

FOKS Guide for Passive sampling technology for water assessment

A tool for identification of key sources of water contamination

Tomas Ocelka, Brano Vrana, Petr Kohout, Grzegorz Gzyl

IPH Ostrava

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Acknowledgement

- Intro words – FOKS relation
- Core Group
- Supplemental group
- Final words

Abstract

To be finished after the final Guide compilation

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 environment³⁶⁻⁴¹.

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 guide, 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. 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.

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 (TWA) 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.

Strategy for identification source of contamination

In a study noticed below (see application **Chyba! Nenalezen zdroj odkazů.**), 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 1), 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.

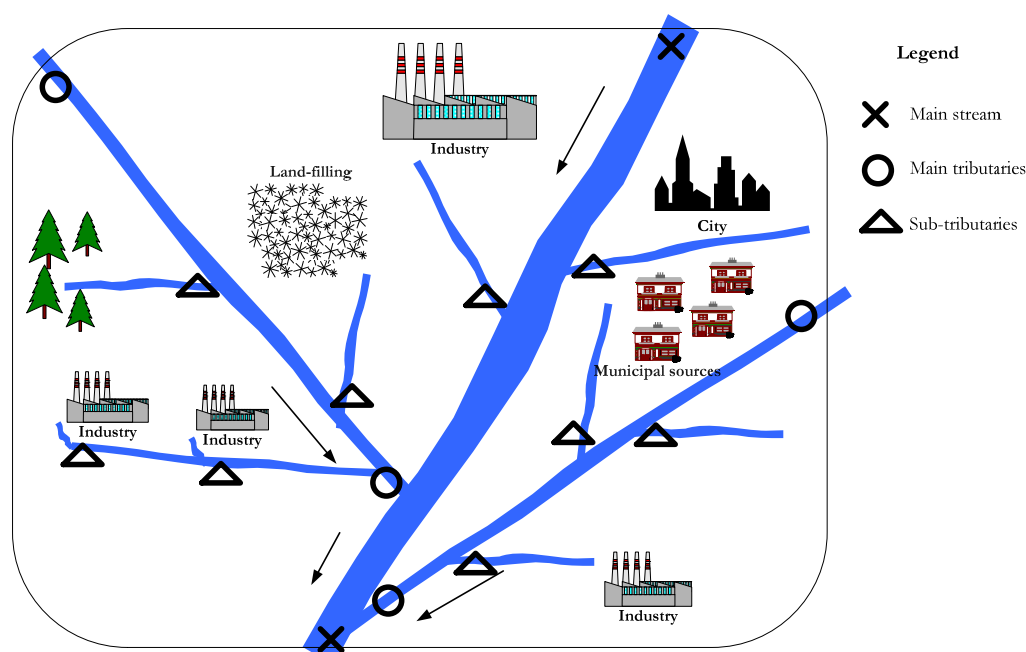


Figure 1 Identification of sources of POPs by SPMDs

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.

Arrangement of deployment devices

All arrangements described below, were adopted from various applications. Practical arrangements using standard membranes can be seen in the Figure 3. 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 2.

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 project).

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 intervals¹.

Fully equipped sampling system was mounted into secured place, by rod or rope (see Figure 3). 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.

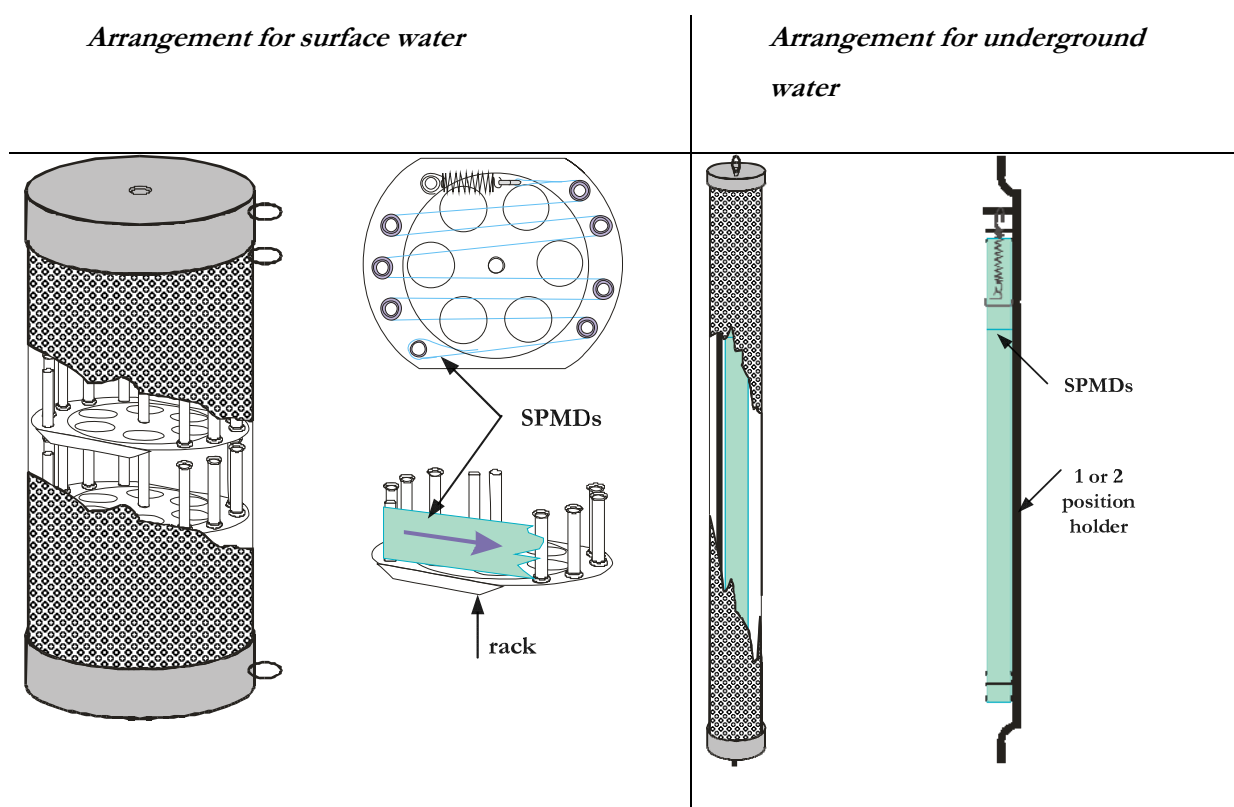


Figure 2 Sampling arrangements – protective shrouds for SPMDs

For surface water: SMPDs are inserted and stretched in sampling racks (left); for underground water SPMDs they are inserted and stretched in sampling holder (right)

¹ Mostly 1-2 hours. In some cases (see application **Chyba! Nenalezen zdroj odkazů.** below), passive sampling devices for heavy metals and polar organics were used (such DGTs or POCIS respectively), and mounted to protective shroud as well.

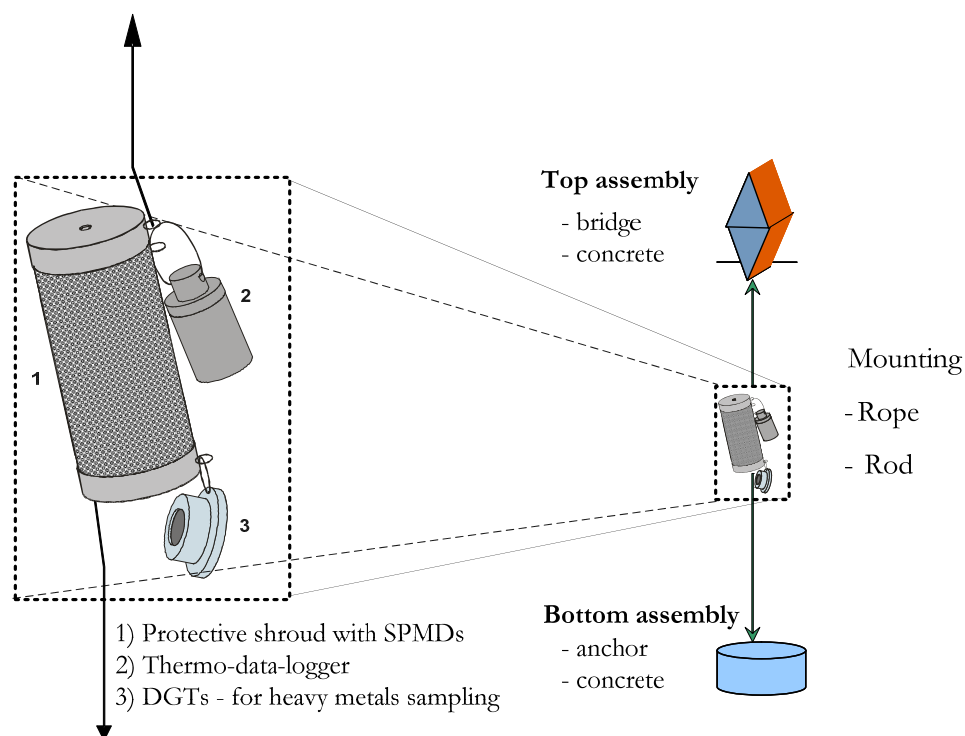


Figure 3 Overall configuration for SPMDs deployment

General sampling strategy for PS

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 project.

Sampling by PS

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.

Rationale and design of selected PS

1.1.1. Non-polar organics - SPMDs

Passive sampling methodology for POPs operates on the partitioning principle of particular contaminant with passive 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).

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.

Since the development of SPMDs, there were various arrangement used. Applications described in this project 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-octacenoyl]glycerol) of high purity (>97%), which makes a thin film in membrane. Exploded view of overall SPMDs is given in Figure 4. 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 project) 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.

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}.

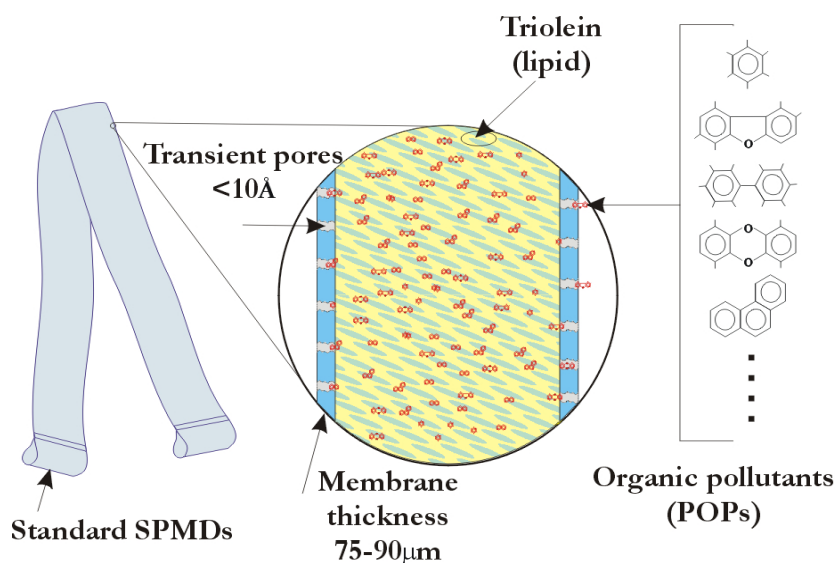


Figure 4 Exploded view of standard SPMDs

→ Applicability of SPMDs

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 sequestrant, 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 sequestrant, 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 sequestrants optimized the design, which was introduced in 1989, and consisted of 99% triolein as sequestrant, filled in layflat LDPE^{99, 126}. Just triolein has been selected as a standard sequestrant for SPMDs design. However, fish lipid and silicone fluids exhibited good results for sequestering non-polar compounds¹⁰⁰.

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.

→ Uptake modelling

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 5).

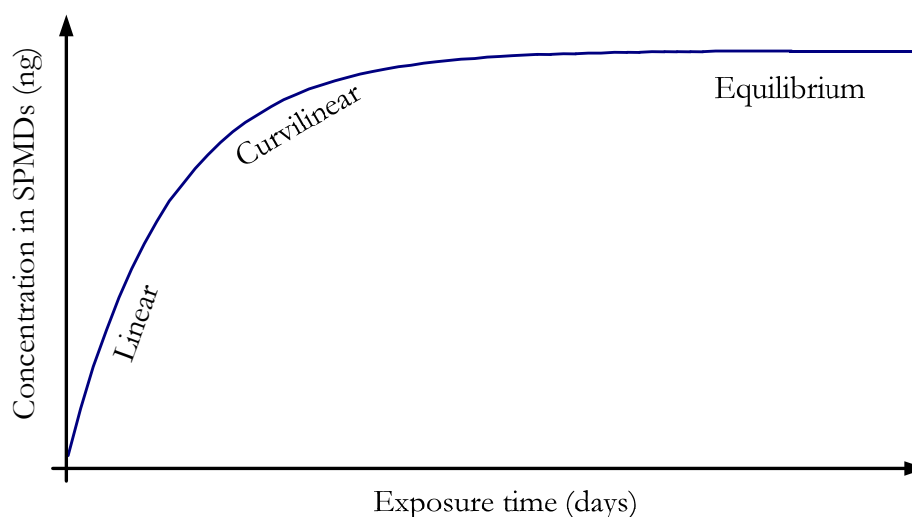


Figure 5 Typical uptake of sampled POPs in SPMDs

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 $L \cdot d^{-1}$, 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±4°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 project, both approaches were used. However, for SPMDs is only PRCs alternative. In use DGTs, POCIS or CD, current research and applications count with calibration data (DGT, POCIS) and theoretical model (CD).

→ 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 project, 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:

$$C_{w,i} = \frac{n_{S,i}}{k_{D,PRC} K_{SW} t V_S}$$

Eq. 10

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

1.1.2. Polar organics - POCIS

Industry developed less persistent, more water soluble polar or hydrophilic organic compounds (HpOCs), which generally have low bioconcentration factors. However, evidence is growing that the large fluxes of these seemingly more environmentally friendly compounds (e.g., pesticides, prescription and non-prescription drugs, personal care and common consumer products, industrial and domestic-use chemicals, and their degradation products) into aquatic systems on a world-wide basis may be responsible for incidents of acute toxicity and sublethal chronic abnormalities.

Within this project, various polar pesticides are focused into main concern of applications, occurred in Jaworzno dump site.

The classification of a compound as an HpOC is based on the presence of one or more polar functional groups (e.g., hydroxyls) or a significant molecular dipole moment. The n-octanol–water partition coefficient (Kow) provides a convenient but somewhat arbitrary means of discriminating between HpOCs and HOCs. For example, volatile organic compounds may have relatively low Kow values but they are generally non-polar. In this chapter, we use a log Kow value of 3.0 as the cutoff point between HOCs and HpOCs. However, it is important to have some overlap in the compounds sequestered by samplers for HOCs and HpOCs to ensure holistic sampling of organic contaminants.

The POCIS has been shown to sample a wide variety of HpOCs as well as some HOCs with log Kow values between 3.0 and 4.0. The POCIS consists of a disk-like configuration of a solid-phase sorbent or a mixture of sorbents sandwiched between two microporous polyethersulfone (PES) membranes. Stainless steel rings are used to form a compression seal to prevent sorbent loss. Exploded overview of POCIS is given on the Figure (ref).

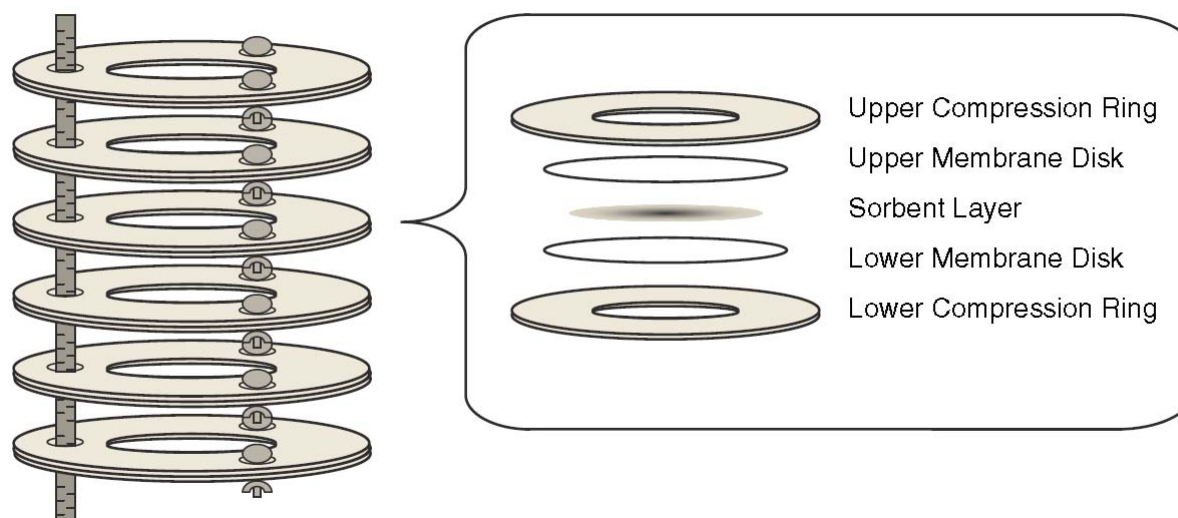


Figure 6 The POCIS exploded view.

The microporous PES membrane acts as a semipermeable barrier between the sorbent and the surrounding environment. It allows dissolved HpOCs to pass through to the sorbent, while particulates, microorganisms, and macromolecules with cross-sectional diameters greater than 100 nm are selectively excluded. Without the protection of the membrane, biofouling of POCIS sorbents is very likely during extended exposures (42 weeks) in surface waters.

For a typical POCIS disk used in field studies, the effective surface area of the membranes in contact with exposure waters is 41 cm² and the sorbent mass is about 228 mg. Herein we have used a standard POCIS having a surface area to sorbent mass ratio of E180 cm²/g

→ Uptake modelling

Accumulation of chemicals by passive samplers generally follows firstorder kinetics, which is characterized by an initial integrative phase, followed by curvilinear and equilibrium partitioning phases. For all phases of uptake sampling rates (R_s ; units of L or mL/day) and sorbent–water (SW) partition coefficients (K_{sw}) are independent of exposure concentrations. During the integrative phase of uptake, a passive sampling device acts as an infinite sink for contaminants, and assuming constant exposure concentrations, residues are accumulated linearly relative to time. Based on results to date, POCIS remains in the integrative phase of sampling during exposure periods of at least 30 days. An advantage of integrative samplers over equilibrium partition samplers is that TWA concentration of contaminants can be determined from sampler concentration data (assuming appropriate calibration data are available). Unlike samplers that rapidly achieve equilibrium (characterized by very high surface area to sorbent volume or mass ratios), chemical residues from episodic release events are retained by integrative samplers

at the end of the exposure period. Thus, integrative samplers have very small analyte loss rates and times to reach equilibrium are very large.

Huckins et al. (Ref) formulated equation (7) for integrative (i.e., linear) sampling by a passive sampling device (similar for SPMDs, see above, derived for POCIS):

$$C_{w,i} = C_{s,i} M_S / R_{s,i} t \quad \text{Eq. 11}$$

Where M_S is the mass of the sorbent. The R_s is dependent, how the uptake is controlled. Sampling rates for selected chemicals have been determined using a static renewal scheme. Specific details regarding the calibration procedure have been described by Alvarez et al. (Ref) and from sources (Ref).

Handling, sampling and analysis – the protocol for PS

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 project were analysed. The scheme showing application relevant for quality data production can be seen in following Figure 7, with detailed description in the Table 1.

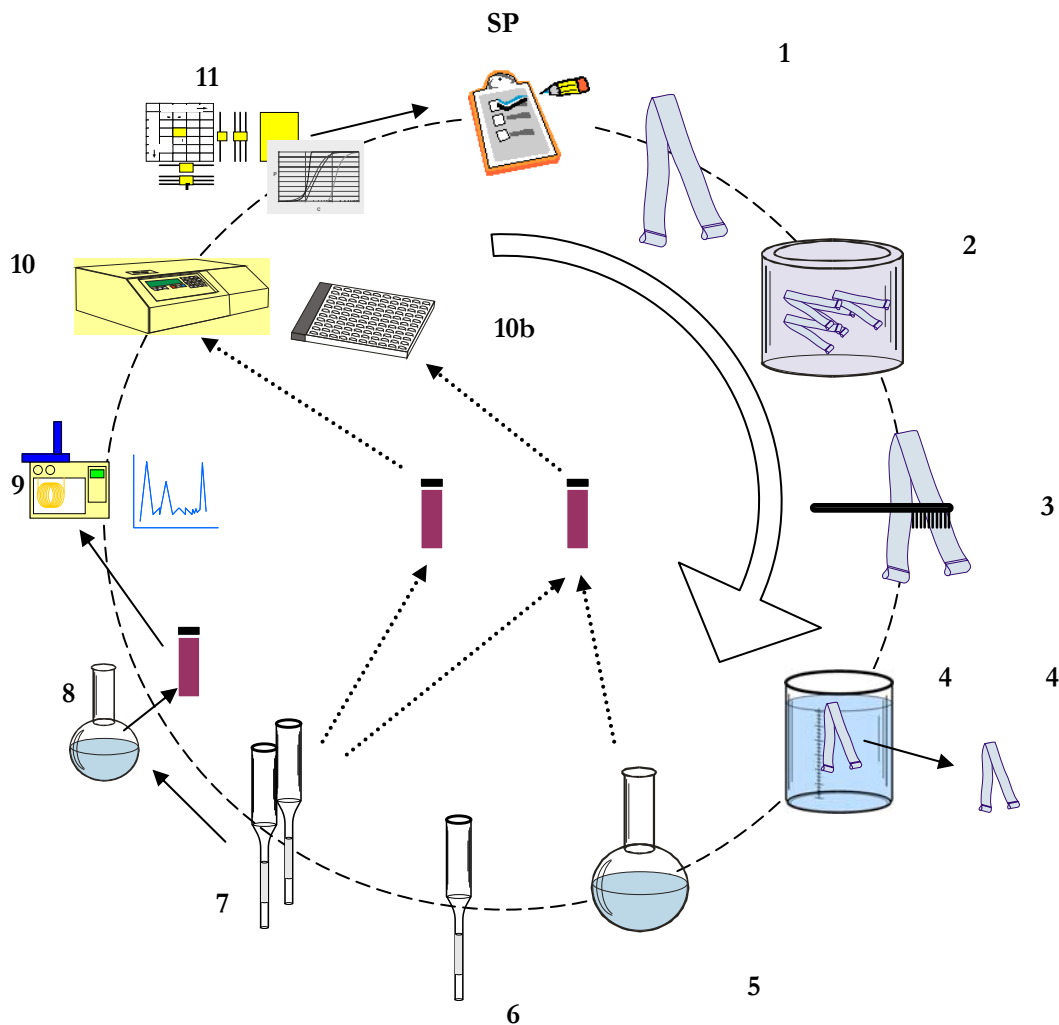


Figure 7 Overall SPMDs processing (the “SPMDs clock”)

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).

<i>Ref</i>	<i>Operation</i>	<i>Description/ Requirements /Blank (frequency) site/project/lot/sample set * = if applicable; see details in the Appendix ()</i>
1	Fabrication of SPMDs	Defined QA/QC conditions / FaBI (0/1*/1*/0)
1	Storage	--/Air-tight can, about -10°C/PrBI (0/1*/1*/0)
2	Transport	--/Air-tight can, ≈0°C/ TrBI (1/1*/0/0)
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/ReBI (0/1*/0/0)
4a	Dialysis	250 ml solvent, 2 exchange/24 hour per one solvent/FaBI*, PrBI (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 + ReBI (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

Ref	Operation	Description/ Requirements /Blank (frequency) <i>site/project/lot/sample set</i> *= <i> if applicable; see details in the Appendix ()</i>
		purposes/given from 11

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

→ QA/QC

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 project, 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.

→ Sampling procedures

SPMDs

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 $^{12}\text{C}_{13}$ 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.

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 μl 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 H_2SO_4 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 $^{12}\text{C}_{13}$ 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 ^{13}C -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 μ m) was used for separation of PAHs under following instrument set up: splitless injection of 1 μ l 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 $10^{\circ}\text{C}/\text{min}$ 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. Monoortho 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 1 μl 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.

Applications on pilot sites

Data treatment, evaluation

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 project, both statistical multivariate and gnostic univariate and Multivariate methods were used for supporting evaluation along with all tested parameters, from various monitoring data.

1.1.3. 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-Cox112, 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.

1.1.4. 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:

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

where the X is a model for source data. The first part of the right hand side ($\bar{1}x'$) 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.

1.1.5. 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 combinations 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.

1.1.6. 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 Eucliden 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.

1.1.7. 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.

1.1.8. Gnostic analysis

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 project) 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 project¹⁵⁸⁻¹⁶¹, 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 criterions of estimation	Formal (e.g. least squares or max. likelihood)	Minimum information loss or entropy increase determined by variation principle
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

1.1.9. 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 project, 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 dimensionless. 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^{®2} - 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.

² 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.

References

1. Harrad, S., Persistent organic pollutants: Environmental behaviour and pathways of human exposure, Springer Netherlands, 2001.
2. Borgå, K., Wolkers, H., Skaare, J. U., et al., Environmental Pollution, 2005, 134, 397-409.
3. de Wit, C. A., Herzke, D. and Vorkamp, K., Science of The Total Environment, In Press, Corrected Proof.
4. Kelly, B. C., Ikonomidou, M. G., Blair, J. D., et al., Science of The Total Environment, 2008, 401, 60-72.
5. Moser, G. A. and McLachlan, M. S., Chemosphere, 2002, 46, 449-457.
6. Paustenbach, D. J., Regulatory Toxicology and Pharmacology, 1989, 10, 204-243.
7. Takenaka, S., Todaka, T., Nakamura, M., et al., Chemosphere, 2002, 49, 161-172.
8. Černá, M., Bencko, V., Brabec, M., et al., Chemosphere, 2010, 78, 160-168.
9. Čupr, P., Bartoš, T., Sáňka, M., et al., Science of The Total Environment, 2010, 408, 486-494.
10. Čupr, P., Klánová, J., Bartoš, T., et al., Environmental Pollution, 2006, 144, 406-413.
11. Dvorská, A., Lammel, G. and Holoubek, I., Atmospheric Environment, 2009, 43, 1280-1287.
12. Holoubek, I., Dušek, L., Sáňka, M., et al., Environmental Pollution, 2009, 157, 3207-3217.
13. Klánová, J., Kohoutek, J., Hamplová, L., et al., Environmental Pollution, 2006, 144, 393-405.
14. Kukučka, P., Klánová, J., Sáňka, M., et al., Environmental Pollution, 2009, 157, 3255-3263.
15. Přibyllová, P., Klánová, J. and Holoubek, I., Environmental Pollution, 2006, 144, 248-254.
16. Randák, T., Žlábek, V., Pulkrabová, J., et al., Ecotoxicology and Environmental Safety, 2009, 72, 737-746.
17. Shegunová, P., Klánová, J. and Holoubek, I., Environmental Pollution, 2007, 146, 257-261.
18. Taniyasu, S., Kannan, K., Holoubek, I., et al., Environmental Pollution, 2003, 126, 169-178.

19. ČSN ISO 5667-4: Jakost vod. Odběr vzorků. Část 4: Pokyny pro odběr vzorků z vodních nádrží (Water quality - Sampling - Part 4: Guidance on sampling from lakes, natural and man-made), ČNI/UNMZ Prague, 1994-03-01.
20. ČSN ISO 5667-10: Jakost vod. Odběr vzorků. Část 10: Pokyny pro odběr vzorků odpadních vod (Water quality. Sampling. Part 10: Guidance on sampling of waste waters), ČNI/UNMZ Prague, 1996-02-01.
21. ČSN ISO 5667-11: Jakost vod. Odběr vzorků. Část 11: Pokyny pro odběr vzorků podzemních vod (Water quality. Sampling. Part Guidance on sampling of groundwaters), ČNI/UNMZ Prague, 1996-02-01.
22. ČSN ISO 5667-18: Jakost vod - Odběr vzorků - Část 18: Pokyny pro odběr vzorků podzemních vod na znečištěných místech (Water quality - Sampling - Part 18: Guidance on sampling of groundwater at contaminated sites), ČNI/UNMZ Prague, 2002-05-01.
23. ČSN EN ISO 5667-1: Jakost vod - Odběr vzorků - Část 1: Návod pro návrh programu odběru vzorků a pro způsoby odběru vzorků (Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques), ČNI/UNMZ Prague, 2007-09-01.
24. ČSN ISO 5667-5: Jakost vod - Odběr vzorků - Část 5: Návod pro odběr vzorků pitné vody z úpraven vody a z vodovodních sítí (Water quality - Sampling - Part 5: Guidance on sampling of drinking water from treatment works and piped distribution systems), ČNI/UNMZ Prague, 2008-06-01.
25. ČSN ISO 5667-6: Jakost vod - Odběr vzorků - Část 6: Návod pro odběr vzorků z řek a potoků (Water quality - Sampling - Part 6: Guidance on sampling of rivers and streams), ČNI/UNMZ Prague, 2008-06-01.
26. ČSN EN 27828: Jakost vod. Metody odběrů biologických vzorků. Pokyny pro odběr vzorků makrozoobentosu ruční sítkou (ISO 7828:1985) (Water quality. Methods of biological sampling Guidance on handet sampling of aquatic benthic macro-invertebrates (ISO 7828:1985)), ČNI/UNMZ Prague, 1996-07-01.
27. ČSN EN 28265: Jakost vod. Konstrukce a použití kvantitativních vzorkovačů makrozoobentosu z kamenitých substrátů mělkých vod (ISO 8265:1988) (Water quality. Design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters (ISO 8265:1988)), ČNI/UNMZ Prague, 1996-07-01.
28. ČSN EN ISO 9391: Jakost vod. Odběr vzorků makrozoobentosu v hlubokých vodách. Pokyny pro použití kolonizačních, kvalitativních a kvantitativních vzorkovačů (Water quality. Sampling in deep waters for macro-invertebrates. Guidance on the use of colonization, quantitative and qualitative samplers), ČNI/UNMZ Prague, 1997-01-01.

29. ČSN ISO 5667-12: Jakost vod - Odběr vzorků - Část 12: Pokyny pro odběr vzorků dnových sedimentů (Water Quality - Sampling - Part 12: Guidance on sampling of bottom sediments), ČNI/UNMZ Prague, 1998-01-01.
30. ČSN EN 14011: Jakost vod - Odběr vzorků ryb pomocí elektrického proudu (Water quality - Sampling of fish with electricity), ČNI/UNMZ Prague, 2003-10-01.
31. ČSN EN ISO 5667-3: Jakost vod - Odběr vzorků - Část 3: Návod pro konzervaci vzorků a manipulaci s nimi (Water quality - Sampling - Part 3: Guidance on the preservation and handling of water samples), ČNI/UNMZ Prague, 2004-10-01.
32. ČSN EN ISO 5667-19: Jakost vod - Odběr vzorků - Část 19: Návod pro odběr vzorků v mořských sedimentech (Water quality - Sampling - Part 19: Guidance on sampling in marine sediments), ČNI/UNMZ Prague, 2005-02-01.
33. ČSN EN 15110: Jakost vod - Návod pro odběr vzorků zooplanktonu ze stojatých vod (Water quality - Guidance standard for the sampling of zooplankton from standing waters), ČNI/UNMZ Prague, 2007-01-01.
34. ČSN EN 14962: Jakost vod - Pokyny pro oblast použití a výběr metod pro odběr vzorků ryb (Water quality - Guidance on the scope and selection of fish sampling methods), ČNI/UNMZ Prague, 2007-01-01.
35. ČSN EN ISO 5667-15: Jakost vod - Odběr vzorků - Část 15: Pokyny pro konzervaci a manipulaci se vzorky kalu a sedimentu (Water quality - Sampling - Part 15: Guidance on the preservation and handling of sludge and sediment samples), ČNI/UNMZ Prague, 2010-04-01.
36. Boehm, P. D., Page, D. S., Brown, J. S., et al., Marine Pollution Bulletin, 2005, 50, 740-750.
37. Ellis, S. G., Booij, K. and Kaputa, M., Chemosphere, 2008, 72, 1112-1117.
38. Gustavson, K. E. and Harkin, J. M., Environmental Science & Technology, 2000, 34, 4445-4451.
39. MacRae, J. D. and Hall, K. J., Environmental Science & Technology, 32, 3809-3815.
40. Pablos, M. V., Fernandez, C., Garcia-Hortiguera, P., et al., Toxicol. Environ. Chem., 1999, 70, 115-127.
41. Shoeib, M. and Harner, T., Environmental Science & Technology, 2002, 36, 4142-4151.
42. Billet, S., Abbas, I., Le Goff, J., et al., Cancer Lett, 2008, 270, 144-155.
43. Butterworth, F. M., Pandey, P., McGowen, R. M., et al., Mutat Res, 1995, 342, 61-69.
44. Cachot, J., Geffard, O., Augagneur, S., et al., Aquat Toxicol, 2006, 79, 257-267.
45. Clement, B., Devaux, A., Perrodin, Y., et al., Ecotoxicology, 2004, 13, 323-333.

46. Evandri, M. G., Mastrangelo, S., Costa, L. G., et al., *Environ Mol Mutagen*, 2003, 42, 85-90.
47. Knerr, S. and Schrenk, D., *Crit Rev Toxicol*, 2006, 36, 663-694.
48. Nagayama, J., Nagayama, M., Haraguchi, K., et al., *Fukuoka Igaku Zasshi*, 1995, 86, 190-196.
49. Silberhorn, E. M., Glauert, H. P. and Robertson, L. W., *Crit Rev Toxicol*, 1990, 20, 440-496.
50. Jones, K. C. and de Voogt, P., *Environmental Pollution*, 1999, 100, 209-221.
51. Spurgeon, D. J., Jones, O. A. H., Dorne, J.-L. C. M., et al., *Science of The Total Environment*, In Press, Corrected Proof.
52. Wania, F. and Mackay, D., *Environmental Pollution*, 1999, 100, 223-240.
53. Van der Ven, L. T. M., van de Kuil, T., Leonards, P. E. G., et al., *Toxicology Letters*, 2008, 179, 6-14.
54. Marca Schrap, S. and Opperhuizen, A., *Science of The Total Environment*, 1993, 134, 381-385.
55. Volný, D., *Stochastic Processes and their Applications*, 1993, 44, 41-74.
56. Seoh, M. and Puri, M. L., *Journal of Statistical Planning and Inference*, 1989, 22, 271-294.
57. Chareka, P., *Statistics & Probability Letters*, 2009, 79, 1456-1462.
58. Dudzinski, M., *Statistics & Probability Letters*, 2008, 78, 347-357.
59. Kovanic, P. and Humber, M. B., *The Economics of Information---Mathematical Gnostics for Data Analysis*, Unpublished, 2003.
60. Kovanic, P. and Vlachy, J., *Czechoslovak Journal of Physics*, 1986, B 36, 71-76.
61. Kovanic, P., *Lecture Notes in Control and Information Sciences*, 1990, 158, 61-70.
62. Kovanic, P., *Problems of Control and Information Theory*, 1984, 13, 259-274.
63. Kovanic, P., *Kybernetika*, 1972, 8, 367-383.
64. Kovanic, P. and Humber, M. B., Portland, Oregon, USA.
65. Johnson, D., in *Encyclopedia of Soils in the Environment*, ed. H. Daniel, Elsevier, Oxford, Editon edn., 2005, pp. 264-271.
66. Miniero, R. and Iamiceli, A. L., in *Encyclopedia of Ecology*, eds. J. Sven Erik and F. Brian, Academic Press, Oxford, Editon edn., 2008, pp. 2672-2682.
67. Domingo, J. L., *Environment International*, 2006, 32, 121-127.
68. Rose, R. L., Hodgson, E. and Roe, R. M., in *Toxicology*, eds. M. Hans, G. S. Siegfried, M. Roger and W. Frank, Academic Press, San Diego, Editon edn., 1999, pp. 663-697.

69. Scippo, M.-L., Eppe, G., Saegerman, C., et al., in *Comprehensive Analytical Chemistry*, ed. P. Yolanda, Elsevier, Editon edn., 2008, vol. Volume 51, pp. 457-506.
70. Gray, L. E., Jr., *Toxicol.Lett.*, 1998, 102-103, 331-335.
71. Garritano, S., Pinto, B., Calderisi, M., et al., *Environ.Health*, 2006, 5, 9.
72. Xu, Y., Yu, R. M., Zhang, X., et al., *Chemosphere*, 2006, 63, 772-784.
73. Steinberg, R. M., Juenger, T. E. and Gore, A. C., *Horm.Behav.*, 2007, 51, 364-372.
74. Yang, M., Park, M. S. and Lee, H. S., *J.Environ.Sci.Health C.Environ.Carcinog.Ecotoxicol.Rev.*, 2006, 24, 183-224.
75. Roy, J. R., Chakraborty, S. and Chakraborty, T. R., *Med.Sci.Monit.*, 2009, 15, RA137-RA145.
76. Schlatter, C., *Science of The Total Environment*, 1994, 143, 93-101.
77. Holoubek, I., Adamec, V., Bartoš, M., et al., *Národní inventura persistentních organických polutantů v České republice. Projekt GF/CEH/01/003. Rok 2003. Ovzduší, hydrosféra, RECETOX - TOCOEN & Associates, Brno, 2003.*
78. Covaci, A., Gheorghe, A. and Schepens, P., *Chemosphere*, 2004, 56, 757-766.
79. Dvorská, A., Lammel, G., Klánová, J., et al., *Environmental Pollution*, 2008, 156, 403-408.
80. Guglielmo, F., Lammel, G. and Maier-Reimer, E., *Chemosphere*, 2009, 76, 1509-1517.
81. Shegunova, P., Klánová, J. and Holoubek, I., *Environmental Pollution*, 2007, 146, 257-261.
82. Breivik, K., Alcock, R., Li, Y.-F., et al., *Environmental Pollution*, 2004, 128, 3-16.
83. Calamari, D., *Toxicology*, 2002, 181-182, 183-186.
84. Kemmlein, S., Herzke, D. and Law, R. J., *Environment International*, 2003, 29, 781-792.
85. Lerche, D., van de Plassche, E., Schwegler, A., et al., *Chemosphere*, 2002, 47, 617-630.
86. Vallack, H. W., Bakker, D. J., Brandt, I., et al., *Environmental Toxicology and Pharmacology*, 1998, 6, 143-175.
87. Kalantzi, O. L., Martin, F. L., Thomas, G. O., et al., *Environmental Health Perspectives*, 2004, 112, 1085-1091.
88. Wang, Y. W., Zhao, C. Y., Ma, W. P., et al., *Chemosphere*, 2006, 64, 515-524.
89. Ceccatelli, R., Faass, O., Schlumpf, M., et al., *Toxicology*, 2006, 220, 104-116.
90. Costa, L. G. and Giordano, G., *Neurotoxicology*, 2007, 28, 1047-1067.
91. Fernlöf, G., Gadhasson, I., Pödra, K., et al., *Toxicology Letters*, 1997, 90, 189-197.

92. Hardy, M. L., *Chemosphere*, 2002, 46, 717-728.
93. Madia, F., Giordano, G., Fattori, V., et al., *Toxicology Letters*, 2004, 154, 11-21.
94. Naert, C., Van Peteghem, C., Kupper, J., et al., *Chemosphere*, 2007, 68, 977-987.
95. Marker, D. A. and Stevens Jr, D. L., in *Handbook of Statistics*, ed. C. R. Rao, Elsevier, Editon edn., 2009, vol. Volume 29, Part 1, pp. 487-512.
96. Seethapathy, S., Górecki, T. and Li, X., *Journal of Chromatography A*, 2008, 1184, 234-253.
97. ČSN EN ISO 5667-13: Jakost vod - Odběr vzorků - Část 13: Pokyny pro odběr vzorků kalů z čistíren a úpraven vod (Water Quality - Sampling - Part 13: Guidance on sampling of sludges from sewage and water treatment plants), ČNI/UNMZ Prague, 1999-03-01.
98. ČSN ISO 5667-14: Jakost vod - Odběr vzorků - Část 14: Pokyny k zabezpečování jakosti odběru vzorků vod a manipulace s nimi (Water quality - Sampling - Part 14: Guidance on quality assurance of environmental water sampling and handling), ČNI/UNMZ Prague, 2001-06-01.
99. Huckins, J. N., Manuweera, G. K., Petty, J. D., et al., *Environmental Science & Technology*, 1993, 27, 2489-2496.
100. Huckins, J. N., Tubergen, M. W. and Manuweera, G. K., *Chemosphere*, 1990, 20, 533-552.
101. Ocelka, T., Grabic, R., Kočí, V., et al., in *Ekoanalytika a Testy toxicity*, Editon edn., 2001, pp. 138-147.
102. Ocelka, T., Grabic, R., Hapala, P., et al., in *Dioxyny*, Editon edn., 2001, pp. 133-141.
103. Kočí, V., Ocelka, T., Dragoun, D., et al., *Environ Sci Pollut Res Int*, 2007, 14, 94-101.
104. Gramatica, P., Consolaro, F. and Pozzi, S., *Chemosphere*, 2001, 43, 655-664.
105. Gramatica, P. and Papa, E., *Environmental Science & Technology*, 2007, 41, 2833-2839.
106. Tuppurainen, K. and Ruuskanen, J., *Chemosphere*, 2000, 41, 843-848.
107. Liu, H., Papa, E. and Gramatica, P., *Chem.Res.Toxicol.*, 2006, 19, 1540-1548.
108. Liu, H., Papa, E. and Gramatica, P., *Chemosphere*, 2008, 70, 1889-1897.
109. Li, J. and Gramatica, P., *Mol.Divers.*, 2009.
110. Mills, G. A., Greenwood, R., Vrana, B., et al., *J Environ Monit*, 2009, 11, 1911-1914.
111. Ocelka, T., Vrana, B., Chmelova, M., et al., IPH Ostrava, Czech Republic, Ostrava, Editon edn., 2010, pp. 2-6.
112. Meloun, M., Militký, J. and Forina, M., *Chemometrics for analytical chemistry. Volume 1: PC-aided statistical data analysis*, Ellis Horwood, Chichester 1992, 1992.

113. Meloun, M., Militký, J. and Forina, M., Chemometrics for analytical chemistry. Volume 2: PC-aided regression and related methods, Ellis Horwood, Chichester 1992, 1994.
114. Kovanic, P., IIASA, Laxenburg, Austria, Laxenburg, 1991.
115. Bergqvist, P. A., Strandberg, B., Hjelt, M., et al., Organohalogen Compounds, 1990, 2, 103-106.
116. Bergqvist, P. A., Strandberg, B., Bergek, S., et al., Organohalogen Compounds, 1993, 11, 41-44.
117. Meadows, J., Tillitt, D., Huckins, J., et al., Chemosphere, 1993, 26, 1993-2006.
118. Petty, J. D., Huckins, J. N. and Zajicek, J. L., Chemosphere, 1993, 27, 1609-1624.
119. Ellis, G. S., Huckins, J. N., Rostad, C. E., et al., Environ Toxicol Chem, 1995, 14, 1875-1884.
120. Petty, J. D., Huckins, J. N., Martin, D. B., et al., Chemosphere, 1995, 30, 1891-1903.
121. Prest, H. F., Jacobsen, L. A. and Huckins, J. N., Chemosphere, 1995, 30, 1351-1361.
122. Baussant, T., Sanni, S., Jonsson, G., et al., Environ Toxicol Chem, 2001, 20, 1175-1184.
123. Vrana, B. and Schuurmann, G., Environmental Science & Technology, 2002, 36, 290-296.
124. Louch, J., Allen, G., Erickson, C., et al., Environmental Science & Technology, 2003, 37, 1202-1207.
125. Huckins, J. N., Petty, J. D., Orazio, C. E., et al., Environmental Science & Technology, 1999, 33, 3918-3923.
126. Huckins, J. N., USGS, Columbia, Editon edn., 2000, vol. 2.
127. Huckins, J. N., Petty, J. D. and Booij, K., Monitors of Organic Chemicals in the Environment, Springer, 2006.
128. Vrana, B., Allan, I. J., Greenwood, R., et al., TrAC Trends in Analytical Chemistry, 2006, 25, 704-715.
129. Booij, K., Vrana, B. and Huckins, J. N., in Comprehensive Analytical Chemistry Passive Sampling Techniques in Environmental Monitoring, ed. R. Greenwood, Elsevier, Editon edn., 2007, vol. Volume 48, pp. 141-169.
130. Bennett, E. R., Metcalfe, T. L. and Metcalfe, C. D., Chemosphere, 1996, 33, 363-375.
131. Charlestra, L., Courtemanch, D. L., Amirbahman, A., et al., Chemosphere, 2008, 72, 1171-1180.
132. Kočí, V., Lukavský, J., Mlejnek, M., et al., Algological Studies, 2004, 111, 173-186.
133. Ocelka, T., Czajka, K., Grabic, R., et al., USGS Columbia Environmental Research Center.

134. Ocelka, T., Grabic, R., Hapala, P., et al., in *Industrial Toxicology*, Bratislava, Editon edn., 2001, pp. 36-44.
135. Ocelka, T. and Kočí, V., in *Aktuální otázky vodárenské biologie*, Editon edn., 2001.
136. Verweij, F., Booij, K., Satumalay, K., et al., *Chemosphere*, 2004, 54, 1675-1689.
137. Zheng, J., Qu, S., Pan, L., et al., *Haiyang Huanjing Kexue*, 1999, 18, 19-23.
138. United States Pat., 5098573, 1992.
139. United States Pat., 5395426, 1995.
140. United States Pat., 20060086665, 2006.
141. Herve, S., Prest, H. F., Heinonen, P., et al., *Environmental Science and Pollution Research*, 1995, 2, 24-30.
142. Peven, C. S., Uhler, A. D. and Querzoli, F. J., *Environ Toxicol Chem*, 1996, 15, 144-149.
143. Yim, U. H., Oh, J. R., Hong, S. H., et al., *Environmental Forensics*, 2002, 3, 357-366.
144. Abad, E., Perez, F., Llerena, J. J., et al., *Environmental Science & Technology*, 2003, 37, 5090-5096.
145. Camus, L., Birkely, S. R., Jones, M. B., et al., *The Science of The Total Environment*, 2003, 308, 221-234.
146. Ohe, T., Watanabe, T. and Wakabayashi, K., *Mutat Res*, 2004, 567, 109-149.
147. Meadows, J. C., Echols, K. R., Huckins, J. N., et al., *Environmental Science & Technology*, 32, 1847-1852.
148. Prest, H. F., Riquelme, F., Jacobson, L. A., et al., *Marine Pollution Bulletin*, 1995, 30, 543-554.
149. Richardson, B. J., Tse, E. S. C., De Luca-Abbott, S. B., et al., *Marine Pollution Bulletin*, 2005, 51, 975-993.
150. Wang, Y., Wang, C. X. and Wang, Z. J., *Chemosphere*, 1998, 37, 327-339.
151. Echols, K. R., Gale, R. W., Schwartz, T. R., et al., *Water-Resour.Invest.Rep.(U.S.Geol.Surv.)*, 1999, 99-4018B, U.S. Geological Survey Toxic Substances Hydrology Program, 1999, Vol. 2, 35-40.
152. Crunkilton, R. L. and Devita, W. M., *Chemosphere*, 35, 1447-1463.
153. Zajicek James, L., Tillitt Donald, E., Huckins James, N., et al., in *Environmental Immunochemical Methods*, American Chemical Society, Washington, DC, Editon edn., 1996, pp. 307-325.
154. Gale, R. W., Huckins, J. N., Petty, J. D., et al., *Environmental Science & Technology*, 1997, 31, 178-187.
155. Folsvik, N., Brevik, E. M. and Berge, J. A., *J.Environ.Monit.*, 2000, 2, 281-284.

156. van der Oost, R., Beyer, J. and Vermeulen, N. P. E., *Environmental Toxicology and Pharmacology*, 2003, 13, 57-149.
157. Huckins, J. N., Geological Survey (U.S.). Water Resources Division. and Columbia Environmental Research Center, [USGS Biological Resources Division, Central Region, Columbia Environmental Research Center, Columbia, MO, Editon edn., 2001.
158. Ocelka, T., Kovanic, P. and Grabic, R., SETAC Europe, Annual Meeting, Book of Abstracts, 2004, 278-278.
159. Ocelka, T., Kovanic, P., Grabic, R., et al., Píšťovy 820, 537 01, Chrudim, 2004.
160. Kovanic, P., Ocelka, T., Grabic, R., et al., 9-th World Multi-Conference on Systemics, Cybernetics and Informatics WMSCI 2005, Orlando, Florida, USA, 2005.
161. Ocelka, T., Kovanic, P., Grabic, R., et al., International Passive Sampling Workshop and Symposium, 3.-6.5.2006, Bratislava, Slovakia, Bratislava, 2006.
162. Gilli, G., Schilirò, T., Pignata, C., et al., *Chemosphere*, 2005, 61, 1691-1699.
163. Isidori, M., Ferrara, M., Lavorgna, M., et al., *Chemosphere*, 2003, 52, 121-126.
164. Kočí, V., Ocelka, T. and Bergqvist, P. A., International Passive Sampling Workshop and Symposium, Book of Abstracts, 2004, 19-19.
165. Lebo, J. A., Huckins, J. N., Petty, J. D., et al., *Chemosphere*, 2000, 40, 811-819.
166. Ma, M., Wang, Y. and Wang, Z., *Huanjing Kexue*, 1999, 20, 80-83.
167. Sabaliunas, D., Ellington, J. and Sabaliuniene, I., *Ecotoxicol. Environ. Saf.*, 1999, 44, 160-167.
168. Sabaliunas, D., Lazutka, J. R. and Sabaliuniene, I., *Environ. Pollut. (Oxford, U. K.)*, 109, 251-265.
169. Ashby, J., *Toxicol. Pathol.*, 2000, 28, 432-437.
170. Tondeur, Y. and Hart, J., *TrAC Trends in Analytical Chemistry*, 2009, 28, 1137-1147.
171. Miličký, J. and Meloun, M., *Analytica Chimica Acta*, 1993, 277, 215-221.
172. Meloun, M. and Miličký, J., *Chemical Papers-Chemicke Zvesti*, 1994, 48, 158-163.
173. Meloun, M. and Miličký, J., *Chemical Papers-Chemicke Zvesti*, 1994, 48, 151-157.
174. Meloun, M. and Miličký, J., *Chemical Papers-Chemicke Zvesti*, 1994, 48, 164-169.
175. Meloun, M. and Miličký, J., *Chemical Papers-Chemicke Zvesti*, 1995, 49, 68-75.
176. Meloun, M., Sanka, M., Nemeč, P., et al., *Environmental Pollution*, 2005, 137, 273-280.
177. Meloun, M., Miličký, J. and Hill, M., *Počítačová analýza vícerozměrných dat v příkladech*, Academia, Pardubice, 2005.
178. Kovanic, P., *Mathematical Theory of Networks and Systems*, Regensburg, 1993.
179. Kovanic, P., *Problems of Control and Information Theory*, 1984, 13, 259-274.

180. Kovanic, P., The Institute of Information Theory and Automation of Czechoslovak Academy of Sciences, 1990.
181. Rantalainen, A. L., Ikonomidou, M. G. and Rogers, I. H., *Chemosphere*, **37**, 1119-1138.
182. Lebo, J. A., Gale, R. W., Petty, J. D., et al., *Environmental Science & Technology*, 1995, **29**, 2886-2892.
183. Kupec, J., *Vodní hospodářství*, 2000, **VTEI 3**, 6.
184. Kupec, J., *Vodní hospodářství*, 2007, **VTEI 1**, 4.
185. Bartkow, M. E., Kennedy, K. E., Huckins, J. N., et al., *Environmental Pollution*, 2006, **144**, 371-376.
186. Booij, K., Sleiderink, H. M. and Smedes, F., *Environ Toxicol Chem*, 1997, **17**, 1236-1245.
187. Crunkilton, R. L. and Devita, W. M., *Chemosphere*, 1997, **35**, 1447-1463.
188. Gourlay-Francé, C., Lorgeoux, C. and Tusseau-Vuillemin, M. H., *Chemosphere*, 2008, **73**, 1194-1200.
189. Harman, C., Tollefsen, K. E., Bøyum, O., et al., *Chemosphere*, 2008, **72**, 1510-1516.
190. Miege, C., Ravelet, C., Croue, J. P., et al., *Analytica Chimica Acta*, 2005, **536**, 259-266.
191. Petty, J. D., Jones, S. B., Huckins, J. N., et al., *Chemosphere*, 2000, **41**, 311-321.
192. Prest, H. F., Jacobson, L. A. and Wilson, M., *Chemosphere*, 1997, **35**, 3047-3063.
193. Shaw, M., Tibbetts, I. R. and Müller, J. F., *Chemosphere*, 2004, **56**, 237-246.
194. Söderström, H., Hajšlová, J., Kocourek, V., et al., *Atmospheric Environment*, 2005, **39**, 1627-1640.
195. Strandberg, B., Gustafson, P., Soderstrom, H., et al., *Journal of Environmental Monitoring*, 2006, **8**, 257-262.
196. Vrana, B., Paschke, A. and Popp, P., *J. Environ. Monit.*, 2001, **3**, 602-609.
197. Vrana, B., Paschke, A., Popp, P., et al., *Environ. Sci. Pollut. Res. Int.*, 2001, **8**, 27-34.
198. Prest, H. F., Jarman, W. M., Burns, S. A., et al., *Chemosphere*, 1992, **25**, 1811-1823.
199. Hofelt, C. S. and Shea, D., *Environmental Science & Technology*, 1997, **31**, 154-159.
200. Waagman, N., Strandberg, B. and Tysklind, M., *Organohalogen Compounds*, 1998, **35**, 212.
201. Rantalainen, A. L., Cretney, W. J. and Ikonomidou, M. G., *Chemosphere*, 2000, **40**, 147-158.
202. Zhu, X., Pfister, G., Henkelmann, B., et al., *Chemosphere*, 2007, **68**, 1623-1629.
203. Wang, J., Bi, Y., Pfister, G., et al., *Chemosphere*, 2009, **75**, 1119-1127.
204. Ke, R. H., Xu, Y. P., Huang, S. B., et al., *Environmental Toxicology and Chemistry*, 2007, **26**, 1258-1264.
205. Levy, W., Henkelmann, B., Pfister, G., et al., *Environmental Pollution*, 2009, **157**, 3272-3279.
206. Rantalainen, A. L., Paasivirta, J. and Herve, S., *Chemosphere*, 1998, **36**, 1415-1427.
207. Huckins, J. N., Petty, J. D., Lebo, J. A., et al., *Environmental Science & Technology*, 2002, **36**, 85-91.
208. Bartkow, M. E., Huckins, J. N. and Müller, J. F., *Atmospheric Environment*, 2004, **38**, 5983-5990.
209. Booij, K., van Bommel, R., Mets, A., et al., *Chemosphere*, 2006, **65**, 2485-2492.
210. Cranor, W. L., Alvarez, D. A., Huckins, J. N., et al., *Atmospheric Environment*, 2009, **43**, 3211-3219.
211. Strandberg, B., Wagman, N., Bergqvist, P. A., et al., *Environmental Science & Technology*, 1997, **31**, 2960-2965.
212. Bergqvist, P. A., Strandberg, B., Ekelund, R., et al., *Environmental Science & Technology*, 1998, **32**, 3887-3892.

213. ČSN EN 12305: Biotechnologie - Modifikované organismy pro použití v životním prostředí - Pokyny pro strategie vzorkování při záměrném uvolňování geneticky modifikovaných rostlin (*Biotechnology - Modified organisms for application in the environment - Guidance for the sampling strategies for deliberate releases of genetically modified plants*), ČNI/UNMZ Prague, 1999-04-01.
214. ČSN EN 12686: Biotechnologie - Uvádění modifikovaných organismů do prostředí - Návod pro strategie vzorkování při záměrném uvolňování geneticky modifikovaných mikroorganismů včetně virů (*Biotechnology - Modified organisms for application in the environment - Guidance for the sampling strategies for deliberate releases of genetically modified microorganisms, including viruses*), ČNI/UNMZ Prague, 2000-03-01.
215. Almeida, C., Serodio, P., Florencio, M. H., et al., *Anal.Bioanal.Chem.*, 2007, **387**, 2569-2583.
216. David, A., Fenet, H. and Gomez, E., *Marine Pollution Bulletin*, 2009, **58**, 953-960.
217. Söderström, H., Lindberg, R. H. and Fick, J., *Journal of Chromatography A*, 2009, **1216**, 623-630.
218. Tilghman, A., Coquery, M., Dulio, V., et al., *TrAC Trends in Analytical Chemistry*, 2009, **28**, 1-9.
219. Ocelka, T., Kočí, V., Mlejnek, M., et al., International Symposium on Earth Systems 2004, September 8-10, 2004, Istanbul, Turkey, 2004.
220. Marešová, V., Šucmanová, M., Marvanová, S., et al., *SETAC Europe, Annual Meeting, Book of Abstracts*, 2004, 164-164.
221. Rantalainen, A. L., Ikonomidou, M. G. and Rogers, I. H., *Chemosphere*, 1998, **37**, 1119-1138.
222. Mlejnek, M., Kočí, V., Ocelka, T., et al., *SETAC Europe, Annual Meeting, Book of Abstracts*, 2004, 165-165.
223. Sabaliunas, D. and Soedergren, A., *Environ.Pollut.*, 1997, **96**, 195-205.
224. Devita, W. M. and Crunkilton, R. L., *ASTM Spec.Tech.Publ.*, **STP 1333**, 237-245.
225. Grabic, R., Novák, J. and Pacáková, V., *Journal of High Resolution Chromatography*, 2000, **23**, 595-599.
226. Kovanicová, D. and Kovanic, P., *Poklady skryté v účetnictví - I. díl*, Polygon, Praha, 1995.
227. Kovanicová, D. and Kovanic, P., *Poklady skryté v účetnictví - II. díl*, Polygon, Praha, 1997.
228. Kovanicová, D. and Kovanic, P., *Poklady skryté v účetnictví - III. díl*, Polygon, Praha, 1997.
229. Meloun, M., Hill, M., Miličák, J., et al., *Clinical Chemistry and Laboratory Medicine*, 2001, **39**, 53-61.
230. Kovanic, P., Ocelka, T., Pavliska, L., et al., *Mathematical Gnostics*, <http://www.math-gnostics.com/index.php?a=books>.
231. *The R Project for Statistical Computing*, <http://www.r-project.org/>.