.76	22.61	
95% CI of EC50	7.322 to 90.63	6.083 to 84.05	
Hillslope	0.5169	0.6453	
Vtox50, l.d⁻¹	0.00121	0.00138	0.00011

	Poděbrady		
	Parallel Membrane A	Parallel Membrane B	Standard Deviation
<i>Daphnia magna</i>			
EC50, ml.l⁻¹	9.774	13.58	
95% CI of EC50	7.891 to 12.11	8.476 to 21.77	
Hillslope	1.858	1.361	
Vtox50, l.d⁻¹	0.00319	0.00230	0.00063
<i>Vibrio fischeri</i>			
EC50, ml.l⁻¹	0.06840	0.07274	
95% CI of EC50	0.0580 to 0.0807	0.0631 to 0.0838	
Hillslope	1.394	1.393	
Vtox50, l.d⁻¹	0.45687	0.42961	0.01927

Table 18 Comparison of toxicity response in parallel membranes (Poděbrady sampling site)

Conclusions

All conclusions are formulated from recent studies as well as research projects as mentioned above.

F. General Conclusions

Based on realised experiments, SPMDs can be applied for any compound within the range $\log K_{OW}$ 3.0÷7.5 (mainly non-polar contaminants), in following applications: (i) determination of their presence, (ii) identification of their sources, (iii) estimation of ambient TWA concentrations and time-integrated concentrations, (iv) in-situ toxicity screening (bioassays/biomarkers, immunoassay, toxicity determination); (v) solvent dialysis from lipid samples. From available applications, physicochemical and functional properties, it is possible to proclaim that among other sampling system, SPMDs are becoming globally accepted sampling technology for both individual POPs and complex mixtures.

To simplify overall points, conclusions about conditions about use of SPMDs for research study and monitoring (with reference to fish-bone, see Figure 17) were adopted. There are structured into subheading reflecting major key areas.

Fundamental

1. For underground water, *protective shroud with two compartments (newly developed within this thesis)* should be used. It allows use SPMDs for both chemistry and/or toxicity.
2. Repeatability (in the term of precision) of SPMDs, as used for both Rs and PRCs approach exhibits excellent parameters, mainly with RSD within the range of 5-15%. For exceptional cases (with values close to LODs) RSD is within 20-40%.
3. In comparison with biotic organisms, like *Dreissena-polymorpha*, main metabolic changes were found in the less persistent compounds, like PAHs and OCPs. In the case of PCBs, PCBs contributes due to its persistence to its biomagnification from food chain as well as from dissolved phase. Due to molecular similarities and $\log K_{OW}$, similar behaviour for both PCDD/Fs and PBDEs is expected.
4. It has been found that PCB contamination and its occurrence in environment can be dependent on its structure. Further research in this field is needed.
5. For definition of *geogenic background, qualitative profile can be expected for phenanthrene, anthracene, fluoranthene and pyrene*; total concentration to be expected is about 1 ng/L (derived from Rs approach). However, for PCBs, background contamination even in glacial water *does not allow to identify pure geogenic background* (for both Wieliczka and Poděbradka), with apparent dominant Hexa-CB profile. Anthropogenic background for PCBs can be estimated at the level of about 20 pg/L (estimated by Rs approach), as analysed within 95 measurable congeners, as the lowest found concentration. For OCPs (as sum of x-HCHs, HCB and DDTs and their metabolites) geogenic background values can be estimated at the limit 1,5 ng/L, similarly to PAHs; it of course reveals similar fundamental observation about absence of pure geogenic background, due to historical use, like PCBs.
6. Principally, *it is not generally easy to correlate toxicity with chemical composition* of such mixture

- sample, like aliquots from SPMDs containing POPs. If such approach is realised, it is strictly limited for tested systems (based on specific synergism and antagonism). For glacial water Poděbradka, used bioassays *showed no dependence on varying concentrations of PAHs*; changes in PCB concentration induced homogenous changes in toxicity response of all used organisms. The HCH together with DDT caused significant response of bacterium, although crustaceans and algae show no sensitivity to this level of pesticides.
7. The realised observations show that further detailed identification of sources will be needed for application of water management with the aim of source identification and abatement. Within this study, main contamination sources causing river contamination, were identified as follows: Břilina-Ústí nad Labem, Elbe-Valy, Elbe-Hřensko, Elbe-Obřížství, Lužická Nisa, Odra-Bohumín, and Svratka-Židlochovice.
 8. Distributions of data differ not only by mean levels but also by their forms. Distributions are non-Gaussian (“normal”). Domains are finite, densities asymmetric.
 9. Both *statistical and gnostic approach* is suitable for data evaluation in marginal and multivariate data analysis. Special parameters arising from the gnostic theory tests, like LB, UB, as well as the shape of DF and PF are welcomed for definition of expected range of data and comparison of methods.
 10. If correlation is used, robustness of used correlation coefficients shall be tested and those most robust applied; for studies within this thesis, the order of robustness is as follows *Pearson < Kendal < Spearman < Gnostic*. A regression seems to be useful as a complementary tool, as represented by various methods given by statistical.
 11. In the evaluation of toxic effects, within given studies, there were found correlation among PCB (directed by HexaCB), OCPs (DDT and metabolites and HCB) with *Vibrio fischeri*, and *Daphnia magna* with PAHs and PCBs (directed with PentaCB). All correlations are positive.

Other – helpful for applications

12. If SPMDs is used for litigation and comparability with control limits, it is important that SPMDs provides sampling of ONLY dissolved phase and generally yields a *lower concentration* than for grab sampling and treatment of whole samples.
13. If strong biofouling is expected, *PRCs approach shall be used* or *Rs approach* with biofouling corrections (e.g. surface water, waste waters). PRCs approach provides better accuracy and data interpretation.
14. For deployment, *sampling protocol* must be adopted and verified (e.g. based on those adopted within this thesis) for majority cases of environmental assessment. Special attention is paid for using blanks to avoid field or/and cross-contamination.
15. There are very important qualification to the safety in the particular field is to be

- supported by appropriate tools (e.g. safety belts, prams, solid shoes, cloth). No compromises are allowed in extreme conditions (e.g. manipulation at strong streams, deep depths).
16. Sampling places are to be arranged as described above, with the closest approach to experimental conditions of calibration, with elimination of flow velocity (standard protective shrouds). If flowing conditions do not allow to moderate a stream, special vessels are to be used for deployment (e.g. WWTP, industrial outlets, wells outlets),
 17. Deployments are to be provided in matrices/environmental samples by standard PRCs, with own QA/QC to reach the highest level of accuracy. It is not advisable to add any compounds by direct injection into SPMD.
 18. It is advisable to have both biotic and SPMDs for biomonitoring studies. This combination allows estimation intakes of POPs from all sources, as basement for risk assessment from food chain, and from ambient environment in dissolved phase without metabolisation.
 19. Analytical methods are to be under statistical regulation (*control charts*). All analytical methods are to be used in accordance with GLP. If possible, isotope-labelled compounds shall be used for highest quality and traceability to primary standards.
 20. If SPMDs is used as pre-treatment tool for fat reduction, PCBs and PCDD/Fs distribution in two special clusters is to be taken in the account, reflecting various chemical structures and log Kow of those compounds. On the other hand, no correlation was found between any congeners to the amount of treated sample (about 10 grams). Very good reproducibility is expected as derived from reproducibility ranging from 1-12% between sample recoveries. SPMDs is applicable for clean-up of following samples: *feeding stuffs* (premixes), *food* (feeding oil, raw animal (pig) fat, butter, natural and dried eggs, sausages, salami, chocolate, whipped cream, cheese, beef, pork, fish, poultry, kidney, liver, dried milk), *human adipose tissue*, having fat content higher than 1% w/w.
 21. Concept of V_{tox} is able to express value of toxicity even if the SPMD membrane was exposed in natural clean and apparently non-toxic site. If the value of V_{tox} increases above critical level, then the site is toxic even for onsite living organisms.
 22. Grab sampling with combination of commonly available analytical techniques is recommended for direct estimation of POPs contamination of produced sludge, due to unpredictable distribution of POPs between liquid and solid phase.
 23. The SPMDs seem to be a suitable tool for qualitative and quantitative monitoring in key profiles of sewage treatment plants. For these cases, all measured parameters indicate, that the major problem is focused to sludge, represented by solid phase at cleaning process. It underlines necessity to accept special limits for assessed POPs, especially if

- used in agriculture. Here, the toxicity testing plays important role in overall quality assessment of both inlets/outlets and produced sludge due to limited set of known chemical parameters to be accessed.
24. All POPs, including PAHs contributes to increasing the solid phase (sludge). This fact underlines the necessity to accept limits for those POPs in sludge, especially due to its consequent use in agriculture.
 25. As results from total PCBs and PAHs concentrations, exceeding the limit concentration for sludge according to 86/278/EEC proposal is to be expected. For that reason, a special attention must be paid when municipal waste-water treatments plants are used for industrial waters.
 26. PCA methodology can be used for identification of similarities in behaviour of both samples and variables. PCA can be also used for identification of goodness of fit, if one group is considered as reference (the cluster “core”) and other as comparable (the cluster “scatter”), e.g. as for general evaluation of capability of handling with SPMDs for individual POPs.
 27. For complex analyses, R-language is a valuable tool, allowing data analysis based on both statistical and gnostic approach.
 28. The best correlation results by using correlation among individual compounds; however, complementary method of correlation of individual isomers is needed to verify models. In that case, loss of information can be accounted about 10%.
 29. The Guide for gnostic analysis can be followed and/or supplemented on new environmental applications, see the link www.math_gnostics.com (as well as other relevant information in this field).
 30. Cluster analysis is also affected by outliers and non-normality of original data sets. Prior to performing multivariate analyse, it is necessary to check the probability features of the variables under this thesis. High skewness and kurtosis values for most of elements showed that without previous transformation they cannot be analysed. Improved normality features are observed for the Box-Cox transformed data sets. The Box-Cox transformation has significantly reduced the skewness values of data sets, pushing them towards zero. This feature is beneficial to multivariate analysis: if the data set cannot be made normal, it should be near normal or symmetric. The CA was applied to identify different geochemical groups, clustering the sample with a similar element content. The used linkage type was undertaken according to the Ward-algorithmic method.
 31. Effect of Box-Cox transformation, despite rigorousness of the method, results into interdependence of data.

Intentions for future work/research

There is a lot to be studied in near future. Both basic and application research is needed. Following intentions can be understood as a part of my individual research plan, as defined in some keywords (written in *italic*)

Some applications described above are only small part confirming its applicability. By combination of excellent properties of SPMDs and other valuable passive sampling tools, giving representative data, of complex analytical, statistical and gnostic methods supporting valuable interpretation, the existing gap between assessed chemicals and their effects to humans can be bridged over. Further research shall be focused to specific effects reflecting specific PCBs chirality. From many new indices, despite many facts found about POPs, there is a lot to be clarified about PCBs in relation to their *chirality* and *toxicity*. Applicability of POPs within the framework of *remediation* opens a way to satisfaction of further needs and motivation of corresponding studies. Moreover, with respect to *endocrine disrupting chemicals*, further development would be focused to their identification, quantification, identification of their sources and limitations.

As to statistical and gnostic analysis, I consider as necessary to further develop complex methodologies for treatment and interpretation of environmental data. Both these analytical methods shall stand as complementary approaches. Whereas statistical methods of univariate and multivariate data analysis will be used by means of commercially packages (mainly S-Plus®, Statistica®, NCSS® packages, QC.Expert™), gnostic programs has been developed into R-language. The new system shall have following features: complex system, accessible world-wide, having: (i) regular, controlled versioning, (ii) adoption to user-specific needs, (iii) enhancement for other applications, (iv) good mathematical, technical and pedagogical background, mainly in strongly motivated experts. First results shall be available in mid 2010, having first Summer School for MVRDA.

Quantification guidelines of uncertainties for SPMDs, applicable for the complex of passive sampling methods jointly used (e.g. DGTs, POCIS, MESCO...) is intended to be created within the frame of a present granting project for coming 2 years. This also includes newly opened series for intercalibration studies (Round-Robin tests) for those mentioned PS, started by SPMDs due to their widest applications and number of interested laboratories, firstly announced as IPSIC2010 in April 2010. This initiative would support QA/QC issues for the broader use of passive sampling techniques.

One activity, relevant to scientific work has not been so far mentioned: due to personal believe and my strong involvement in further progress in passive sampling in general. I consider as a great challenge to continue in organising the IPSW since 2000 (see www.animaracio.com/ipsw2009 from the most recent meeting), gathering experts around passive sampling systems worldwide. The IPSW, apart of its own programme, used to host several satellite workshops of EU funded programmes, as an expert group meeting dedicated to passive sampling techniques for monitoring the emerging

pollutants, the network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants. The target audience, from present up to near future would comprise experts in the development and application of passive sampling technologies, such as SPMDs, DGTs, POCIS, MESCO and Chemcatcher in environmental monitoring and assessment.

All intentions would of course require a lot of financial sources and time and concrete programmes, for many of which I have already released, newly developed and prepared new programme calls. Other ones, to be launched latter, would be based on current needs.

Projects overview

Below, there is listed overview of projects, thematically oriented as this thesis and their granting agencies or stakeholders are kindly acknowledged with their support. It is a list of both finished and ongoing projects.

Name	Period / Stockholder
Supervision of remediation procedure of dioxin contamination at Spolana-Neratovice chemical plant	2003-2007 / MEnv (through OPV, Ltd).
Assessment of POPs contamination after flood episode in 2002	2002 / Ministry of Health CR
Pilot biomonitoring studies	1997-2002 / CHMI + IPH Ostrava
Biomonitoring of harmful compounds in Czech and Bohemia Rivers	2003-ongoing / MEnv (through CHMI)
Research and validation of new integral sampling methods of persistent organic compounds, heavy metals with toxicity tests for monitoring applications	2004-2006 / GAČR
Management of groundwater at industrially contaminated areas (MAGIC)	2005-2008 / Interreg IIIB / CADSES
Focus on Key Sources of Environmental Risks (FOKS)	2008-2011 / Central Europe / 1CE026P3
Full-chain and uncertainty approaches for assessing health risks in future environmental scenarios (2-FUN)	2007-2010 / 6FP

4 APPENDIXES

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H. Glossary overview

Accreditation: a process in which certification of competency, authority, or credibility is presented. For laboratory operation, analytical quality is directed in accordance with EN ISO/IEC 17025 standard.

Bio-accumulation: the ability to accumulate some compounds in an animal through absorption from its diet and immediate environment.

Bio-concentration: the ability to accumulate compounds in an organism through absorption from its immediate environment.

Bio-magnification: a concentration increase of particular compound in organisms with increasing intensity.

Chronic toxic effect: long-term, low-level exposure effect.

Composite sample: a non-discrete sample composed of two or more aliquots (of equal volume) collected at various sampling points or times.

EC50: the concentration of a chemical, in the ambient environment, that exerts a 50% change of the measured effect on the test organisms when compared to the control under standard conditions and over a specified exposure period.

Endocrine disrupters: synthetic chemicals that can disrupt hormones in humans and animals. These "hormone disrupters" can cause cancer, birth defects, and immune problems. Even incredibly tiny concentrations can interfere with reproduction.

Flow proportional composite: a sample collected proportionally to the flow rate during the compositing period by either a time-varying/constant volume or time-constant/varying volume method.

Grab sample: discrete aliquot from one specific sampling location at a specific point in time.

Gnostics: a mathematical theory of quantitative recognition of an individual item of uncertain data. This theory is based on the theory of measurement, general algebra and Riemannian geometry. It proves deep ties with Einstein mechanics. Fundamentally, it is suitable for small size data sets.

In-situ: means to examine the phenomenon exactly in place where it occurs (i.e. without moving it to some special medium).

LC50: the concentration of a substance that is estimated to be lethal to 50% of the test organisms.

Long-range transport: the potential to travel great distances from the source of release through various media (e.g. air, water, and migratory species).

Migration pathway: runoff from the waste pile, lagoon, drum dump, or agricultural activities; outfall from the lagoon or sewage plant (surface water).

Normal conditions: temperature 25°C and pressure 101325 Pa.

Passive sampling: collection of contaminant in passive sampling device in a completely passive manner. Sampling is directed by the diffusion of chemicals from a material (sampled medium), where chemical fugacity or potential is high, to a material (receiving medium or sorbent), where chemical fugacity or potential is low.

Persistence: the ability to resist degradation in various environmental media (e.g. air, water, soil, and sediments), wildlife and humans (e.g. adipose tissue, blood) for a particular time (months, years).

Potential Sources: site (waste pile, lagoon); drum dump; sewage plant outfall; agricultural activities.

Quality assurance: an integrated system of management activities involving planning, implementation, assessment, reporting, and quality improvement to ensure that a process, item, or service is of the type and quality needed and expected by the client.

Quality control: the overall system of technical activities that measures the attributes and performance of a process, item, or service against defined standards to verify that they meet the stated requirements established by the customer; operational techniques and activities that are used to fulfil requirements for quality. The system of activities and checks used to ensure that measurement systems are maintained within prescribed limits, providing protection against “out of control” conditions and ensuring the results are of acceptable quality.

Quality: the totality of features and characteristics of a product or service that bears on its ability to meet the stated or implied needs and expectations of the user.

Repeatability: precision under *repeatability* conditions, where independent test results are obtained with the same method on identical test item in the same laboratory by the same operator using the same equipment within short intervals of time.

Reproducibility: precision under *reproducibility* conditions, where test results are obtained with the same methods on identical test items in different laboratories with different operators using different equipment.

Standard operating procedure: a written document that details the method for an operation, analysis, or action with thoroughly prescribed techniques and steps and that is officially approved as the method for performing routine or repetitive tasks.

Time composite: (chronological sampling), is composed of a varying number of discrete aliquots collected at equal time intervals during the compositing period.

Traceability: (in a calibration sense) traceability relates measuring equipment to national or international standards, primary standards, basic physical constants or properties, or reference materials.

Validation: confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use have been fulfilled. In design and development, validation concerns the process of examining a product or result to determine conformance to user needs.

Variability: observed difference attributable to heterogeneity or diversity in a population/observation. Sources of variability are the results of natural diversifying processes and stem from environmental differences among elements of the population/observation. Variability is not usually reducible by further measurement but can be better estimated by increasing sampling.

Variance (statistical): a measure or dispersion of a sample or population distribution.

I. List of abbreviations and symbols

AMAP	Arctic Monitoring and Assessment Programme
BAF	Bio-accumulation Factor
BAT	Best Available Techniques
BCF	Bio-concentration Factor
BEP	Best Environmental Practises
BMOs	Bio-monitoring Organisms
BLOD	Below of detection limit
CAS	Chemical Abstracts Service
CR	Czech Republic
CHMI	Czech Hydrometeorological Institute
DF	Distribution function
DDD	Dichlorodiphenyl dichloroethane
DDE	Dichlorodiphenyl dichloroethylene
DDT	Dichlorodiphenyl trichloroethane
DOC	Dissolved organic carbon
DGT	Diffusive Gradient in Thin films
DQO	Data quality objectives
EA	Executing Agency
EDA	Exploratory Data Analysis
EDF	Empirical Distribution Function
ELDF	Estimating Local Gnostic DF
EGDF	Estimating Global Gnostic DF
EST	Environmental Sampling Technology
FA	Factor Analysis
FAO	Food and Agricultural Organisation
FLD	Fluorescence detection
GA	Gnostic Analysis
GACR	Grand Agency of Czech Republic
GC	Gas chromatography
GEF	Global Environment Facility
GIWA	Global International Waters Assessment

GUI	Graphical User Interface
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPLC	High Performance Liquid Chromatography
HRGC	High-resolution gas chromatography
HRMS	High-resolution mass spectrometry
IA	Implementing Agency
IARC	The International Agency for Research on Cancer
IFCS	Inter-Governmental Forum on Chemical Safety
IGO	Intergovernmental organisations
INC	Intergovernmental Negotiating Committee
IOMC	Inter-Organisational Programme for the Sound Management of Chemicals
IPCS	International Programme on Chemical Safety
IPH	Institute of Public Health (Ostrava)
IPSIC	International Passive Sampling Intercalibration Comparison
IPSW	International Passive Sampling Workshop and Symposium
IUPAC	International Union of Pure and Applied Chemistry
K _{ow}	Octanol-water partition coefficient
LDPE	Low-density polyethylene
LRMS	Low-resolution mass spectrometry
LRTAP	Long-Range Transboundary Air Pollution Convention
LOD	Limit of detection
ME _{env}	Ministry of Environment CR
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MVDA	Multivariate data analysis
MVRDA	Multivariate robust data analysis
NGOs	Non-Governmental Organisations
NRL - POPs	National reference laboratory for POPs, IPH Ostrava
ND	Non-detected (without specification of numerical value LOD)
OCPs	Organochlorine pesticides
OP	Operational Programme
PAHs	Polycyclic Aromatic Hydrocarbons
PBDE	Polybrominated Diphenyl Ethers

PBT	Persistent, Bioaccumulative and Toxic
PC	Principal Component
PCA	Principal Component Analysis
PCBs	Polychlorinated Biphenyls
PCDDs	Polychlorinated dibenzo- <i>p</i> -dioxin (often abbreviated as dioxins)
PCDFs	Polychlorinated dibenzofurans (often abbreviated as furans)
PCNs	Polychlorinated naphthalenes
PCP	Pentachlorophenol
PD	Probability density
PDF	Project Preparation and Development Facility
PLS	Partial Least Squares Projection into Latent Structures
POCIS	Sampling system for hydrophilic organic (e.g. pesticides, hormones, etc...)
POPs	Persistent Organic Pollutants
PP	Polypropylene
PRCs	Performance reference compounds
PS	Passive sampler
PTS	Persistent Toxic Substances
PVC	Polyvinylchloride
Q ²	Cross-validated explained variance
QSAR	Quantitative Structure Activity Relationship
R ²	Explained variance
RBA	Regionally based assessment
RSD	Relative standard deviation
SPMD	Semipermeable Membrane Device
SOP	Standard Operational Procedure
TEF	Toxic equivalency factor
TEQ	Toxic equivalent concentration
TOC	Total organic carbon
TWA	Time weighted average
UN	United Nations
UNCED	United Nations Conference on Environment and Development
UNECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme ?

UNEP	United Nations Environmental Programme?
UNIDO	United Nations Industrial Development Organization
US EPA	United States Environmental Protection Agency
WWTP	Waste water treatment plant
WHO	World Health Organisation
V _{TOX}	toxicological factor for toxicity evaluation (based on EC50)

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