A Strategy for Chemical Analysis of Halogenated Environmental Pollutants

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Introduction

Organic chemicals, pesticides and products used in technical applications, were first identified as environmental pollutants some 30-40 years ago. Major improvements in the instrument developments, i.e. the mass spectrometer and the electron capture detector to be used for gas chromatography allowed the analytical chemist to obtain structural information, and to determine pollutants in biota at much lower levels than before. Much effort was then put into the development of analytical methods and analysis for monitoring of what today may be called traditional anthropogenic compounds. As a result, a large number of environmental pollutants were identified in the environment in the years that followed. Among them were chemical classes such as pesticides like DDT and its metabolites, hexachlorohexanes (HCHs), polychlorinated biphenyls (PCBs), naphthalenes (PCNs) dioxins (PCDDs), dibensofurans (PCDFs). Many other compounds were detected but have gained much less interest. Lately, increasing analytical activity can be observed for e.g. PCB methyl sulfones ($MeSO_2$-PCBs) (1), phenolic organohalogen substances (OHS), including phenols and biphenylols (OH-PCBs) (1,2), polybrominated diphenyl ethers (PBDEs) and a few other brominated flame retardants (BFR) (3), polychlorinated bornanes and paraffins and some miscellaneous OHS e.g. bis(4-chlorophenyl) sulfone (BCPS) (4).
The aim of this presentation is to describe a strategy for chemical analysis, extraction, clean-up, identification and quantification, of environmental contaminants, particularly OHS, in different matrixes. Some examples are given.

Material and Methods

Chemicals: Chemicals for clean-up as described elsewhere (5,6). Please see "Results and Discussion" for references to synthesis authentic reference standards. Internal standards have been synthesized as part of the projects in synthesis.
Instruments: High performance liquid chromatography HPLC), gas chromatography (GC) and mass spectrometry (MS) were performed on the instruments described elsewhere (e.g. 5,6). The instruments were equipped and operated as stated in these references.
Samples: The methodological development for analysis of so far unknown environmental contaminants has been concentrated to pollutants in biota at high trophic levels. Tissue samples including adipose tissue, muscle, individual organs and blood from wildlife have been carefully selected by and obtained from the Swedish Environmental Specimen Bank (ESB) through close collaboration with prof. Mats Olsson (Swedish Museum of Natural History, Stockholm). The sample selection has been based on similar age, sex, sampling time, time periods, considering geographical areas of interest etc. Humans samples have been obtained via collaboration with

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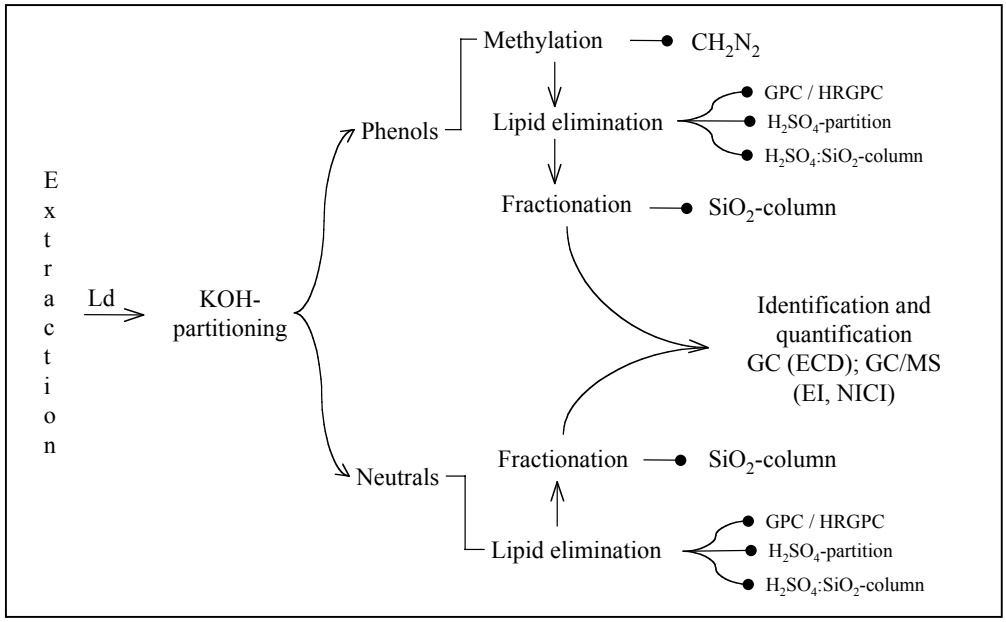
epidemiologists working with occupational and environmental exposures to anthropogenic compounds, in particular Prof. Lars Hagmar (Lund University, Lund).
Clean-up and analysis: The extractions of tissues were based on the method described by Jensen and coworkers (7) and a recently developed method for extraction of blood analysis of phenolic OHS (8). The methodologies for clean-up and analysis of neutral and phenolic OHS are further discussed below.

Results and Discussion
The concept for clean-up, identification and quantification of environmental contaminants is a tiered strategy using *i)* adequate biological samples, *ii)* synthesized authentic reference standards and *iii)* relevant clean-up methodology of adipose tissue, organs and blood samples prior to detection by well known instrumental techniques. The analytical steps may either be targeted towards specific compound analysis or non-discriminating analysis.
The approach to identify potential "new" environmental contaminants is based on: *i:* knowledge of substances produced as pesticides, in chemical technical applications, as additives in manufacturing processes and in goods or being formed as process byproducts, *ii:* knowledge of physicochemical and reactivity properties of the compounds, *iii:* metabolite formation *in vivo* and *iv:* indications of unidentified compounds present in human and wildlife samples. The synthesis of four classes of OHS are referred to below.

Synthesis of relevant standards: A few highly chlorinated polychlorinated naphthalene (PCN) congeners, indicated in the environment, were synthesized via reduction reactions of octaCN (9). In this way two environmentally relevant hexaCN congeners, 1,2,3,4,6,7-hexaCN and 1,2,3,5,6,7-hexaCN, were shown to both be present in the environment but that they also coelute on all GC columns tested so far. These two isomers were possible to separate on a pyrene derivatized silica gel column (7).
Approximately 30 $MeSO_2$-PCBs, all relevant environmental standards and for which 10 form enantiomeric pairs, have been synthesised via diaryl coupling reactions of relevant methyltio- or metylsulfonyl-substituted starting materials and polychlorinated anilenes or benzenes, respectively (1). Also, methylthiolate may be used in a NAS for preparation of $MeSO_2$-PCBs.
Methoxylated PCBs are primarily synthesized via the Cadogan reaction to obtain, after demethylation, polychlorobiphenylols that may be detected in blood from wildlife and humans (1)
More than 30 PBDE congeners have been synthesized via direct bromination of diphenyl ether, the Ullman diaryl ether synthesis or via diaryl iodonium salt reactions (10,11).

Methods for analysis: The basis for identification of hitherto unknown environmental pollutants is to use non-destructive extraction and clean-up steps. If the analysis is targeted on a specific type of substances it is possible to include destructive methods in the clean-up procedure. The key-issue to improve the possibilities for identification, and quantification, of OHS is to remove matrix-related



Ld = Lipid determination

Figure 1. General clean-up scheme for unprejudiced analysis of OHS in biota.

substances from the OHS analytes without degradation or descrimination of analytes. A general scheme, based on the partitioning of neutral and phenolic OHS to permitthe detection of compounds not only accumulating in lipids,for the clean-up of tissue or blood samples is shown in Figure 1.

Extraction procedure and partitioning: The classic extraction method suggested by Bligh and Dyer (12) was compared with blood plasma extraction using hexane:methyl *tert*-butyl ether (MTBE) (1:1) as extracting solvent, after denaturation of proteins by the addition of 2-propanol and hydrochloric acid to the plasma (8). The gravimetrical lipid weight determination showed improved lipid yield in the new method. Further clean-up of the plasma extracts is similar to clean-up of tissue extracts Thus, separation of acidic and neutral analytes is obtained by partitioning of an alkaline aqueous phase (0.5 M KOH in 50% ethanol) and an organic solvent phase (Figure 1). The phenolic OHS present in this fraction are methylated prior to further clean-up for several reasons; the availability of methoxy-standards, avoidance of adsorption to glass, and improved chromatographic behaviour.

Removal of extracted matrix-related material: Several non-destructive methods have been tested to improve the elimination of endogenic material from the extracts.; e.g. gel permeation chromatography (GPC), high-resolution chromatography (HR-GPC) and separation on silica and modified silica have been tested. As GPC is non-destructive, it was applied in metabolite analysis (13) but also a valuable tool for purification neutral OHS. HR-GPC, initially used for PCN and PCB separations with tetrahydrofuran as mobile phase (14), has been used for lipid removal using hexane:dichloromethane (7:3) as the mobile phase (8). Silica and particularly silica/sulfuric acid (2/1) columns, with DCM as mobile phase, have been shown to be another valuable tool for removal of matrix related interference's leading to highly improved gas chromatographic results.

Separation of chemical classes of substances: GPC were shown to be useful for isolating e.g. chlorinated paraffins from other analytes as well as HR-GPC separations of analytes such as PCNs and PCBs (14). PBDEs are efficiently separated from pesticides and PCBs on nitrophenylpropyl derivatized silica (15). Similar separations may also be obtained on pure silica using stepwise elution with different solvent mixtures. Methoxylated PCBs and anisols are separated from dimethoxylated compounds on silica and also methoxylated PBDEs may be isolated this way.
Identification and quantification: More than 120 phenolic OHS in total have been indicated in plasma samples from humans, and of these up to 30 were found to be OH-PCB congeners (2). The compounds are indicated by GC/MS (EI and NICI) and their identities are confirmed by comparisons to the synthesized standards. However, coelution problems call for additional studies using GC columns of different polarities. Proper adjustments of GC conditions and selection of GC column has lead to identification and quantification of the perbrominated decaBDE and other PBDEs (6). MeO- and OH-PBDEs have been detected in salmon (5) and in humans (unpublished).

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