A REVIEW OF SHORT CHAIN CHLORINATED PARAFFINS (SCCPS) IN THE AQUATIC ENVIRONMENT AND THE DEVELOPMENT OF AN ANALYTICAL TECHNIQUE FOR THEIR ANALYSIS IN ENVIRONMENTAL SAMPLES

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SUMMARY

1. Due to their persistence, potential for long-range atmospheric transport, high bioaccumulation and toxicity, short chain chlorinated paraffins (SCCPs) are listed on the OSPAR List of Chemicals for Priority Action and are classified as priority hazardous substances under the Water Framework Directive (WFD).

2. SCCPs have been found in the OSPAR Convention area in both sediment and fish and in areas remote from known sources, indicating they are widespread environmental contaminants. Although not produced in Scotland, SCCPs have been produced within the UK and have been detected in the UK marine environment.

3. A method for the analysis of SCCPs in environmental samples was developed at FRS using Pressurised Liquid Extraction (PLE), clean-up by column chromatography followed by analysis by gas chromatography with electron capture negative ionisation mass spectrometry (GC-ECNIMS).

4. Few individual SCCP standards are currently available, so technical mixtures of 51.5%, 55.5% and 63% chlorination were used as calibration standards. SCCP isomers cannot be separated, and an unresolved complex mixture (UCM) is observed in the GC chromatogram. 13C Hexachlorobenzene (HCB) was selected as injection standard, as it could be separated from the UCM and is not present in environmental samples.

5. Calibration standards at concentrations ranging from 2 ng µl⁻¹ to 75 ng µl⁻¹ (5 point calibration) were analysed. Linear calibration curves gave correlation coefficients of 0.992, 0.995 and 0.997 for the 51.5%, 55.5% and 63% technical mixtures, respectively. The response increased with the chlorination level of the technical mixture.

6. The middle chlorination level (55.5%) was used for spiking experiments and determination of precision. The method was validated for the analysis of SCCPs in biota and sediment using the middle chlorination level. Replicate analysis of the high and low standards on separate days by GC-MS gave CV% of 13%. Recoveries ranged between 111% and 121% for sediment and from 66 to 107% for biota.
7. Limits of detection (LoDs) for SCCPs were around 9.2 μg kg\(^{-1}\) wet weight for mussels (8 g sample extracted) and 2.9 μg kg\(^{-1}\) dry weight for sediment (20 g sample extracted).

8. Although the validation data from the analysis of standards and spiked samples was acceptable, the analysis of environmental samples is less straightforward. The calculated concentrations are dependent on the chlorination level of the technical mixture used in the calibrations and can vary by as much as 100%. Before quantification of SCCPs in environmental samples is achieved, chromatograms must be classified according to the chain length and degree of chlorination and quantified against the appropriate technical mixture. However, this is not easy as mixtures of SCCP formulations are likely to be present in the environment. As such it was concluded that although the extraction and clean-up method was good, the dependence of the calculated concentration on the chlorination level of the calibration standards means that for quantifying environmental sample the method is at best semi-quantitative.

9. Preliminary investigations were undertaken into an alternative methodology where the chlorinated compounds are hydrogenated to produce \(n\)-alkanes which can be detected either by GC with flame ionisation detection (FID) or GC with electron impact mass spectroscopy. Although good results were obtained, details of the chlorination pattern are lost and rigorous clean-up is required due to the ubiquitous nature of \(n\)-alkanes. Further research will be required to conclude on the relevant merits of the two detection methods.

INTRODUCTION

The marine environment acts as a sink for many persistent organic pollutants (POPs). Many of these compounds are known to bioaccumulate and biomagnify and some have been found in areas remote from sources or emissions as a result of long-range atmospheric transport. In recent years a number of these substances have been highlighted as a cause for concern and have been the subject of extensive study and international regulation. Monitoring of hazardous substances in the Scottish marine environment is required to enable Scotland to assess what action is required in order to enable the Scottish vision of clean and safe seas to be delivered. In addition, such monitoring is required in order to ensure that Scotland fulfils its international obligations to OSPAR and in respect of EU Directives (e.g. Water Framework Directive (WFD)). The WFD objective for priority hazardous substances (PHS) is for the cessation of discharges, emissions and losses within 20 years of the adoption of the Directive (i.e. by 2020). The OSPAR Hazardous Substances Strategy requires the progressive reduction of discharges, emissions and losses of hazardous substances to the marine environment with the aim of achieving concentrations of near background for naturally occurring substances or close to zero for man-made chemicals.

A contaminant group that is not currently monitored in Scotland, but may be an issue on the basis of known discharges and the limited data available to date, is the short chain chlorinated paraffins (SCCPs). SCCPs are listed on the OSPAR List of Chemicals for Priority Action and are classed as PHS under the WFD. Therefore, data on this contaminant group is required for Scotland to fulfil its international obligations under both OSPAR and the WFD and to assess whether Scotland is moving towards the Scottish
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Government’s vision of achieving clean, healthy, safe, productive and biologically diverse oceans and seas.

Chlorinated paraffins (CPs) are formed by the chlorination of \( n \)-paraffins, with carbon chain lengths of between 10 and 30 carbon atoms and with a chlorine content of between 30 and 70% by weight\(^1,2 \). CP formulations are highly complex mixtures that contain a large number of structural isomers, theoretically more than 10,000 diastereoisomers and enantiomers. These complex mixtures can be divided into three groups depending on their chain length and degree of chlorination; short, medium and long chain CPs. Short chain CPs (SCCPs) are defined as C\(_{10}\)-C\(_{13}\) paraffins with greater than 48% chlorination by weight. The most abundant chain lengths are C\(_{11}\) (33%) and C\(_{12}\) (38%). Medium chain CPs (MCCPs) have carbon chain lengths of C\(_{14}\)-C\(_{17}\) and long chain CPs (LCCPs) have carbon chain lengths of > C\(_{17}\). SCCPs are of greatest interest as they have the highest toxicity and the greatest potential for release into the environment and are therefore included on the OSPAR List of Chemicals for Priority Action and are classed as PHS under the WFD. SCCPs have been produced over the last 30 years for use in metal working fluids (70%), as plastisics in paints, coatings (9%) and sealants (5%) and as flame retardants in rubbers (10%) and textiles (1.5%) and in leather processing (3%)\(^1,2 \). The main sources of SCCPs are from production sites of these compounds, production sites for the formulation of metal working fluids and leather finishing plants and via waste disposal. SCCPs can reach the marine environment via rivers and via atmospheric deposition. In the UK, CPs are manufactured by ICI (Runcorn, Cheshire).

SCCPs are considered to be relatively persistent. They can undergo bacterial breakdown with the more highly chlorinated compounds being more persistent than the less chlorinated compounds\(^1-3 \). SCCPs degrade faster than the medium and long chain CPs. Half-lives have been estimated at between 0.85 to 7.2 days in the atmosphere. SCCPs have high octanol water partition coefficients (Log \( K_{ow} > 4 \)), which increase with increasing chlorine content; Log \( K_{ow} \) ranges from 4.39 to 8.01 \(^1-4 \). Therefore, they adsorb to sediment and have the potential to bioaccumulate. SCCPs are bioaccumulated to a greater extent than the medium or longer chain CPs but less so than chlorobiphenyls (CBs)\(^2 \). Uptake of higher molecular weight CPs may be inhibited by their high molecular weight. Bioconcentration factors range from 7 to 7,155 for fish and from 223 to 138,000 for mussels\(^2 \). Toxicity in fish was found to be related to chain length with the longer chain CPs being less toxic\(^5-7 \). However, neurotoxic effects have been observed with SCCPs. This includes loss of motor function and immobilisation\(^8-10 \). SCCPs have been shown to be carcinogenic to rats and mice\(^11 \). An environmental quality standard (EQS) of 0.1 \( \mu g l^{-1} \) has been derived for coastal and territorial waters and 198 \( \mu g kg^{-1} \) dry weight for marine sediment.

**Environmental Concentrations**

There are few data available on SCCPs in the OSPAR Maritime Area. The lack of environmental data on SCCPs reflects the lack of sensitive, quantitative analytical methods available for measuring these compounds. The presence of SCCPs in biological samples from the Arctic suggests that these chemicals can be transported through the atmosphere\(^1,2,12 \). SCCPs were detected in two species of fish (cod and Arctic char) from the European Arctic with concentrations ranging from 0.007 to 0.07 mg
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kg⁻¹ wet weight¹². CP concentrations have been measured in UK waters (water and sediment samples) from both remote and industrialised areas¹³. This survey included some sites in Scotland (Clyde, Loch Linnhe and the Minches). However, only sediment from the Minches was analysed and concentrations were below the detection limit. However, detection limits were high (0.05 mg kg⁻¹ wet weight). Highest concentrations of CPs (C₁₀ – C₂₀) were 0.5 mg kg⁻¹ wet weight in Barmouth harbour¹³. Concentrations in freshwater sediments close to an industrial source ranged from 1 to 10 mg kg⁻¹ wet weight. Higher molecular weight CPs (C₂₀ – C₃₀) were not found in aquatic organisms and C₁₀ – C₂₀ CPs were only occasionally found but at low concentrations. SCCPs have been detected in the atmosphere of the UK, with the hexa- and hepta-chlorinated C₁₂ SCCPs dominating the profiles¹⁴. Air samples collected from a semi-rural location in Lancaster in 1997 and 1998 gave concentrations similar to those found in Canada and indicated the potential of SCCPs to undergo long range atmospheric transport. Nichols et al. looked at SCCPs and MCCPs in environmental samples from England and Wales¹⁵. Sites were selected to target industries known to be using CPs in their products or manufacturing process such as metal working lubricant producers, and a CP production site at Runcorn. Concentration ranges for fish and sediment were < 0.1 – 5.2 mg kg⁻¹ wet weight and <0.2 – 65.1 mg kg⁻¹ dry weight, respectively¹⁵. SCCPs and MCCPs were measured by electron capture negative ionization- mass spectrometry (ECNI-MS) in sediment from the North and Baltic Sea, concentrations ranged from 8 – 63 µg kg⁻¹ dry weight for the North Sea and between 0.0022 – 0.149 mg kg⁻¹ dry weight for the Baltic Sea¹⁶. SCCP concentrations in sediment close to industrial discharges in the Czech Republic ranged from 0.0046 – 0.18 mg kg⁻¹ dry weight¹⁷. Marine sediments from Barcelona were analysed for a range of contaminants, including SCCPs. Their concentrations in coastal sediments ranged from 0.21 to 1.17 mg kg⁻¹ dry weight and from 1.25 to 2.09 mg kg⁻¹ dry weight at the mouth of Besos River, close to a submarine emissary¹⁸.

METHOD DEVELOPMENT AND VALIDATION

Extraction and Clean-up Methods

The extraction and clean-up of environmental samples for SCCPs is relatively straightforward and methods used for other persistent organic pollutants can be applied to the extraction of SCCPs. Extracts will always contain many compounds in addition to the compounds of interest and therefore, a suitable clean up is necessary to remove those compounds which may interfere with the subsequent analysis. A range of methods have been used for the extraction of SCCPs from biota and sediment. Bayen et al. published a review of analytical methods for the determination of CPs in 2006¹⁹. Extraction methods for CPs in environmental samples include the more traditional methods such as Soxhlet or Ultra Turrax homogenization. Only two publications were found utilising newer automated methods such as pressurised liquid extraction (PLE). Tomy and Stern used PLE to extract MCCPs from sediment samples²⁰. Pellizzato et al. used PLE extraction, followed by a Florisil column clean-up and analysis by GC-MS equipped with a palladium liner (see last section) for the determination of SCCPs in environmental samples²¹.

An advantage of using PLE extraction is that it is possible to combine the clean up with the extraction, especially where mass spectrometry is being used as the method of detection. Methods have been developed for online clean-up and fractionation of
dioxins, furans and polychlorinated biphenyls (PCBs) with PLE for food, feed and environmental samples\textsuperscript{22}. One method utilises a fat retainer for the on-line clean-up of fat. Silica impregnated with sulphuric acid, alumina and Florisil have all been used as fat retainers. A non-polar extraction solvent such as hexane should be used if fat retainers are required during PLE.

PLE methods are routinely used at FRS for the extraction of organic contaminants such as PBDEs and CBs in both sediment and biota\textsuperscript{23}. Addition of 5% deactivated alumina (biota, 30 g; sediment 15 g) to the PLE extraction tubes allows the extraction and clean-up steps to be combined.

The PLE method, using fat retainers, was investigated at FRS ML for the extraction of SCCPs. Initially no matrix (mussel or sediment) was used and the PLE cell was filled with sodium sulphate and alumina (5% deactivated) and spiked with the three commercially available SCCP technical mixtures (51.5%, 55.5% and the 63% chlorination). iso-Hexane was used as the extraction solvent with an extraction temperature of 100°C (Table 1). Recoveries of greater than 90% were obtained using 5g of alumina.

There is a requirement to further clean-up the initial extract obtained by PLE. Specifically, there was a need to assess the separation of SCCPs from other organic contaminants such as PBDEs and CBs which may also be present in the initial extract. The column clean-up procedure that was investigated was based on a method used by Reth et al.\textsuperscript{16}, but using acetone instead of dichloromethane to elute the SCCPs. A Florisil column (1.5% deactivated, 4 g) was conditioned with hexane (10 ml) and the SCCP technical mixtures applied to this. The column was eluted with iso-hexane. The first fraction (0 – 15 ml column eluate) should contain any CBs and toxaphenes that are co-extracted with the SCCPs. Acetone was then applied to the column and the second fraction (15 – 17 ml column eluate) collected. The third fraction (17 – 40 ml column eluate) was found to contain SCCPs.

To test the efficiency of the Florisil column in separating SCCP mixtures from possibly interfering compounds in the environment, a mixed standard solution, containing a 51.5% SCCP technical mixture, polybrominated diphenyl ethers (9 congeners) and chlorobiphenyls (23 congeners) was prepared and applied to the Florisil column. Fractions were collected as above and analysed by GC-MS. The first fraction contained most of the CB congeners; the rest were in fraction 2. The PBDEs were found to split between the 2\textsuperscript{nd} and 3\textsuperscript{rd} fraction. However, PBDEs do not contain any ions that will interfere with the GC-MS analysis of SCCPs since the main ions used to detect PBDEs are the bromine ions (m/z = 79/81) and their molecular weight is quite different to those ions used to detect the SCCPs.

**Instrumental determination of SCCPs**

The most challenging aspect of the determination of SCCPs in environmental samples is the quantification. Currently there are no fully validated methods published that are suitable for routine monitoring. Most published methods are qualitative or semi-quantitative; there are very few quantitative methods. The lack of certified standards, lack of sensitive and selective analytical methods and the lack of a formal quality assurance scheme have hindered the development of the analysis of SCCPs in environmental samples.
Few individual SCCP standards are available and, therefore, commercial, technical mixtures are generally used as calibration standards. As stated earlier, SCCP formulations consist of a range of molecular chain lengths and degrees of chlorination resulting in more than 10,000 structural isomers with a wide range of physical properties. Due to the complexity of these mixtures, analysis of CPs is difficult. Early work used thin layer chromatography (TLC) to investigate the presence of CPs in the environment. However, more recent methods utilise extensive column clean-ups followed by gas chromatography - mass spectrometry (GC-MS), normally in electron capture negative ionisation (ECNI) mode. Electron impact (EI) mode results in increased fragmentation of all CPs and therefore only total CPs (sum of SCCPs, MCCPs and LCCPs) can be reported. Gas chromatography with electron capture detection (GC-ECD) has also been used, but as with GC-MS in EI mode, results can only be reported for total CPs. Therefore, GC-MS in ECNI, monitoring the \([M-Cl]^-\) or \([M-Cl]^+\), is the most commonly applied detection method for SCCPs. However, concentrations may be overestimated due to the presence of compounds with similar molecular weights, which are difficult to separate during clean up, being present. CP congeners with five carbon atoms more and two chlorines less present ions and fragments with the same nominal mass; for example, the MCCP, \(C_{15}H_{28}Cl_4\) and the SCCP, \(C_{10}H_{16}Cl_6\) have molecular weights of 350 and 349 Da. However, little mass overlap was found for the major components of the SCCPs (\(C_{11}\) and \(C_{12}\)) from other CPs. Mass overlap may be overcome using high resolution GC-ECNIMS.

Randegger-Vollrath used gas chromatography with electron capture detection (GC-ECD) and GC-MS in conjunction with electron capture negative ionisation (ECNI) mode to analyse CPs in cutting fluids and lubricants. Sample chromatograms were classified according to their chain length and degree of chlorination and quantified against the appropriate technical mixture. The concentration obtained is largely dependent on the chlorination level of the technical mixture used in the quantification. Thus a common method for the quantification is to select the technical mixture closest to pattern obtained in a sample chromatogram and to use this in the calibration. Nicholls et al. also used GC-ECNIMS for the analysis of SCCPs and MCCPs, made a qualitative identification of the formulation type in the samples from each site, then quantified the samples using this formulation. Where no particular formulation can be identified, a mid range formulation (45 – 65%) has been used for the calibration. Žencak et al. compared four mass spectrometric methods for the analysis of SCCPs. High and low resolution ECNIMS, electron ionisation tandem mass spectrometry (EI-MS/MS) and low resolution ECNIMS using methane and dichloromethane as the reagent gas. The use of dichloromethane in the reagent gas favours the formation of chlorine adduct ions, but requires modifications to be made to the GC-MS. However, due to the use of dichloromethane, the MS source requires more regular cleaning and maintenance and therefore this technique may not be suitable for routine monitoring. Both high and low resolution ECNI methods were more dependent on the chlorination level of the technical mixture used in the calibrations compared to the EI-MS/MS and low resolution ECNIMS, using methane and dichloromethane as the reagent gas methods.

GC-GC combined with time of flight ECNIMS was shown to improve the separation of CPs. GC-GC utilises a modulator to focus the eluate from the first column on to a second, enabling further separation of all relevant components. This enabled the separation of SCCPs, MCCPs and LCCPs. CPs with the same chain length could be separated based on the number of chlorines, however, there was significant overlap.
The selection of the GC column is less critical as individual SCCP isomers cannot be separated and an unresolved complex mixture is obtained. As it is impossible to separate all SCCP isomers a short GC column may be used. Stejnariva et al. recommended the use of a very short column (1.3 m x 0.15 mm id) which gave a single peak for all isomers\textsuperscript{17}. However, this method requires a very thorough clean-up to remove all potentially interfering substances, such as CBs.

High-performance liquid chromatography combined with chloride enhanced atmospheric pressure chemical ionisation (CACI) mass spectroscopy was also used for the quantification of CPs\textsuperscript{28}. Methane/dichloromethane is used as the reagent gas and has the advantage that the MS response is not dependent on the degree of chlorination, and therefore the technical mixtures used in the calibration will not have an effect on the quantification. An LoD of 1 ng µl\textsuperscript{-1} was achieved using this method.

A GC-ECNIMS method was developed at FRS for the analysis of SCCPs using an Agilent HP5 MS column (30.0 m x 250 µm x 0.25 µm). The carrier gas was helium, set at a constant pressure of 15 psi. Methane was used as the reagent gas at a pressure of 1.6 bar. The transfer line was held at 280°C and the ion source at 150°C. Injections were made at 120°C and the oven temperature held constant for 2 minutes. Thereafter the temperature was raised at 10°C min\textsuperscript{-1} up to a final temperature of 260°C and held at this temperature for 30 minutes. The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. The ions monitored are shown in Table 2. The GC chromatogram (Fig. 1) gave a UCM similar to published chromatograms, with the response being dependent on the chlorination level of the technical mixture used. A range of injection standards were investigated; 2, 4-dichlorobenzyl alkyl hexyl ether with C\textsubscript{6} and C\textsubscript{16} alkyl chains (D\textsubscript{6D16}), γ-chlordane and 13C hexachlorobenzene (HCB). 13C HCB was selected as this was separated from the UCM in the chromatogram and will not be found in environmental samples.

**Method Validation for SCCPs (PLE followed by a Florisil Column Clean-up and Analysis by GC-ECNIMS)**

For the validation of the method the following was investigated:

- linear response range
- precision of standards
- limit of detection
- recovery
- blank values

**Linear Response Range**

The linear response range was assessed by the triplicate analysis of five standards for each of the three SCCP technical mixtures. The injection volume was 1 µl. Calibration standards at concentrations of 75 ng µl\textsuperscript{-1}, 50 ng µl\textsuperscript{-1}, 25 ng µl\textsuperscript{-1}, 10 ng µl\textsuperscript{-1} and 2 ng µl\textsuperscript{-1} were analysed. Each of the calibration levels contained 0.2 ng µl\textsuperscript{-1} of 13C HCB. Linear calibration curves gave correlation coefficients of 0.992, 0.995 and 0.997 for the 51.5%, 55.5% and 63% technical mixtures, respectively (Fig. 2). The response increased with...
the chlorination level of the technical mixture. The retention time also changed, with the UCM shifting to the right (retention time increasing) as the chlorination level increased.

**Precision of Standards**

For the determination of analytical precision, replicate low (10 ng µl⁻¹) and high (67.5 ng µl⁻¹) standards of the working range were analysed by GC-ECNIMS on separate days. CV% for the both high and low standard was 13%.

**Limits of Detection**

The limits of detections (LoD) of SCCP were determined through the repeat analysis (n =7) of a low spiked sample consecutively on the same day. Biota (mussels, 8 g) or sediment (20 g) were spiked with 250 ng of the 55.5% SCCP technical mixture. Following PLE extraction and clean up by Florisil column, samples were analysed by GC-ECNIMS in one batch. The mean concentration and standard deviation for SCCPs were calculated and the LoDs calculated from 4.65 x standard deviation (Table 3). The LoDs for biota samples was 9.2 µg kg⁻¹ wet weight and for sediment 2.9 µg kg⁻¹ dry weight. Few publications give method LoDs. Reth and Oehme reported an instrument LoD of 1 ng µl⁻¹ for a SCCP technical mixture at a signal-to-noise ratio of 3:1 and an Limit of Quantification (LoQ) of 2 ng µl⁻¹ at a signal-to-noise of 10:1²⁴. Zencak et al. also reported an instrument LoD of 1 ng µl⁻¹ for a SCCP technical mixture (55.5%) using both high and low resolution GC-ECNIMS²⁶. Castells et al. reported a LoD for sediment of 1.8 ng g⁻¹ dry weight using GC-ECNIMS/MS (ion trap)¹⁶. Nicholls et al. reported a LoD of 200 ng g⁻¹ for sediment using GC-ECNIMS¹⁵.

**Procedural Blanks**

With each batch of samples a procedural blank was analysed. The matrix/sodium sulphate mixture was replaced with sodium sulphate and extracted by PLE, cleaned-up by Florisil column, concentrated by Syncore and Rotary Evaporator and analysed by GC-ECNIMS. Concentrations of compounds with equivalent retention times in the procedural blanks were below the method LoD.

**Recovery**

Recoveries were calculated through replicate analysis of SCCPs in mussel and sediment samples. For a sediment sample (10 g) spiked with 2.5 µg of 55% SCCP technical mixture, recoveries ranged from 111 – 121% (n = 7) with a CV% of 2.4%. For a sediment sample (20 g) spiked with 250 ng of 55% SCCP technical mixture, recoveries ranged from 69 - 82% (n = 8) with a CV% of 6.9% (Table 3). For biota (mussels, 10g) spiked with 2.5 µg of 55% SCCP recoveries ranged from 66 to 107% (n = 7) with a CV% of 19.8% while a mussels spiked with 250 ng gave recoveries of 68 to 95% (CV% = 11.5%) (Table 3). Nicholls et al. reported recoveries for sediment spiked with SCCPs of 50 – 119% (n = 7) and for fish 59 and 97% (n = 2)¹⁵. Reth et al. reported recoveries in fish tissue and fish oil of between 80 and 100%¹⁶. The validation data for the FRS method appears to be as good as most published methods.
Quantification

The spiking experiments were carried out using a known technical mixture and the calibrations were carried out using the same technical mixture as used for spiking samples. In the environment it is likely that there will be a combination of technical mixtures present. The calculated concentration is highly dependent on the chlorination level of the technical mixture used in the calibration; therefore quantification of real samples will be less straightforward. The most common method employed is to classify the sample chromatograms according to their chain length and degree of chlorination and quantify against the appropriate technical mixture. Where it was not possible to decide on the SCCP formulation present, or it is likely that a mixture of SCCP formulations are present, a mid range chlorination SCCP technical mixture (55.5%) should be used in the calibration. However, this will increase the uncertainty of the method for quantifying real samples. It has been reported that the concentration of a sample can vary by as much as 100% depending on which chlorination level is used in the calibration. A spiked sediment sample (250 μg kg⁻¹ dry weight of 55.5% SCCP technical mixture) was quantified using three separate 5 point calibrations for the 63%, 55.5% and 51.5% technical mixtures. The calculated concentrations were 104.5, 295.5 and 648.2 μg kg⁻¹, respectively.

ANALYTICAL METHODOLOGY

Cleaning of Glassware, PLE tubes, filters and sodium sulphate

At FRS ML glassware was washed and dried in a GW 4000 glassware washer (Camlab Ltd., Cambridge, UK). Prior to use, all glassware was rinsed twice with acetone and then twice with iso-hexane, the latter being allowed to evaporate before proceeding. Anhydrous sodium sulphate was washed ultrasonically with dichloromethane (DCM) (2 x 500 ml) followed by iso-hexane (2 x 500 ml) and dried overnight at 150°C.

Glass fibre filters were wrapped in aluminium foil and placed in a muffle furnace set to 200°C for 12 hours. All pressurised liquid extraction (PLE) cells, caps and collection bottles were solvent washed with acetone followed by iso-hexane, with the latter being allowed to evaporate before proceeding. The lids of the collection bottles were fitted with ultra low bleed septa which were first solvent washed with iso-hexane.

Preparation of Standard Solutions

Three commercially available SCCP technical mixtures (100 ng µl⁻¹ of 51.5%, 55.5% and 63% chlorine) were used as calibration standards. Five calibration standards were prepared by diluting 1500 µl, 1000 µl, 500 µl, 200 µl and 40 µl of each technical mixture to 2 ml in iso-hexane to give 75, 50, 20, 10 and 2 ng µl⁻¹, respectively. ¹³C HCB, at a concentration of 0.2 ng µl⁻¹, was also included in the calibration standards. All calibration standards were analysed by gas chromatography - electron capture negative ionisation mass spectrometry (GC-ECNIMS).
Preparation for Pressurised Liquid Extraction (PLE)

For biota extractions, an appropriate amount of tissue (equivalent to 300 mg lipid) was mixed with sodium sulphate (~40 g), and left overnight before being ground using a mortar and pestle. Solvent washed PLE cells (100 ml) were packed as follows: solvent washed filter paper, pre-washed sodium sulphate (10 g), 5% deactivated alumina (30 g), solvent washed filter paper and the biota/sodium sulphate mixture prepared as above or freeze dried sediment (20 g). The cell was finally filled to the top with more sodium sulphate then packed down and topped up if required and another filter paper placed on top. It was essential that the cell was tightly packed, in order to minimise the dead volume.

Pressurised Liquid Extraction (PLE)

Samples were extracted using an oven temperature of 100°C and a pressure of 1500 psi (Table 1). Five minutes heating was followed by 2 x 5 min static cycles. The cell flush was 50% total cell volume (i.e. 25% of the cell volume for each flush = 25 ml per flush) with a 60 second purge (using nitrogen) at end of each sample extraction. The extraction solvent used was iso-hexane. Following PLE extraction, the extracts were transferred to Syncore tubes and the volume reduced to ~0.5 ml.

Extract Clean-up and Concentration

Any remaining co-extractives and other organic contaminants (e.g. CBs) were separated from the SCCPs using a Florisil column. Florisil was heated to 600°C prior to cooling and deactivation with distilled water, on a percentage weight basis, to 1.5%. Glass chromatography columns (22 cm x 1 cm, l x d) were packed with deactivated Florisil (4 g). The column was conditioned with hexane (5 ml) and the extracts were transferred to columns and iso-hexane (15 ml) added. The first fraction (0 – 15 ml) containing CBs and toxaphenes, was discarded. Acetone (25 ml) was then applied to the column and the second fraction (15 - 17 ml) also discarded. The third fraction (all the following eluent, 17 – ~40 ml), containing the SCCPs, was collected. The collected fraction was concentrated to approximately 1 ml by rotary evaporator, solvent exchanged with 50 ml iso-hexane and concentrated again to approximately 0.5 ml. 13C HCB (30 µl of a 0.1 ng µl⁻¹ solution) was added to a GC vial, to which the concentrated extract was then added gradually whilst concentrating further under nitrogen to approximately 50 µl prior to analysis by GC-ECNIMS.

Analysis by Gas Chromatography - Electron Capture Negative Ionisation Mass Spectrometry (GC-ECNIMS)

The concentrations of SCCPs were determined by GC-ECNIMS using an HP6890 Series gas chromatograph interfaced with an HP5973N MSD, fitted with a cool on-column injector. A medium polarity column was used for the analyses (HP 5MS, 30.0 m x 250 µm x 0.25 µm film thickness; Agilent, Stockport, UK). The carrier gas was helium, set at a constant pressure of 15 psi. Methane was used as the reagent gas at a pressure of 1.6 bar. The transfer line was held at 280°C and the ion source at 150°C. Injections were made at 120°C and the oven temperature held constant for 2 minutes. Thereafter the temperature was raised at 10°C min⁻¹ up to a final temperature of 260°C.
and held at this temperature for 30 minutes. The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. The ions monitored are shown in Table 2.

The GC-MSD was calibrated by a series of five SCCP standards (2 – 75 ng µl⁻¹), prepared from technical mixtures, that contain ¹³C-HCB (0.2 µg ml⁻¹) as an injection standard.

**PRELIMINARY INVESTIGATIONS OF AN ALTERNATIVE METHODOLOGY**

CPs have also been analysed by gas chromatography with flame ionisation detection (GC-FID) or GC-MS following dechlorination and hydrogenation to the corresponding n-alkane. Individual n-alkane standards can be used for quantification. The n-alkane concentrations will correspond to the SCCP concentration. Koh et al. used a palladium catalyst to dechlorinate before analysis by GC-FID²⁹. This method was used to determine the CP content of cutting fluids and sealing materials. A recent discussion paper on SCCP analysis also recommended the hydrogenation method using GC-MS for the analysis of n-alkanes²¹. The authors used a palladium modified liner, and hydrogen as the carrier gas, for the hydrogenation of SCCPs. An LoD of 1 nmol g⁻¹ was achieved.

The hydrogenation method was investigated at FRS, using palladium as the catalyst in the GC liner and then analysing for the n-alkanes produced using GC-FID. The GC injector was kept at 300°C and the GC oven temperature programme was 3 minutes at 50°C, then raised at 10°C min⁻¹ to 280°C then held for 25 minutes; this gave a total run time of 51 minutes. A split/splitless injector was used. Individual, well separated, n-alkanes (nC₁₀ – nC₁₃) were quantified using the corresponding n-alkane standards. Figure 3 shows the chromatogram of n-alkanes (nC₁₀ – nC₁₄) following the hydrogenation of the 55.5% SCCP technical mixture. nC₁₄ is a minor component of this SCCP technical mixture. This method is promising, however any information on chlorination level will be lost and a very thorough clean up will be required to ensure all n-alkanes present in the sample are removed before hydrogenation.

**CONCLUSIONS**

1. A GC-ECNIMS method, following extraction by PLE and clean-up on Florisil, was developed for the analysis of SCCPs, this is the most commonly used technique for this analysis of SCCPs. However, there are some pitfalls with this analysis, including overestimation of the concentration due to mass overlap and the dependence on the GC-MS response on the chlorination level of the technical mixtures.

2. The PLE extraction and clean-up method for SCCPs was based on equivalent methodology developed at FRS for other organic contaminants (PBDEs and CBs). One advantage of using PLE is that the extraction and clean-up may be combined by adding fat retainers to the extraction cell. For SCCPs, using 5% deactivated alumina in the PLE cell, it was possible to extract and clean-up sediment and biota (= 300 mg lipid) samples. However, a further clean-up and separation step was required, using a Florisil column, before analysis by GC-ECNIMS.
3. The linearity of the three commercially available technical mixtures analysed by GC-ECNIMS were assessed, correlation coefficients of at least 0.99 were obtained. A UCM was obtained for all three mixtures, the response increases with chlorination level. Replicate analysis of high and low standards on separate days by GC-ECNIMS gave CV% of 13% for SCCPs.

4. Recoveries for SCCPs (2.5 μg) from spiked biota ranged from 66 to 107% and for sediment from 111 to 121%. For a 250 ng spike recoveries ranged from 69 – 82% for sediment and from 68 – 95% for biota. The LoD was calculated using a low spiked sample. For an 8 g mussel sample this was 9.2 μg kg⁻¹ wet weight and for a 20 g sediment sample 2.9 μg kg⁻¹ dry weight.

5. The analysis of real samples is complicated as it is necessary to first classify sample chromatograms according to their chain length and degree of chlorination of the SCCPs and quantify against the appropriate technical mixture. The calculated concentration can vary by as much as 100% depending on which SCCP technical mixture is used for the calibration.

6. In environmental samples it is likely that a combination of SCCPs will be present. Therefore, the method developed at FRS, and similar to other published methods, is at best semi-quantitative.

7. An alternative technique involves a hydrogenation step; hydrogenation of SCCPs gives \( n \)-alkanes which can then be accurately quantified by either GC-FID or GC-MS using individual \( n \)-alkane standards (\( nC_{10} \) to \( nC_{13} \)) for the calibration. This method was briefly investigated at FRS and looks promising. However, information on the chlorination level will be lost using this technique and a very effective clean-up will be required to remove all \( n \)-alkanes present in the sample before hydrogenation.

REFERENCES


11. **S. Hallgren, and P. O. Darnerud**, 1998. Effects of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) on thyroid hormone levels and enzyme activities in rats. *Organohalogen Compounds*, 35, 391-394.


TABLE 1

Pressurised Liquid Extraction (PLE; ASE 300) settings for the extraction of SCCPs from sediment and biota.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td>1500 psi</td>
</tr>
<tr>
<td>Temperature</td>
<td>100°C</td>
</tr>
<tr>
<td>Heat</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Static Time</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Flush %</td>
<td>50</td>
</tr>
<tr>
<td>Purge</td>
<td>60 seconds</td>
</tr>
<tr>
<td>Number of Cycles</td>
<td>2</td>
</tr>
<tr>
<td>Extracting solvent</td>
<td>iso-hexane</td>
</tr>
</tbody>
</table>
TABLE 2

Mass-to-charge ($m/z$) ratios of the [M-Cl]$^-$ ions monitored for SCCP congeners

<table>
<thead>
<tr>
<th>SCCP congener</th>
<th>Most abundant isotope</th>
<th>Second abundant isotope</th>
</tr>
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<tbody>
<tr>
<td>$C_{10}H_{15}Cl_7$</td>
<td>347</td>
<td>349</td>
</tr>
<tr>
<td>$C_{10}H_{14}Cl_8$</td>
<td>381</td>
<td>383</td>
</tr>
<tr>
<td>$C_{11}H_{20}Cl_4$</td>
<td>257</td>
<td>259</td>
</tr>
<tr>
<td>$C_{11}H_{19}Cl_5$</td>
<td>291</td>
<td>293</td>
</tr>
<tr>
<td>$C_{11}H_{18}Cl_6$</td>
<td>327</td>
<td>329</td>
</tr>
<tr>
<td>$C_{11}H_{17}Cl_7$</td>
<td>361</td>
<td>363</td>
</tr>
<tr>
<td>$C_{12}H_{20}Cl_6$</td>
<td>341</td>
<td>343</td>
</tr>
<tr>
<td>$C_{12}H_{19}Cl_7$</td>
<td>375</td>
<td>377</td>
</tr>
<tr>
<td>$C_{12}H_{18}Cl_8$</td>
<td>409</td>
<td>411</td>
</tr>
<tr>
<td>$C_{13}H_{21}Cl_7$</td>
<td>389</td>
<td>391</td>
</tr>
</tbody>
</table>

TABLE 3

Summary of validation data for the analysis of SCCPs in sediment and biota. Initial extraction was by PLE. This was followed by a Florisil column cleanup and analysis by GC-ECNIMS.

<table>
<thead>
<tr>
<th></th>
<th>Sediment</th>
<th>Biota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (2.5 μg)</td>
<td>111 – 121%</td>
<td>66 – 107%</td>
</tr>
<tr>
<td>CV%</td>
<td>2.4</td>
<td>19.8</td>
</tr>
<tr>
<td>Recovery (250 ng)</td>
<td>69 – 82%</td>
<td>68 - 95</td>
</tr>
<tr>
<td>CV%</td>
<td>6.9</td>
<td>11.5</td>
</tr>
<tr>
<td>Limit of Detection (LoD)</td>
<td>2.9</td>
<td>9.2</td>
</tr>
</tbody>
</table>
Figure 1 GC-ECNIMS chromatogram of three SCCP technical mixtures (51.5%, 55.5% and 63% chlorination). The response increases with chlorination level.
**Figure 2** Calibration curve, using $^{13}$C-HCB as an injection standard, for three SCCP technical mixtures. The response increases with chlorination level which is indicated as a percentage on the graph.
Figure 3 GC-FID chromatogram showing the $n$-alkanes produced from the hydrogenation of the 55.5% SCCP technical mixture.