FROZEN ENVIRONMENTAL HISTORY: THE GERMAN ENVIRONMENTAL SPECIMEN BANK

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Abstract
The German Environmental Specimen Bank (ESB) is a monitoring instrument of the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. The ESB is managed by the Federal Environment Agency and operated by contracted research institutes and university groups with special competencies in the particular fields (e.g., sampling of human, biological, and abiotic material, trace analysis of pollutants, cryobank operation). Routine operation of the German ESB started in 1985. Human specimens are taken annually from students at four German universities and are archived as individual samples. Environmental specimens are also taken annually from representative marine, fresh water and terrestrial ecosystems. After pooling and homogenizing, environmental samples are stored at temperatures below -150°C. After two decades of operation the ESB provides now a continuous historical record of the state of the environment in Germany in this period. It allows the retrospective monitoring of pollutants to identify temporal trends and spatial load differences. Target compounds may be those which had not yet been recognized as hazardous when the specimens were archived (emerging pollutants) or which could not be analyzed with the desirable precision at that time. The ESB makes it possible to analyze samples from the past using the analytical methods of the future.

Introduction
The monitoring of time trends and sources of environmental contamination and the fate and behaviour of chemicals requires special strategies. In the past in most cases materials were analysed that accumulate particular substances and which were relatively easy to date. Such matrices under study included glacier ice, tree rings, bones, teeth, hair, and organisms from natural history collections.
In the 1970s within the strategy for harmonised European chemicals legislation the German government decided to set up an archive for environmental specimens as a part of their precautious policy on human health and the environment. This archive was designated to preserve samples under virtually stable conditions for investigation in future years. In Germany, there were already repositories and archives which inspired these ideas, such as a collection of seed samples from cultivated plants. In 1979, Germany began extensive systematic research into the feasibility of an environmental specimen bank. On basis of a pilot project the German government took over the bank as a permanent facility in 1985.[1]
The ESB is now a part of the environmental observation network in Germany and the backbone of the federal human health related environmental monitoring strategy. It serves to recognize ecosystems health changes and the nature and extent of possible damage. Thus the German ESB provides scientific knowledge for political decision making and elaborates fundamental concepts for precautionary politics. Within the framework of this specimen bank, ecologically representative environmental samples are collected, analyzed for environmentally relevant substances and then stored. Thus, the samples are available for a retrospective monitoring which can include new pollutants as well as those which were not yet analyzable at the time of sampling or which were not considered to be important at that time.

Material and Methods
The ESB is founded on a high degree of continuity in all work steps. Therefore, all important work is regulated by ESB-specific standard operating procedures (SOPs). The SOPs describe all main operations in detail and were published by the Federal Environment Agency.[2] Currently all ESB SOPs are in the process of revision. Already revised versions of the SOPs are available via the internet site of the ESB (www.umweltprobenbank.de; in German and English language).
Sampling: Sampling of environmental specimens is performed annually in 13 ecologically representative areas which reflect the environmental situation in Germany. The selected specimen are algae (*Fucus vesiculosus*),

common mussel (*Mytilus edulis*), eelpout (*Zoarces viviparous*), and eggs of herring gull (*Larus argentatus*) in marine ecosystems, as well as zebra mussel (*Dreissena polymorpha*), bream (*Abramis brama*), and suspended particulate matter in fresh water ecosystems. In terrestrial ecosystems spruce (*Picea abies*), pine (*Pinus sylvestris*), beech (*Fagus sylvatica*), roe deer (*Capreolus capreolus*), eggs of pigeon (*Columbia livia f. domestica*), earthworm (*Lumbricus terrestris*), and soil are sampled regularly.[3,4] Monitoring sites are described elsewhere.[5,6,7]

The human specimens blood, urine, and hair are taken from living persons at selected sites. It was decided to use voluntary students from the Universities of Muenster, Halle, Greifswald, and Ulm as donors. Human specimens are taken every once a year with a sample size of around 100 students at each sampling site. The students come usually from different regions in Germany to the universities and are selected for non-specific exposure. With individuals and groups moving home frequently in a mobile society, it is assumed that almost the entire country is represented.[8]

Sample processing: For sample preparation of the environmental specimens, a cryomill is used that has been proven to be appropriate for the treatment of ESB-sample material.[9,10] After grinding and homogenization under clean-air conditions in a laminar flow area, the sample material is subdivided in approx. 10 g portions into individually labelled 20 mL-vials. Human specimens are portioned if required and stored as individual samples.

Storage: The archive for environmental samples of the ESB is operated by Fraunhofer IME in Schmallenberg. Currently, it consists of 45 cryostorage containers made of stainless steel (internal volume: 1 m^3 or 1.4 m^3). All samples are stored in the gaseous phase above liquid nitrogen.

The filling of the containers is regulated by an automatic system which keeps the liquid nitrogen level between a minimum and maximum level. The maximum temperature in the cryostorage containers is -150°C. These low temperatures ensure that chemical processes in the samples are minimized. Atmospheric oxygen is excluded through the inert gas layer resulting from the evaporation of liquid nitrogen so that oxidative reactions are prevented. Since the temperature is below the glass transition temperature of water of approx. -135°C physical processes are stopped (e.g., no growth of ice crystals which may change the morphology of the sample material). The cryogenic storage conditions should ensure that no changes in the state of the sample material occur over a period of several decades.

Fig.1 Archive for environmental samples

The human specimen archive is located on the premises of the University of Muenster and consists of two walk-in cooling chambers with a total volume of 65 m^3, where the specimens are kept at a temperature of around -85°C. A graduated cooling system with numerous safety mechanisms ensures that the storage chambers are kept at the required low temperature. Since 2004 samples are stored under cryogenic conditions as described for environmental species. Currently a new building is set up for the cryogenic storage of human specimens.

Results and Discussion

Upon completion of sampling and sample processing sub samples of all specimens are routinely analyzed for a fixed set of substances (real-time monitoring). As a basis for political measures or for the evaluation of the efficacy of legislative restrictions retrospective monitoring studies are performed.

Real-time monitoring: The routine analyses for several well-known pollutants like metals/elements, polycyclic aromatic hydrocarbons (PAH), and chlorinated hydrocarbons allow both a characterization of the sampled material and the assessment of the appropriateness of the specimens. Furthermore, it results in information on the actual contamination level in the respective sampling areas and enables an identification of possible trends. The fixed set of substances includes Pb, Hg, and Cd, eighteen individual PAHs, and six (aldrin, dieldrin, heptachlor, HCB, PCB, DDT) of the twelve POPs initially targeted by the Stockholm convention. Data from these routine measurements are compiled in a public data bank available via internet (www.umweltprobenbank.de).

Retrospective monitoring: The archived specimens have been used to investigate the effectiveness of imposed reduction measures regarding the use of alkylphenol ethoxylates (APEO)[6], to show the increasing contamination of fresh water fish with polycyclic musk fragrances[11], and to give an overview of temporal trends and spatial distribution of dioxins, furans, dioxin-like PCBs in terrestrial, fresh water, and marine ecosystems.[12,13,14]

Recently, a former retrospective study regarding the occurrence of tin organic compounds in marine ecosystems has been amended to verify several restrictions on the use of tributyltin (TBT) in antifoulings. While TBT concentrations remained relatively constant in the period 1985 to 2003 significant decreases became obvious since 2004 in mussel and fish samples from the German North Sea and Baltic Sea coast. Although existent concentrations in mussels exceed threshold values defined by OSPAR it can be assumed that the total ban of organotin compounds in antifoulings in the countries of the European Union (Commission Directive 2002/62/EC) became effective.

Another retrospective study investigated the internal phthalate exposure of humans over the last two decades.[15] For this purpose archived urine samples were analyzed for the concentrations of primary and/or secondary metabolites of di-n-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP), butylbenzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP) and di-iso-nonyl phthalate (DiNP). In more than 98% of the urine samples metabolites of all five phthalates were detectable indicating a ubiquitous exposure of the German population to all five phthalates considered throughout the last twenty years. For the DEHP urinary metabolite levels an increase was observed between 1988 and 1991, but after this a gradual decrease was seen until 2003. In contrast, the urinary levels of the sum of the two DiNP metabolites increased almost continuously between 1988 and 2003.

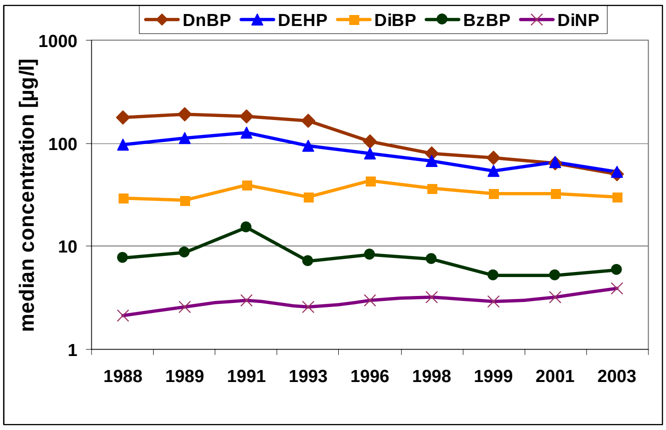

Fig. 2 Phthalate concentrations (µg/l) in urine. DnBP measured as MnBP; DEHP given as sum of the metabolites 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, 2cx-MMHP, and MEHP; DiBP measured as MiBP; BzBP measured as MBzP; DiNP given as sum of the metabolites oxo-MiNP and OH-MiNP

Based on the urinary metabolite excretion daily intakes of the parent phthalates were estimated. For a considerable 14% of the subjects daily DnBP intakes above the tolerable daily intake (TDI) value deduced by the European Food Safety Authority could be observed. However, the frequency of exceedance decreased during the years and was beneath 2% in the 2003 subset.

At present, human blood is investigated for the temporal trend of contamination with perfluorinated compounds, fish samples are screened for ingredients of sunscreens (UV filters), and environmental as well as human specimens are examined for a set of brominated flame retardants (PBDE, HBCD, TBBPA).

Conceptual progress: Apart from the undoubted value of the German ESB as a tool for the retrospective monitoring of chemical substances some limitations of the program and first steps towards an optimization should be mentioned.

At present, processing of environmental specimens is routinely performed by grinding at low temperature leading to a set of homogenous sub-samples with low standard deviation in chemical analyses. However, grinding destroys information on tissue structure. Therefore specific microanalyses, morphological and histological studies are not possible. For biological analyses it has to be guaranteed that as much morphological information as possible is conserved. The samples should be suitable for assessment with the methods available in about 20 or 30 years. It is expected that with future methods information from structural modifications will provide information on environmental changes. Besides, the size of the sample has to be small as storage capacity is limited. To combine these both demands, methods were elaborated to cut the frozen biological samples in slices or cubes with dimensions of about 1 - 2 mm. Care has to be taken that no thawing occurs during the preparation. Samples of interest are bladder wrack, mussels, liver and muscle of fish, liver of deer, earthworms as well as leaves and needles from trees.

Analyzing chemical substances only gives information about the concentrations in the tested organisms themselves but not whether any exposure directly influences the organism, e.g. by changing gene expression. First investigations into the detection of effects on a cellular level showed that the analysis of molecular biomarkers can successfully be used for a retrospective monitoring. By comparison of gene sequences with zebrafish (*Danio rerio*), specific genes could be identified which are regulated in bream (*Abramis brama*) by different stressors. It was possible to detect genes which are expressed in an agent-specific or agent-unspecific way. Heat shock protein (HSP) was identified as a marker to be regulated agent-unspecifically. HSP will be over-expressed fast by the influence of different stressors and can therefore be used as a health indicator. Agent-specific genes like metallothionein and vitellogenin represent genes which are influenced by heavy metals or endocrine disrupters, respectively. If it is possible to identify molecular biomarkers even in other ESB samples, an effective tool will be available which extends the retrospective monitoring by including the genetic information in an outstanding way.

When establishing the human part of the ESB it was decided to acquire healthy collegiate volunteers aged 20 to 29 years. Students are regarded as a proper subgroup for analyzing general trends of exposure in Germany because they are a relatively homogenous group with similar socio-demographic features, high mobility, and little occupational or accidental exposure. Restriction on the aforementioned age interval results in exclusion of exposures of children as well as age- and/or disease-influenced body burdens in most instances. However, in regard to precautionary policy the observation of more vulnerable stages in human development would be desirable. For this purpose a research project was initiated to verify the suitability and availability of perinatal matrices like cord blood and placenta as possible specimens for the ESB.

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