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Identification and determination of chlorinated paraffins using multivariate evaluation of gas chromatographic data

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A B S T R A C T

Chlorinated paraffins (CPs) were found in the biodegradable fraction of source separated waste from Uppsala, Sweden. We identified and quantified the CPs by multivariate evaluation of gas chromatography-electron capture detection chromatograms. Using principal component analyses (PCA) we identified different types of CP-formulations and also obtain quantitative data. PCA yielded better identifications of individual CP-formulations than visual comparison of chromatograms. Partial least squares regression gave good calibration curves of the standards, but did not work for the waste samples. No source of CPs could be identified in the waste collection chain, and as the waste samples seemed to contain at least two different CP-formulations the source was probably to be found in the waste material itself. The method was used to determine CPs in additional environmental samples, demonstrating that multivariate methods may be developed into a powerful tool for identification and quantification of complex mixture.

1. Introduction

Chlorinated paraffins (CPs) have found many applications, but the main uses are as flame-retardant plasticizers in vinyl plastics and as high-temperature lubricants in metal-processing industry (Tomy et al., 1998; EuroChlor, 2010). Production of CPs commenced in 1930 and today the total world production is approximately 300 kt/year (EuroChlor, 2010). CPs are produced by chlorination of petroleum n-alkane fractions under UV irradiation. Depending on the number of carbons in the petroleum alkane fraction they are characterized as short (C_{10}–C_{13}), medium (C_{14}–C_{17}) or long (C_{20}–C_{30}) chain, and further into subcategories depending on their mass percentage of chlorine into low (30–50%), medium (50–60%), or highly (60–70%) chlorinated. Carbon chain length and chlorine content determine the properties of the individual CP product and vary depending on the specific use. The reaction has low positional selectivity and gives complex mixtures of alkanes with varying carbon chain length and varying and unspecific chlorination (Tomy et al., 1998), why CPs are among the most complex mixtures of environmental pollutants; a single sum formula can give rise to a very large number of isomers (Shojania, 1999).

Several countries have regulated the use of CPs as they are persistent in the environment and bioaccumulate (Stockholm Convention, 2010). The complex mixture of homologues and isomers is problematic as it renders the determination of CPs complicated (Tomy, 2010); any CP product will elute over a wide retention time window without baseline resolution (Figures S1-S2 in electronic supplement). In addition, other commonly found environmental contaminants will act as interferences as they overlap and obscure the chromatographic pattern of the CPs (and *vice versa*). Consequently, information on levels and fate of CPs in the environment is limited compared to other persistent organic pollutants, e.g., polychlorinated biphenyls (PCB), the production of which started at about the same time as CPs (Tomy et al., 1998). CPs have been reported from both terrestrial and aquatic ecosystems, and both high and low trophic levels and they occur in both biota and in abiotic compartments such as sediment and soil (Bayen et al., 2006; Santos et al., 2006).

Many different extraction and clean-up procedures for the determination of CPs have been described (Bayen et al., 2006; Santos et al., 2006). Because of the chromatographic behaviour of CPs, a particular complication is to remove compounds that obscure the identification of the CP product and interfere with the quantification. Short columns that allow the CPs to elute within a shorter retention time span have been used to achieve lower detection limits (Coelhan, 1999; Fridén et al., 2004), but will also exacerbate the problems with interfering compounds. Generally, most clean-up procedures consist of several liquid chromatographic steps (Bayen et al., 2006; Santos et al., 2006), including gel permeation and adsorption

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chromatography, sometimes with addition of, e.g., liquid chromatography with stationary phases with modified selectivity (Nilsson et al., 2001; Fridén et al., 2004), or ultraviolet (UV) irradiation (Friedman and Lombardo, 1975; Fridén et al., 2004). For the final qualitative and quantitative determination gas chromatography (GC) with electron capture detector (ECD) has often been used, but, due to the complexity of the CPs, GC coupled to high resolution mass spectrometry (HRMS) has become increasingly popular (Bayen et al., 2006; Santos et al., 2006; Tomy, 2010). However, a drawback with HRMS is the cost, why methods that allow good determinations with GC-ECD have been explored (Fridén et al., 2004). The lower cost of GC-ECD determinations would make it possible to obtain more environmental data, potentially increasing the understanding of the environmental fate of CPs by obtaining a higher statistical power. The drawback, however, is that in many cases an understanding of the distribution within homologue classes is desirable. A GC-ECD instrument will not yield this type of information, and multidimensional GC-HRMS instruments may be necessary (Tomy, 2010).

In many parts of Europe composting and anaerobic digestion are increasingly important methods of handling the biodegradable fraction of household waste (Nilsson, 2000; Brändli, 2006). Not only is it a means of reducing the amount of waste that needs to be deposited or incinerated, but it is also a potential means of recirculating nutrients from urban areas to agriculture or horticulture, thus contributing to a sustainable development of society. However, to use the compost or digestate in cultivation a minimum quality must be ensured so that contaminants do not build up in the arable soil, potentially reaching the crops. Developing environmental quality criteria for compost was one of the goals of a multidisciplinary study initiated by the Faculty for Agriculture, Landscape Planning and Horticulture at the Swedish University of Agricultural Sciences. During the project, CPs were found in the biodegradable fraction of household waste from Uppsala, Sweden. As the concentrations were fairly high, it was important to trace the source of the contamination in order to avoid such contamination in the future. As CPs were not expected to be present in food waste in Sweden at the levels detected, the initial hypothesis was that the CPs originated from somewhere within the collection chain from the households to the waste handling plant. As this would be a technical source, we expected that the CP profile in the samples should be possible to attribute to a technical CP-formulation. We, therefore, undertook a project to determine the identity of the CP-formulation contaminating the household waste, to track the source of the contamination, and to map the circulation and fate of CPs in the waste handling system in Uppsala.

Here we report on experiments to use multivariate processing of GC-ECD data for the qualitative and quantitative determination of CPs. We investigated if it was possible to use principal component analysis (PCA) to explore differences between different types of CPs, and if it was possible to develop partial least square regression (PLSR) models to quantify CPs.

2. Materials and methods

2.1. Solvents and chemicals

Different commercial CP products of short and medium chain length, with chlorine content between 42 and 70% (Table 1), were used as standards. Cyclohexane, acetone, and dichloromethane (LabScan, Stockholm, Sweden) and *n*-hexane (Merck Eurolabs, Spånga, Sweden) were of pesticide grade. Hydromatrix, (Varian, Harbor City, CA, USA), was bought from Scantec Lab (Partille, Sweden). Sodium sulphate (Merck Eurolabs) was heated to 600 °C over night, cooled and stored in a desiccator until used. Silica gel 60 (Merck Eurolabs) was washed with dichloromethane, dried at 130 °C for at least 5 h, cooled in a desiccator, deactivated with 1.5% (w/w) deionised water, and stored under *n*-hexane. Concentrated sulphuric acid was from Merck Eurolabs.

Standard solutions of CPs were prepared in cyclohexane (Table 1). Internal standards were 1,2,5,6,9,10-hexabromocyclododecane (HBCD), α,α,α′,α′-tetrachloro-*o*-xylene, 2,2′,5,6′-Tetrachlorobiphenyl (PCB 53), and decachlorobiphenyl (PCB 209) (Sigma–Aldrich, Stockholm, Sweden). HBCD, being a halogenated alkane as are the CPs, was added as surrogate standard prior to extraction to estimate the total recovery. The other standards were added after clean-up and used 1) to calibrate the retention times and 2) to estimate the recovery of HBCD and thus also the CPs. A retention time standard consisting of technical grade DDT (app. 76% *p,p′*-DDT [1,1,1-trichloro-2,2-*bis*-(4-chlorophenyl)-ethane] and 20% *o,p′*-DDT [1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)-ethane], Merck Eurolabs) in *n*-hexane was prepared to calibrate the liquid chromatographic fractionation (see below).

Table 1

Standard solutions of chlorinated paraffins (CP) with different carbon chain length (C) and chlorine content (% Cl). The individual CP products were designated with letters A-H. The standard solutions were fortified with 1, 2, 5, 6, 9, 10-hexabromocyclododecane (0.185 ng/mL) and α,α,α′,α′-tetrachloro-*o*-xylene (0.019 ng/mL) as surrogate and internal standards and were also used to calibrate the retention times using the auxiliary programme ChromPro.

Chlorinated paraffin							
A[a]	B[a]	C[b]	D[b]	E[c]	F[c]	G[c]	H[c]
C_{14-17}	C_{10-13}	C_{10-13}	C_{10-13}	C_{10-13}	C_{14-17}	C_{14-17}	C_{14-17}
52%	56%	60%	70%	55.5%	42%	52%	57%
Concentration (μg/mL)							
26.38	25.07	19.05	6.61	25.6	32.0	25.6	25.6
19.33	12.53	9.52	3.31	12.8	25.6	12.8	12.8
9.67	6.27	4.76	1.65	6.4	12.8	6.4	6.4
4.83	5.48	4.17	1.45	5.6	6.4	5.6	5.6
4.23	3.92	2.98	1.03	4.0	5.6	4.0	4.0
3.02	2.35	1.79	0.62	2.4	4.0	2.4	2.4
1.81	0.78	0.60	0.21	0.8	2.4	0.8	0.8
0.60	0.39	0.30	0.10	0.4	0.8	0.4	0.4

[a] product from ICI Chlor-Chemicals (Milbank, UK).
[b] product from Hüls (Marl, Germany).
[c] Dr. Ehrenstorfer reference material (Augsburg, Germany).

2.2. Samples

The biodegradable fraction of fresh source separated household waste from Uppsala, Sweden, was collected in the last week of February 1995 (Eklind et al., 1997). The subsamples were divided into three groups WA, WB and WC, with slight differences in the methods of extraction and clean-up. The waste samples were partially dried at ambient temperature before extraction. Homemade orange marmalade made in January 1995 was obtained from three separate households in the vicinity of Uppsala.

2.3. Extraction and clean-up

Detailed information on the clean-up procedures have been given by Nilsson (2000), and Nilsson et al. (2001). In summary, WA samples were extracted in a Soxhlet apparatus and WB samples in a Soxtec Avanti 2050 Auto System (Foss Tecator, Höganäs, Sweden), both with dichloromethane and with addition of the recovery standard HBCD prior to extraction. After extraction the solvent was changed to cyclohexane and the extract treated with concentrated sulphuric acid after which the organic phase was filtered (Acrodisc CR PTFE filter, 0.45 μm, 25 mm, Gelman Sciences, Ann Arbor, MI, USA). The filtered extract was further purified using size exclusion chromatography (SEC) with Bio-Beads S-X3 (200–400 mesh, Bio-Rad Laboratories, Hercules, CA, USA) as stationary phase. The SEC-fraction containing the CPs was cleaned-up further on a Nucleosil 5 NO_2 nitrophenylsilica column (250 mm, 4.6 mm ID, 5 μm phase thickness, Jones Chromatography, Hengoed, Mid Glamorgan, Wales) coupled to a Nucleosil 5 NH_2 (10 mm, 4 mm ID, 5 μm phase thickness) guard column, with n-hexane as eluent. The eluent flow was reversed between the peaks of *o,p′*-DDT and *p,p′*-DDT and a backflush fraction was collected containing the CPs without any PCBs (Kylin et al., 1996) and α,α,α′,α′-tetrachloro-o-xylene was added.

WC samples were ground with hydromatrix and extracted in a Soxtec Avanti 2050 Auto System with acetone:dichloromethane (3:1) with addition of PCB 53. After size exclusion chromatography as above, final clean-up was fractionation on deactivated (1.5%) silica gel where the least polar organochlorines were eluted with *n*-hexane and the CPs with *n*-hexane:toluene (65:35). PCB 209 was added prior to quantification.

The marmalade samples, two from each household, were macerated with an Ultra-Turrax (IKA, Staufen, Germany) until smooth. Subsamples of each marmalade sample were taken out and dried at 105 °C until stable mass readings were obtained. The marmalade samples (2 g) were mixed with concentrated sulphuric acid (6 g) in a test tube with Teflon-lined screw cap, left standing for 24 h, and then extracted with *n*-hexane (3 × 6 ml) after addition of the recovery standard HBCD. After phase separation with centrifugation, the *n*-hexane extracts were pooled and the volume reduced under a slow stream of nitrogen, after which the extracts were processed further as sample groups WA and WB above.