ANALYSIS OF CHLORINATED PARAFFINS IN DIFFERENT BIOLOGICAL AND NON-BIOLOGICAL MATRICES - AN OVERVIEW

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Introduction

Chlorinated paraffins (CPs) consist of a complex mixture of polychlorinated *n*-alkanes containing thousands of different isomers, enantiomers and diastereomers. The chlorination degree of CPs can vary between 30 and 70%. CPs are subdivided according to their carbon chain length into short chain CPs (SCCPs, C_{10-13}), medium chain CPs (MCCPs, C_{14-17}) and long chain CPs (LCCPs, $C_{>17}$).[1] Commercial CPs find application as flame retardants and plasticisers and are widely used as additives in plastics, paints, coatings, and in metal working fluids.[2] SCCPs are classified as persistent and their physical properties imply a high potential for bioaccumulation.[1] Furthermore, CPs are toxic to aquatic organisms and carcinogenic to rats and mice. Nevertheless, limited information are available about metabolic pathways and toxicokinetics of CP congeners compared to other persistent organochlorine compounds like PCBs or toxaphenes.[1] Main reasons are the complex composition of the technical mixtures and the small number of laboratories, who are able to analyse CPs.[3] Until recently there has been a lack of simple analytical methods.

This work presents an unified analytical approach suitable for the determination of CPs in various matrices based on methods developed in previous studies for fish, sea birds, sediment, and human milk samples.[4,5,6,7] The different matrices are extracted by specific extraction techniques which are followed by a standardised clean-up applicable to all kind of samples. Furthermore, for the first time compost, needle, and soil samples could be integrated in this approach.

Materials and Methods

Chemicals. Cyclohexane, dichloromethane, and *n*-hexane for pesticide residue analysis were obtained from Scharlau (Barcelona, Spain). Technical SCCP (chlorine contents of 51.5, 55.5 and 63.0%) and technical MCCP mixtures (chlorine contents of 52.0 and 57.0%) with concentrations of 100 ng/µl in cyclohexane as well as ε-hexachlorohexane (ε-HCH, solution in cyclohexane, 10 ng/µl) were purchased from Ehrenstorfer GmbH (Augsburg, Germany). $^{13}C_{10}$-*trans*-chlordane (100 ng/µl, solution in *n*-nonane, purity 99%) was supplied by Cambridge Isotope Laboratories (Andover, USA). Florisil® PR (60-100 mesh), sodium sulphate (Pestanal®; Fluka, Buchs, Switzerland) and silica gel 60 (230-400 mesh, 0.045-0.063 mm; Merck KGaA, Darmstadt, Germany) were dried over night at 600 °C and then kept at 130 °C until usage.

Extraction.

For all analysis 10 ng of $^{13}C_{10}$-*trans*-chlordane in 10 µl of cyclohexane was used as internal standard (ISTD).

Sediment, soil, compost: 10-25 g of dried samples were spiked with ISTD and soxhlet extracted with 200 ml of dichloromethane and *n*-hexane (DCM/nHex, 1+1, v/v) over night. If necessary, activated copper powder was added to eliminate sulphur. Then, extracts were concentrated to 1 ml using a Turbo Vap 500 (Zymark, Hutchinson, USA).

Spruce needles: 200 ml of DCM/nHex (1+1, v/v) and ISTD were added to 20 g of fresh spruce needles placed into a flask. The solution was shaken by hand for 3 minutes and was left standing over night. The extract was paper filtrated directly into a Turbo Vap flask. The needles were washed with an additional volume of 50 ml of DCM/nHex (1+1, v/v). The solution was shaken and after filtration both extracts were combined. Subsequently, extracts were concentrated to 1 ml with a Turbo Vap 500.

Animal tissues (liver, muscle and eggs): Between 5 and 10 g were homogenised with a tenfold amount of anhydrous sodium sulphate. The mixture was dry-packed into a glass column (30 cm long, 2.0 cm i.d.), a 1 cm layer of water free sodium sulphate was placed on top, and ISTD was added. The lipids were eluted with 250 ml of DCM/nHex (1+1, v/v). The extracts were concentrated to 1 ml using a Turbo Vap 500.

Human milk: Ca. 50 g of milk were centrifuged (3000 rpm, 4 °C, Varifuge 3.0 R, Heraeus Instruments, Germany) for ten minutes. The creamy lipid fraction on top was separated and melted in a water bath at 50 °C. Afterwards, anhydrous sodium sulphate was added and the extract was stirred until dryness. The extract was covered with nHex (1-2 cm). Lipids were dissolved by stirring and filtrated through a glass funnel filled with a pre-cleaned piece of cotton wool and sodium sulphate. The extraction procedure was repeated 3-4 times. Solvents were removed at first with a speed vac (Laborota 4001 efficient, Heidolph Instruments, Germany, water bath at 50 °C, 250-300 mbar) and then in a preheated sand bath (50 °C) under a gentle stream of nitrogen for 5-10 minutes to evaporate residues of solvent. ISTD was added to the lipid extract.

Clean-up. The following clean-up method was applied to all sample extracts, independently of the matrix. Further details are described elsewhere.[4,5,6]
1. Clean-up step: A glass column containing 1 g of anhydrous sodium sulphate, 20 g of silica gel impregnated with concentrated sulphuric acid (44%) and 1 g of anhydrous sodium sulphate was conditioned with DCM/nHex (1 + 1, v/v). After transferring the extract to the column, CPs were eluted with 70 ml of DCM/nHex (1+1, v/v). The eluate was evaporated to 0.5 ml with a Turbo Vap 500, then diluted with 10 ml of nHex and reduced to 0.5 ml, each twice.
2. Clean-up step: Further fractionation was carried out on 16 g of Florisil® (1.5% water content; conditioned with nHex) by eluting with 75 ml of nHex and 5 ml of DCM (prefraction) and 60 ml of DCM (main fraction). The main fraction contained all CPs and was concentrated to 0.5 ml, then diluted with 10 ml of nHex and reduced to 100 µl, each twice. Finally, 10 ng of ε-HCH in 10 µl of cyclohexane were added as recovery standard prior to analysis.

Instrumentation. Parameters for the HRGC-EI-MS/MS analysis are published in detail elsewhere and hence only briefly described.[8] Instrumental analysis was performed on a gas chromatograph CP-3800 coupled to a 1200L triple quadrupole mass spectrometer (Varian, Walnut Creek, USA). The gas chromatograph was equipped with a split/splitless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with 0.25 µm of crosslinked 5% phenyl-methylpolysiloxane (DB5-MS, J&W Scientific, Folsom, USA). Helium (99,999 %, Carbagas, Basel, Switzerland) was employed as carrier gas at a constant flow of 2 ml/min. The injector temperature was set to 275 °C. Splitless injections (3.0 min) of 2.5 µl volume were carried out with a Combi Pal autosampler (CTC Analytics, Zwingen, Switzerland). The temperature program was as follows: 1 min isothermal at 100 °C, increased at 50 °C/min to 300 °C, then isothermal for 4 min. The transfer line temperature was set at 280 °C and the ion source temperature at 200 °C. The EI mass spectra were acquired at 70 eV electron energy with a filament emission current of 150 µA and a scan time of 0.5 s/scan. CID gas pressure (Argon) was set to 1.0 mTorr. The following fragmentations and collision energies were used for the determination of the total CP amount: *m/z* 91 $[C_7H_7]^+$ → *m/z* 53 $[C_4H_5]^+$ (collision energy: -10 V), *m/z* 102 $[C_5H_7Cl]^+$ → *m/z* 65 $[C_5H_5]^+$ (-18 V) and *m/z* 102 $[C_5H_7Cl]^+$ → *m/z* 67 $[C_5H_7]^+$ (-10 V). The precursor ion *m/z* 383 $[M\text{-}Cl]^+$ and the product ion *m/z* 276 $[M\text{-}4Cl]^+$ were selected for the internal standard $^{13}C_{10}$-*trans*-chlordane (-28 V).

Results and Discussion
What is necessary to perform routine CP analysis (e.g. for a service laboratory)? Extraction procedures combined with a suitable standardised clean-up applicable to all kind of sample extracts, and a fast determination method (e.g. EI-MS/MS). As shown in figure 1 only the extraction step of the here presented approach is different for each matrix. However, also here coherence is present. Except for milk samples, all shown matrices were extracted with DCM/nHex (1+1, v/v). The extraction procedures were different due to the different sample properties. Soxhlet extraction was preferred for dried samples. Liquid extraction provided optimal results for biota after drying by homogenisation with sodium sulphate. For plant material a solvent bath over night was sufficient to achieve high recoveries of CPs. The extraction of human milk samples is more complex due to the separation of the lipid fraction.
The clean-up procedure was the same for all the samples. It consisted of a silica gel column impregnated with sulphuric acid. Hereby, interfering compounds degradable by sulphuric acid, such as wax, lipids and other matrix, were removed. Finally, a deactivated Florisil® column was applied to separate CPs from other organochlorines, such as toxaphenes and PCBs.

Instrumental analysis was performed using a triple quadrupole in the EI-MS/MS mode.[8] This is a huge advantage compared with other quantification methods, which are either highly complex and time consuming (e.g. ECNI) or too expensive (e.g. high resolution MS). This method allows determining the sum of short, medium and long chain paraffins in one fast measurement within 10 minutes.[9]

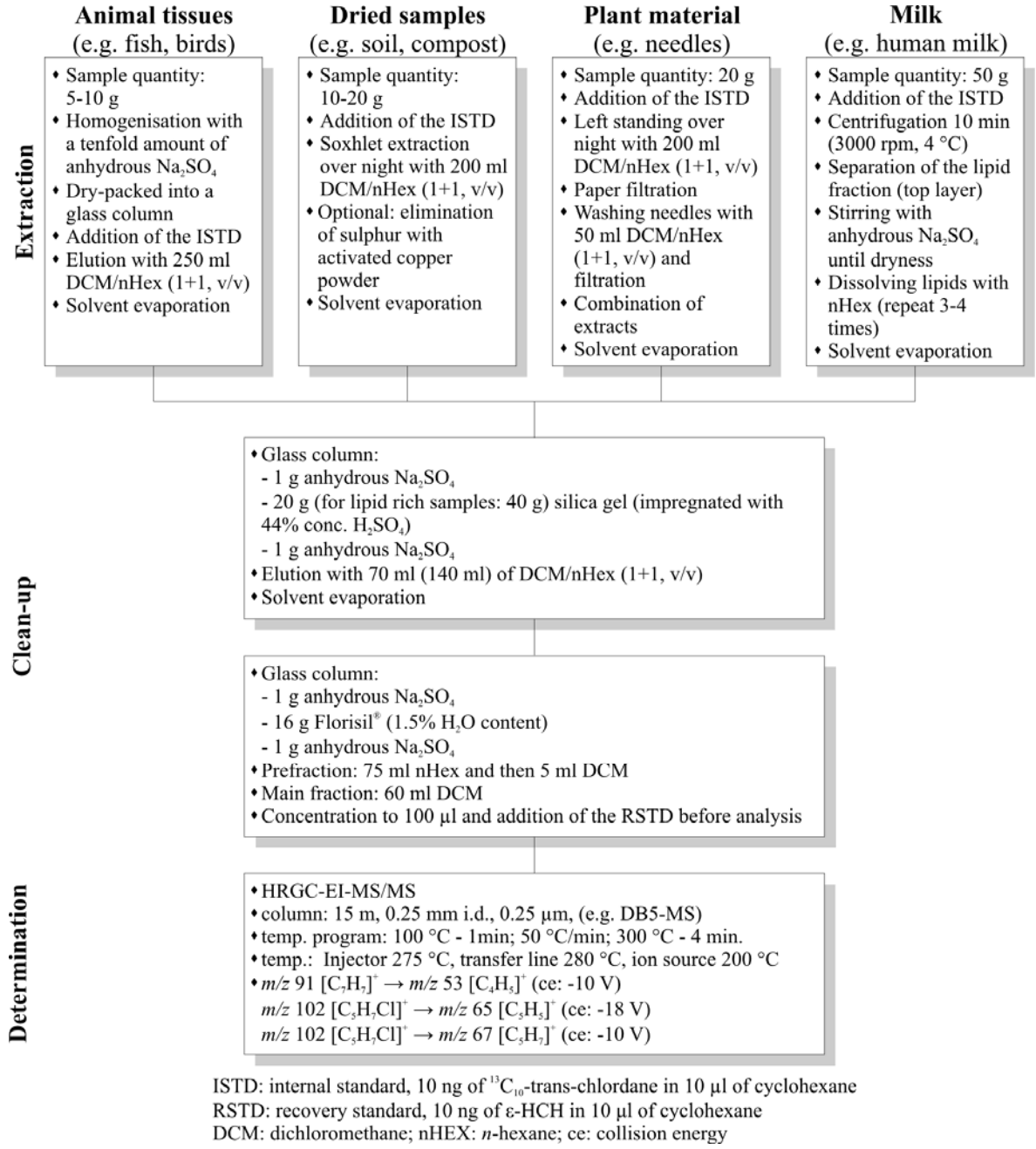

Figure 1: Scheme of the analytical procedure to determine chlorinated paraffins in different kinds of environmental samples.

For the first time pine needle, compost and soil samples were included in this analytical approach. Soil and compost samples were extracted in the same way as sediment samples whereas a new extraction was developed for pine needles. The combination of the here presented extraction and clean-up methodologies allowed to obtain chromatograms with a low background showing the typical CP profile without interferences (Figure 2).

Conclusion. This study shows that a unified analytical approach for the determination of CPs in different sample matrices can be achieved. This should allow to implement routine analysis of CPs more easily.

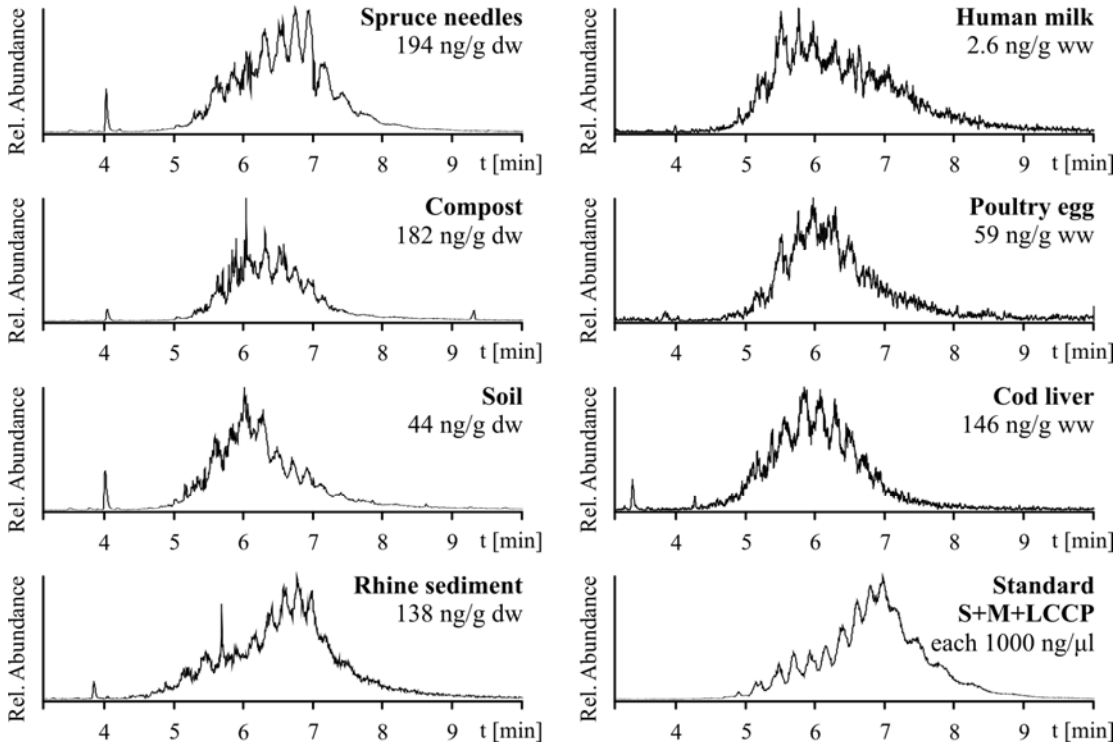

Figure 2: EI-MS/MS chromatograms (*m/z* 102 $[C_5H_7Cl]^+ \rightarrow$ *m/z* 67 $[C_5H_7]^+$) of CPs in different sample matrices and of a standard mixture of short (55% Cl), medium (57% Cl) and long chain (49% Cl) chlorinated paraffins (1+1+1). Poultry egg samples were analysed with the animal tissue extraction.

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References

1. Muir DCG, Stern GA, Tomy GT. *The Handbook of Environmental Chemistry* 2000;3:203, ISBN 3-540-65838-6.
2. WHO. *Environmental Health Criteria 181 - Chlorinated Paraffins* 1996, ISBN 92-4-157-181-0.
3. UNECE. *Short Chain Chlorinated Paraffins (SCCP) Substance Dossier, Final Draft II* 2003.
4. Reth M, Zencak Z, Oehme M. *Chemosphere* 2005;58:847.
5. Reth M, Ciric A, Christensen GN, Heimstad ES, Oehme M. *Sci Total Environ* 2006; in press.
6. Hüttig J, Oehme M. *Arch Environ Contam Toxicol* 2005;49:449.
7. Reth M, Kypke K, Schächtele J, Oehme M. *Organohalogen Comp* 2005;67:1671.
8. Zencak Z, Reth M, Oehme M. *Anal Chem* 2004;76:1957.
9. Zencak Z, Oehme M. *Trends Anal Chem* 2006;25:310.