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Treball Final de Grau

Determination of short-chain chlorinated paraffins in gull egg samples by gas chromatography-mass spectrometry.

Determinació de parafines clorades de cadena curta en mostres d'ou de gavina per cromatografia de gasos-espectrometria de masses.

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Science is not only a disciple of reason but, also, one of romance and passion.

Stephen Hawking

M'agradaria dedicar unes línies a totes aquelles persones que m'han envoltat durant aquesta etapa de la meva vida, a tots aquells que han acabat formant part del meu dia a dia, han contribuït a la meva evolució com a persona i m'han ajudat a créixer com a científic, amb la passió intrínseca que aquesta professió comporta. M'agradaria també agrair especialment les persones involucrades en aquest treball i sense les quals aquestes pàgines no haurien arribat a existir, començant pel Dr. Francisco Javier Santos per la seva paciència i dedicació en les seves escasses hores lliures, la Pauline Angelic per les extenses i intenses sessions de treball al laboratori i la resta de gent al grup de recerca CECEM del departament de Química Analítica per fer-m'hi un lloc durant la meva curta estada. Finalment, m'agradaria dedicar també una petita menció a la meva família, per entendre la meva passió i donar-me l'oportunitat de satisfer la meva curiositat a través de la ciència.

REPORT

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1. SUMMARY

Chlorinated paraffins (CPs), otherwise known as polychlorinated *n*-alkanes, are chlorinated derivatives of paraffinic hydrocarbons used for years in a wide variety of industrial applications such as lubricating additives and fire retardants, among others. They are very complex mixtures composed by a large number of congeners, homologues and isomers, resulting in a great variety of physical and chemical properties. Among CP mixtures, short-chain chlorinated paraffins (SCCPs, C_{10}-C_{13}) are well-known ubiquitous organic pollutants of great concern because they exhibit a high toxicity and persistence and cause adverse effects in the environment and in living beings. Fish-eating birds are exposed to these contaminants through their diet and can accumulate in their bodies and transfer to their offspring, causing toxic effects at individual level or to the whole colony. For this reason, they are in the spotlight of the international community since the United Nations Environment Programme Governing Council proposed them as a new candidate to the list of Persistent Organic Pollutants (POPs).

In the present work, an analytical method for the determination of SCCPs in gull eggs was optimised and validated according to international guidance. The proposed method consists of a pressurised liquid extraction followed by a clean-up procedure with Florisil and quantification by gas chromatography-electron-capture negative ionisation-mass spectrometry (GC-ECNI-MS). The method provides good figures of merit with a high sensitivity, trueness and precision, achieving low limits of detection (0.04 ng/g wet weight). The established methodology was applied to the analysis of SCCPs in egg samples from two gull species (*Larus michahellis* and *Larus audouinii*) collected in several natural parks and protected areas from Spain with the aim of evaluating the real impact of these pollutants in the environment using a non-invasive way of monitoring, and to propose measures to minimise their adverse effects on the living organisms.

Keywords: polychlorinated *n*-alkanes, short-chain chlorinated paraffins, persistent organic pollutants, gull egg analysis, electron-capture negative ionisation, gas chromatography-mass spectrometry, method validation.

2. RESUM

Les parafines clorades (CPs), altrament conegudes com *n*-alcans policlorats, són derivats clorats d'hidrocarburs parafínics utilitzats durant anys en una gran varietat d'aplicacions industrials tals com additius per lubricants i retardants de flama, entre d'altres. Són mescles molt complexes compostes per un gran nombre de congèneres, homòlegs i isòmers, resultant en una gran varietat de propietats físiques i químiques. Entre les mescles de CPs, les parafines clorades de cadena curta (SCCPs, C_{10}-C_{13}) són contaminants orgànics ubics ben coneguts i de gran preocupació perquè exhibeixen una alta toxicitat i persistència i causen efectes adversos en l'ambient i en éssers vius. Les aus que s'alimenten de peixos estan exposades a aquests contaminants a través de la seva dieta i poden acumular-se als seus cossos i transferir-se a la seva descendència, causant efectes tòxics a nivell individual o a tota la colònia. Per aquesta raó són el centre d'atenció de la comunitat internacional des que el *United Nations Environment Programme Governing Council* les va proposar com a nou candidat a la llista de contaminants orgànics persistents (POPs).

En aquest treball, un mètode analític per la determinació de SCCPs en ous de gavina s'ha optimitzat i validat d'acord amb directrius internacionals. El mètode proposat consisteix d'una extracció per líquids pressuritzats seguida d'un procediment de neteja amb Florisil i quantificació per cromatografia de gasos-ionització negativa per captura electrònica-espectrometria de masses (GC-ECNI-MS). El mètode proporciona bones figures de mèrit amb una alta sensitivitat, veracitat i precisió, aconseguint baixos límits de detecció (0.04 ng/g pes humit). La metodologia establerta ha estat aplicada a l'anàlisi de SCCPs en mostres d'ou de dues espècies de gavina (*Larus michahellis* and *Larus audouinii*) recollides en diversos parcs naturals i àrees protegides d'Espanya amb l'objectiu d'avaluar l'impacte real d'aquests contaminants en el medi ambient utilitzant un mode no invasiu de monitorització i proposar mesures per minimitzar els seus efectes adversos en organismes vius.

Paraules clau: *n*-alcans policlorats, parafines clorades de cadena curta, contaminants orgànics persistents, anàlisi d'ous de gavina, ionització negativa per captura electrònica, cromatografia de gasos-espectrometria de masses, validació d'un mètode.

3. INTRODUCTION

3.1. ENVIRONMENTAL POLLUTION

Environmental pollution is one of the greatest problems that humanity is facing nowadays, increasing every day and causing irreparable damage to the world's ecosystems and living beings, threatening the persistence of life in the Earth in the future.

Contamination can be found in various forms, such as air pollution caused by uncontrolled gas and smoke emissions and volatile substances, water pollution caused by improper disposal of industrial waste products that are released into rivers, lakes and seas or soil pollution as a result of fertilisers, atmospheric deposition, the use of sewage sludge for agricultural purposes, etc. Each and every one of these, among others, contribute to an increase of global contamination that needs to be addressed in a short-term period.

Among the environmental contaminants, Persistent Organic Pollutants (POPs) constitute an important group of compounds that are cause of concern because of their wide distribution in the environment and the harmful effects on wildlife and humans. According to the Stockholm Convention agreement text [1], POPs are organic chemicals that possess a particular combination of physical and chemical properties such that, once released into the environment, they:

- remain intact for exceptionally long periods of time;
- become widely distributed throughout the environment as a result of natural processes involving soil, water and air;
- accumulate in the adipose tissue of living organisms and are found at higher concentrations at higher levels in the food chain; and
- are toxic to both humans and wildlife.

POPs are compounds mainly characterised by a low solubility in water and tend to accumulate in fatty tissues due to their high lipid solubility, allowing bioaccumulation in living organisms and offering great resistance to degradation because of their stability and increasing resistance to hydrolysis with greater halogen substitution ratios. This leads to an over-exposure

for living beings that can trigger serious health issues such as developmental effects, reproductive disorders, immune and nervous system disruptions, other chronic illnesses and even death.

Many of the most well-known POPs were widely used during the industrial boom caused after World War II as a result of the introduction of synthetic chemicals into commercial use due to beneficial properties especially in pest and disease control, crop production and industry. They were mainly used as electrical transformers, hydraulic and heat exchange fluids, additives to paints and lubricants or pesticides, but they can also be by-products of high-temperature processes and pesticide production residues.

Awareness on increasing threats to human health and the environment caused by POPs has been growing in the last few decades, reaching a fundamental point in May 1995, when the United Nations Environment Programme (UNEP) Governing Council requested an international assessment process of an initial list of twelve POPs that concluded in the Stockholm Convention in May 2001. The list encompassed three categories of POPs (pesticides, industrial chemicals and by-products) that included compounds such as chlordane, dichlorodiphenyl trichloroethane (DDT), hexachlorobenzene, toxaphene or polychlorinated biphenyls (PCBs). At its fifth meeting in May 2009, the Conference of the Parties added nine new persistent pollutants to the list, including pentachlorobenzene, lindane or chlordecone, some of them still in use under restricted conditions. A full list of included compounds can be found at the UNEP's Stockholm Convention official website [2].

Any participant taking part in the Convention may submit a proposal to add a new chemical to the list, subject to evaluation by the POPs Review Committee. At the time of writing the present work, six new groups of chemicals were being evaluated as possible new candidates to the list, including hexachlorobutadiene, chlorinated naphtalenes, pentachlorophenol, decabromodiphenyl ether, dicofol and short-chain chlorinated paraffins (SCCPs). The latter will be object of study in the major part of this document.

3.2. POLYCHLORINATED N-ALKANES

Chlorinated paraffins (CPs), otherwise known as polychlorinated *n*-alkanes (PCAs), are industrially prepared chlorinated derivatives of paraffinic hydrocarbons of the general formula $C_nH_{2n+2-z}Cl_z$ with a total chlorine content between 30% and 70% by weight. Production of CPs

involves chlorination of *n*-alkanes of chain lengths between ten and thirty carbon atoms using molecular chlorine [3]. They are produced by addition chlorination of α-olefins, depending on the specificity of the application [4]. The reaction can occur at temperatures between 50ºC and 150ºC, at elevated pressures and/or in presence of UV light. Gas sparging is used in order to separate the product of the solvent, other residues and reaction products, resulting in colourless or yellow, dense and viscous mixtures of up to twenty carbon CP chains or solid phases of longer highly chlorinated chains. According to their carbon chain length, they are classified in three sub-categories: short-chain CPs (SCCPs, C_{10}-C_{13}), medium-chain CPs (MCCPs, C_{14}-C_{17}) and long-chain CPs (LCCPs, over C_{17}). They are very complex mixtures composed by a number of theoretically possible congeners, homologues, diastereomers and enantiomers that by far exceed ten thousand compounds with a great variety of physical and chemical properties, most of them similar to those of other high molecular weight organochlorine pollutants such as polychlorinated biphenyls, PCBs, and DDT. The separation of these compounds by high-resolution gas chromatography using a single-column is extremely difficult because they generally elute over a wide retention time range and the chromatograms are characterised by a broad hump corresponding to a large number of co-eluting peaks.

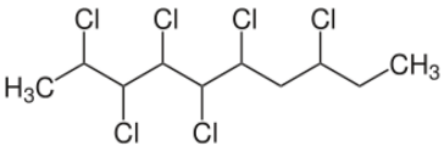

Figure 1. Example of a SCCP (61% Cl by weight): 2,3,4,5,6,8-hexachlorodecane.

CPs began being used in large scale in 1932 as high-pressure additives in lubricants, and since then their global consumption has kept rising from around 38-50kt/year at the beginning of the 1960's decade [5] to over 300kt/year in the middle 1990's [6]. Their main applications were as lubricating additives, fire retardants, plastic and rubber additives, paints and adhesives or sealants.

Until now, CPs have been of much less concern and received less exposure and risk assessment than other POPs because of less mammalian toxicity and lack of exposure-related measurements for humans and animals. Even so, SCCPs have the highest toxicity of CP mixtures and a great potential for environment release. That is the reason why international organisations such as the Environmental Protection Agency of the United States (EPA), the UNEP in the European Union and even the World Health Organisation have recently started to

address the problem by placing SCCPs on the scope of new health and exposure assessments and completely or provisionally listed them as “Priority Toxic Substances”, “Priority Persistent Organic Pollutants” or similar classifications, and have even established restrictions to the usage of this sort of compounds.

3.2.1. Physical-chemical properties and environmental fate

CPs are a group of compounds of special interest because of a series of properties and characteristics that make them relevant, not only in the most physical or chemical way but also from an environmental point of view that leads to further concern about our own health.

Water solubility of CP mixtures varies in the μg L^{-1} to ng L^{-1} range, with a huge dependence on the carbon chain length and chlorine content. That makes them low soluble compounds in water, even though these solubilities are generally ten to one hundred times higher than those of chlorinated aromatic compounds of similar molecular weight, such as PCBs [7]. Even so, the vapour pressures of SCCPs are similar to those of other chlorinated compounds (also PCBs [7] and toxaphene [8]), ranging from 0.066 to 0.001 Pa amongst the tetra- to hexachlorodecanes (classifying them as semi-volatile organics with a significant proportion in the gas phase at room temperature), and from $1.5 \cdot 10^{-3}$ to $1.9 \cdot 10^{-10}$ Pa for C_{12} to C_{20} CPs with a chlorination level over 50%. Henry’s Law Constants (HLC) of SCCPs are also similar to those of PCBs and other pesticides, ranging from 0.8 to 15 Pa m^3 mol^{-1} [9], which implies that low molecular weight CPs may volatilise from water to air at slightly high temperature environments just as PCBs and many pesticides do, but longer chained chlorinated paraffins such as MCCPs and LCCPs are less likely to do so. Moreover, Drouillard et al. [9] found a trend of decreasing HLCs with increasing degree of chlorination.

CPs are very hydrophobic compounds based on their octanol-water partition coefficients (log K_{ow}), which are expected to be under 5.5, but Sijm and Sinnige [10] found a parabolic relationship between the number of carbon and chlorine atoms in the molecule and the value of the K_{ow}, growing as they increase, resulting in values of 5.06-8.12 for C_{10}-C_{13}, 6.83-8.96 for C_{14}-C_{17} and 8.70-12.68 for C_{18}-C_{26} according to the literature [11]. These coefficients represent a fairly reliable indicator of the tendency of a substance to bioaccumulate in living organisms and to penetrate into soils and sediments. Octanol-air partition coefficients have been estimated to range from 8.2 to 9.8 for SCCPs with a chlorine content of 50 to 60%.

CPs, as stated before, are distinguished by a large variety of physical-chemical properties that make them environmentally-harming regarding persistence in the environment, bioaccumulation and biomagnification, as well as potential for long-range transport. Data in the following lines has been fully developed in some of the documents under the Stockholm Convention [1].

Estimated atmospheric half-lives for SCCPs determined through reaction with hydroxyl radicals at different concentrations showed a range from almost 1 to 15 days, and of almost 13 hours in water through a photolysis degradation study (even though it could lead to underestimation of the results due to the extreme use of ultraviolet radiation that may not occur at normal conditions). Persistence in soil using biochemical oxygen demand tests was tested, encountering a full degradation by acclimatised microorganisms of SCCPs of different chlorine content that ranged from 12 to 30 days (depending on the origin of the sample). The half-lives under anaerobic degradation were estimated to be of 450 days in marine sediments and over 1,600 days in fresh water sediments. Therefore, SCCPs meet the criterion for persistence for sediment established by the Stockholm Convention and are sufficiently persistent in air for long-range transport to occur and hydrolytically stable enough, but there is a lack of reports concerning highly chlorinated SCCPs in water or in soil. Moreover, comparison of vapour pressures and Henry's Law Constants demonstrate that SCCPs have characteristic properties in the range of other POPs known to undergo long-range atmospheric transport.

Values of the bioaccumulation factor (BAF) were estimated to be way over 5,000, which means a positive result for bioaccumulation in biota. These reports [12] were supported by other studies regarding bioconcentration factors (BCF) in multiple organisms, mainly fish species from Japan [13], and biomagnification through the food chain that showed accumulation influenced by carbon chain length and chlorine content with biomagnification factors (BMF) greater than 1, implying a potential to biomagnify in aquatic food chains. Based on these data and several other reports [14,15], SCCPs are considered bioaccumulative according to the criteria established by the Stockholm Convention.

3.2.2. Toxicity and exposure

Several exposure and toxicity assessments have been carried out since SCCPs started getting international concern regarding environmental spreading and pollution issues. Reports in different organisms, such as mammals, birds or aquatic species can be found. There are a lot of

reported effects on animal species, many of them summarised on the Stockholm Convention's document regarding SCCPs [1,12].

Based on studies on SCCP mammalian toxicity in rats and mice, and the health and environmental impact, the International Agency for Research on Cancer (IARC) determined in 1990 that there was sufficient evidence to classify short-chain CPs (C_{12}) with an average chlorine content of 60% as "possibly carcinogenic – group 2B", concluding that the liver and the thyroid are target organs in repeated dose studies with damage caused by alterations associated with peroxisome proliferation and altered thyroid hormone status and glucuronyl transferase induction [16], but with a smallest sensitivity expected in humans. More severe effects were seen in mammalian species such as tumours in the liver, thyroid and kidneys as well as mononuclear cell leukaemia and alveolar and bronchiolar carcinomas, with poor survival ratios in some tests. No reproductive organ changes were observed at low concentration exposures, but severe maternal toxicity was observed in rats at higher doses. Some mortality data was reported for rat pups at 74 mg/kg/day and for dams at 100 mg/kg/day, but more information about mortality and fertility in mammals is still needed.

Other assessments in aquatic species, birds and other mammals as well as in microorganisms have also been conducted [1,12]. Reproductive studies in ducks exposed to dietary concentrations of SCCPs were carried out, leading to a significant decrease in the mean eggshell thickness, although still in the normal range. No significant effects were observed in studies with hens other than slight variations in pancreas weight. Other studies conducted in different fish species originated lethargic state in organisms and even severe histopathologies like extensive fibrous lesions and hepatocyte necrosis in highly exposed fishes, although it is not clear if these could be a result of reduced feeding patterns also observed. Finally, developmental malformations and reduced embryo growth were reported in frogs at high concentrations of SCCPs.

Exposure values at sites close to likely sources of release could lead to higher detected levels in living beings and worse health-related effects could occur than in other less significant and remote sites. The way of exposure can also be a decisive factor regarding incidence factors in organisms (i.e. atmospheric exposure, aquatic exposure, etc.), as well as the feeding network to which they belong. That is the reason why migratory species represent a great way of monitoring exposure levels in organisms and affectation of pollutants in different areas. Other

studies and tests related to this matter can be easily found, as well as data extrapolation assessments to the human being.

3.2.3. Legislation and regulation

Given the international concern about this kind of compounds, CP mixtures have been taken into consideration by organisations and regulatory organisms in many countries and state unions. As of April 2012 (publishing date of the last update of the Chlorinated Paraffins Industry Association [17] about the regulatory status of these compounds), the most important restrictions already proposed or applied are the following.

In the United Nations, SCCPs continue to be considered Persistent Organic Pollutants (POPs), but the Stockholm Convention has yet to vote on it since it was voted down in the last five sessions. In the European Union, all CPs are REACH registered, with SCCPs included in the Candidate List of Substances of Very High Concern (SVHC) for Authorisation [18]. Some evaluations are underway regarding SCCPs and MCCPs, but LCCPs are not classified as hazardous to human health or the environment. Even so, the use of SCCPs has been restricted under the European Commission Marketing and Use Directive 76/769/EEC [19], according to which SCCPs may not be marketed or used in concentrations greater than 1% for metalworking and leather finishing, but other products such as paints, coatings and flame retardants are not affected. In 2001, SCCPs were included in the list of priority substances in the field of water policy (European Community, 2001), amending the European Water Framework Directive (WFD) 2000/60/EC [20]. Specifically, they have been listed in the group of priority hazardous substances at maximum admissible levels of 0.4 μg L^{-1} (annual average) and 1.4 μg L^{-1} (maximum allowable concentration) as environmental quality standard in inland surface waters and other surface waters [21]. The US Environmental Protection Agency (EPA) in the USA intends to initiate an action plan under TSCA section 6a [22] to ban or restrict SCCPs, while other CP mixtures are not taken into account, and in Canada C_{10} to C_{13} chloroalkanes (including SCCPs) have already been added to the prohibited substances list and classified as toxic under the Canadian Environmental Protection Act (CEPA) [23].

3.2.4. Chlorinated paraffins in the environment

The release of CPs into the environment could occur during many stages of the production chain and due to release from plastics, paints and sealants in which they are incorporated, but

the major releases are thought to be from production and industrial usage. Some reports included in the Stockholm Convention [1] showed a high release ratio to the environment for metalworking lubricants (around 18% of the total loss), especially in wastewater, soils and sediments.

Several studies and measurements of CPs have been carried out both in biotic and abiotic samples of areas of special interest, such as river sediments, waters, soils, birds and fish species that could be affected by those potentially polluted environments.

Information on the occurrence of SCCPs in different environmental matrices have been reported across the world [24]. Tables 1 and 2 summarise respectively selected data published in the recent years on the concentration levels of CP mixtures found in abiotic and biotic matrices.

CP mixture	Sample	Location	Country	Concentration	Analytical method	Ref
C_{10}-C_{13} 50-70% Cl	Water	Red River, Selkirk (b)	Canada	0.03 µg l^{-1}	GC/ECNI/HRMS	[27]
C_{10}-C_{13} 62% Cl	Water	Sewage plant run-off (a)	Germany	0.12 µg l^{-1}	GC/ECNI/MS	[25]
C_{10}-C_{13} 50-70% Cl	Water	Sewage treatment plant effluents (a)	Canada	0.60-4.48 µg l^{-1}	GC/ECNI/HRMS	[26]
C_{10}-C_{13} 60-70% Cl	Sediment	Lake Ontario, Harbour (a)	Canada	0.007-0.29 µg g^{-1} (d)	GC/ECNI/HRMS	[26]
C_{10}-C_{13} (c)	Sediment	Hamburg, Harbour (b)	Germany	17 µg kg^{-1} (d)	GC/ECNI/MS	[28]
C_{10}-C_{13} 60-70% Cl	Sediment	Lake Nipigon (b)	Canada	18 µg kg^{-1} (d)	GC/ECNI/HRMS	[29]
C_{10}-C_{13} (c)	Sediment	River Main (b)	Germany	25-50 µg kg^{-1} (d)	GC/ECNI/MS	[28]
C_{10}-C_{13} (c)	Sediment	River Lech (a)	Germany	<5-700 µg kg^{-1} (d)	GC/ECNI/MS	[28]
C_{10}-C_{13} 60% Cl	Sewer film	Industrial (a)	Germany	0.5-30 µg g^{-1} (d)	GC/ECNI/MS	[25]
C_{10}-C_{13} 60-70% Cl	Air	Canadian arctic, Alberta (e)	Canada	<1-8.5 pg m^{-3}	GC/ECNI/HRMS	[30]
C_{10}-C_{13} 60-70% Cl	Air	UK, Lancaster (b)	UK	99 pg m^{-3}	GC/ECNI/HRMS	[31]

(a) Sampling site near industrialised area.
(b) Sampling site remote from industrialised area.
(c) No information given on Cl content.
(d) Dry weight.
(e) No information given on industrialisation of the area.

Table 1. SCCP concentration in abiotic samples.

As can be seen, different trends are established in areas near or far from industrialised zones. For instance, Muir et al. [26] reported C_{10}-C_{13} (60-70% Cl) CP concentrations ranging from 0.007 to 0.29 μg g^{-1} in surface sediment samples from harbour areas in Lake Ontario, Canada, where highest concentrations were found at the most industrialised site. C_{10}-C_{13} (60% Cl) CP concentrations in surface films (formed by deposition of organic matter) in waste water sewer pipes of a city in southern Germany were reported by Rieger and Ballschmiter [25], ranging from 0.5 to 30 μg g^{-1} from different industrialised areas where CPs are used in high pressure additives for metal processing, while those samples collected from less industrialised areas range from 0.5 to 15 μg g^{-1}. Other data from water and air samples can also be found in the table above.

There is limited information about CP levels in biota, even though there is a growing interest in this kind of analysis due to recent regulation proposals concerning CP classification as POPs. Some levels found in aquatic biota and terrestrial animals are shown in the table below, and further and more recent information about levels in bird species relevant to the present work can be found in the next paragraph.

CP mixture	Sample	Matrix	Location	Concentration	Analytical method	Ref
C_{10}-C_{13} 52% Cl	Carp	Whole fish	Hamilton Harbour, Canada [b]	2630 μg kg^{-1} [c]	GC-ECNI-HRMS	[26]
C_{10}-C_{13} 60-70% Cl	Catfish	Muscle	Detroit River, USA [a]	305 μg kg^{-1} [c]	GC-ECNI-HRMS	[32]
C_{10}-C_{13} 60-70% Cl	Beluga	Blubber	Canada [b]	164-851 μg kg^{-1} [c]	GC-ECNI-HRMS	[27,30]
C_{10}-C_{13} 60% Cl	Rabbit	Muscle	Revingeshed, Skåna, Sweden [b]	2900 μg kg^{-1} [d]	GC-ECNI-MS	[33]
C_{10}-C_{13} 52% Cl	Human milk	Milk	N. Quebec, Canada [b]	12.8 μg kg^{-1} [d]	GC-ECNI-HRMS	[27]
C_{10}-C_{13} 63% Cl	Gull egg	Whole egg	Ebro Delta, Catalonia, Spain [b]	2.87-7.61 ng g^{-1} [c]	GC-ECNI-MS	[34]
C_{10}-C_{13} [e]	Birds	Muscle	South China [a]	19-340 ng g^{-1} [c]	GC-ECNI-MS	[35]

(a) Sampling site near industrialised area.
(b) Sampling site remote from industrialised area.
(c) Wet weight.
(d) Lipid weight.
(e) Chlorine content not specified.

Table 2. SCCP concentration levels in biota samples.

Some data of special interest for this work can be found in other studies about the SCCP concentration in the same gull species that are on the scope of the present study (*Larus michahellis* and *Larus audouinii*) reported by Morales et al. [34] in Spain (2012) ranging from 2.87 to 7.61 ng g^{-1} (ww), or the SCCP concentration in other terrestrial bird species inhabiting a recycling site in China by Luo et al. [35] in 2015, reported to vary between 19 and 340 ng g^{-1} (ww), showing a higher accumulation in muscles from non-migratory species due to the contamination of the local area.

3.2.5. Analysis of CP mixtures

CP chromatograms present an unresolved broad peak due to the presence of a large group of co-eluting compounds resulting in overlapping individual peaks, hence the challenge that their quantification implies. Single-column gas chromatography is unable to separate all individual congeners and its isomers, so often the only way of reporting results is by presenting CP content as a whole (total short, medium and long CP content), sometimes including interferences such as other halogenated compounds. Therefore, a suitable extraction and clean-up process is needed in order to isolate the CPs from their matrix and other interferences, and a quantification should be carried out by a sufficiently sensitive mass-spectrometry technique. In the next paragraphs special focus has been given to the treatment and quantification of SCCPs in environmental and biologic samples given the general scope of the present work.

3.2.5.1. Sample treatment

Generally, the most commonly used methods for the extraction of CP mixtures from solid environmental samples are Soxhlet, pressurised liquid extraction (PLE) and ultrasonic assisted extraction. Clean-up procedures for SCCP quantification in environmental samples are similar to those used for other persistent organochlorines. Chromatographic columns packed with adsorbents like silica gel, alumina or Florisil after a pressurised extraction of the sample are the most commonly used purification methods. An adequate optimisation of the clean-up process leads to an almost complete separation of the analyte and its most important interferences like PCBs, toxaphene, chlordane, DDT or polybrominated diphenyl ethers (PBDEs). The main solvents used in the extraction, clean-up and fractionation process are dichloromethane (DCM)

and mixtures of other solvents such as *n*-hexane:DCM, *n*-hexane:acetone, *n*-hexane:diethylether, etc.

Tomy et al. [32] reported extraction of CP mixtures from fish and sediments by using DCM and clean-up with Florisil column chromatography followed by a fractionation step eluting with hexane (obtaining a fraction containing PCBs, chlorinated benzenes), *n*-hexane:DCM 85:15 and *n*-hexane:DCM 1:1 (both containing SCCPs, toxaphene, chlordane and other polar organic compounds). Nicholls et al. [36] analysed SCCPs and MCCPs in arable and grasslands soils by using a silica gel column and elution of interferences by fractionation with hexane, recovering the analytes with *n*-hexane:DCM 1:1 and DCM. Reth et al. [37] reported a clean-up process consisting on a similar Florisil-packed column to remove interferences in fish samples, achieving an almost complete removal of PCBs, toxaphene and DDT with hexane as a solvent, and elution of SCCPs with DCM. Luo et al. [35] reported the usage of a multi-layered column containing Florisil, neutral silica and sulphuric acid impregnated silica with similar fractionation solvents in bird samples. Many other studies have been carried out presenting similar sample pre-treatment conditions.

Regarding the present work, the most similar study performed is the one reported by Morales et al. [34] for the quantification of SCCPs in gull eggs from two species from the Ebro delta Natural Park in Spain. Extraction was carried out using a pressurised liquid extraction using acid silica as a retainer and a mixture of *n*-hexane:DCM 1:1. Fractionation with a Florisil column lead to two fractions using *n*-hexane and *n*-hexane:DCM 85:15 (containing PCB and PBDE congeners) and *n*-hexane:DCM 1:1 (containing mainly the SCCPs).

3.2.5.2. Determination of CP mixtures

GC coupled to electron-capture negative ionisation-low-resolution mass spectrometry (GC-ECNI-LRMS) or high-resolution mass spectrometry (GC-ECNI-HRMS) are the more frequently used instrumental techniques for CP analysis. However, other approaches have been reported, such as GC with electron ionisation-tandem mass spectrometry (GC-EI-MS/MS), metastable atom bombardment-high resolution mass spectrometry (MAB-HRMS) and comprehensive two-dimensional gas chromatography-time of flight mass spectrometry (GCxGC-ToF-MS).

SCCPs themselves don't self-interfere in the determination due to the high-resolution of the mass spectrometer, but other compounds like toxaphene, chlordane and PCBs can interfere with the analytes. However, despite being a highly selective method, many laboratories don't

have access to such equipment or it turns to be too expensive for routine analysis, so LRMS is more widely used. Despite having an appropriate sensitivity, the main drawback is the lack of selectivity and thus an increase of possible interferences that require an enhanced clean-up process, and also a possible mass overlapping between congeners with the same mass. Reth and Oehme [38] concluded, though, that most abundant congeners with C_{10}-C_{14} chain lengths are not affected by this issue. Other ECNI-based ionisation methods have been developed, but all of them suffer from a strong and increasing dependence from the response factors on the degree of chlorination and the position of the chlorine atoms in the carbon chain. That is the reason why CP mixtures with lower chlorine contents (usually under five chlorine atoms) are not sensitively detected by ECNI-MS.

3.3. METHOD VALIDATION

Method validation has been defined by the Organisation for Standardisation (ISO) in the ISO/IEC 17025 document [39] as "the confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled", among many other definitions. It is basically a process that has been established in response to the necessity to assess the extent to which a method under consideration has capabilities consistent with the application it is intended to fulfil. An immediate result of that is the evaluation of the method's performance through extensive testing of its analytical parameters (such as selectivity, precision, trueness or ruggedness) in order to set a framework that meets all the specific requirements that demonstrate adequacy to its purpose. But aside from demonstrating fitness for purpose it also supplies other benefits even to the laboratory that performs the analysis, such as a solid knowledge of the method's capabilities, a better confidence when working with it and awareness of its limitations, boundaries and critical steps.

All of these benefits matter in a society that has established itself in such a way that examination and measuring of properties related to everything that we produce and surrounds us is the key issue to everything from stating a product quality to ensure safe conditions, providing evidence in biological and medical examinations, confirming the adequacy of material capabilities or analysing the properties of environmental samples. Providing reliable results and being able to prove that they are correct is a major concern for analytical laboratories since the customer has to be able to assume a certain proficiency level in the laboratory's performance. Analytical examinations play an important role in innumerable processes and are part of huge

decision-making networks on which many other processes depend. Therefore, a result of an analysis needs to be reliable enough to allow decisions based on it to be taken with enough confidence. Moreover, reliable analytical methods are required for compliance with national and international regulations regarding any industry and service areas.

3.3.1. Approaches to method validation

Given the relevance of method validation, many international bodies and organisations have addressed the matter and have established protocols and guidelines focused on method performance studies and validation parameters, proficiency testing and quality control. In some sectors the validation of methods has been prescribed by legislation and required in any process, such as in the analysis of foodstuffs.

As stated in ISO/IEC 17025, "validation is always a balance between costs, risks and technical possibilities" so, besides the diverse needs of each one of the different applications, that is why there is no particular way of carrying out a full validation for an analytical method. The laboratory has to define a scope of the analytical work to carry out and dispose a series of tests in accordance to a detailed validation plan, deciding the extent and detail to which the validation should be performed, always taking into account its capabilities and limitations.

There are two widely used main approaches to method validation: the interlaboratory comparison approach and the single-laboratory approach. Which one to use depends on the own laboratory and the purpose of the method being validated. Regardless of the selected approach, the responsibility of assuring the fitness for purpose of the validation process relies completely on the laboratory unless established in related regulation, and so does the commitment to ensure the appropriate interpretation of protocols and performance characteristics of the validation.

The interlaboratory approach refers to cooperative and collaborative studies involving various laboratories for validation of widely applicable methods in order to provide a robust and reliable result. This approach has been extensively reported and established in several protocols and international standards that will not be further explained in this text.

The single-laboratory approach aims to cover those occasions where a specific method is needed but it has not been fully developed or not enough data or standards have been published regarding its capabilities, such as a specific modification of a previously existing

method, a method that generates almost no interest among the analytical community or when intending to ensure the viability of a method before undergoing a formal collaborative trial. In these cases, multiple assumptions have to be carefully made regarding the applicability of established protocols and guidelines in the area of interest and specific attention has to be paid to define the characteristics that require extensive testing. Anyway, a single-laboratory approach is normally accepted when properly performed even though it might require further verification.

3.3.2. Validation of SCCP quantification methods

CP mixtures, and specifically SCCPs, are a group of highly produced compounds over the globe, but they actually get a low level of relevance regarding exposure assessments and environmental level regulations. As a result of that and due to the little demand on quantification methods for this kind of compounds, very few validation studies have been fully carried out and no standard method has been defined and settled as the main way to proceed. Every time a laboratory faces a CP analysis, assurance of optimal analytical conditions for the method is a main concern in order to set a reliable framework for the assessment. Moreover, future studies will require better analytical methods and reference certified materials, and those will set a need for extensive method testing and further validation guidance. Nowadays, validation studies applied to SCCP determination and quantification consist on a vaguely defined revision of certain parameters depending on the needs and capabilities of the laboratory in charge of the study.

The overarching element in the present work is the establishment of a validation plan for an analytical method for SCCP quantification in gull egg samples. Special attention will be paid to define the essential parameters for an adequate demonstration of purpose fitness for the already existing method.

4. OBJECTIVES

The main objective of the present research work is to develop and validate an analytical method for the determination of short-chain chlorinated paraffins in gull eggs as bioindicators of the environmental contamination based on selective pressurised liquid extraction combined with gas chromatography-mass spectrometry. To accomplish this main objective, specific sub-objectives concerning the analysis and quantification of short-chain chlorinated paraffins were defined as follows:

- To establish an analytical method capable to separate the main interfering analytes from short-chain polychlorinated paraffins that allows their reliable determination and quantification.
- To ensure, through a method optimisation procedure, the adequate conditions for the whole process in order to achieve an optimal performance during the sample treatment and determination of the analytes.
- To determine the quality parameters of the method to be assessed in order to prove the method's suitability for its intended use and to validate it in accordance to the present regulations and laboratory guidelines.
- To evaluate the applicability of the present method to the analysis of short-chain chlorinated paraffins in biota samples by studying its performance.

All these objectives will be further explained and detailed in the following sections, as well as the procedures used in order to achieve them, the obtained results and their discussion.

5. EXPERIMENTAL SECTION

This section covers the experimental procedures and instrumental parameters of the techniques needed for the method's optimisation and validation, as well as a brief description of the assessed parameters and the used resources in the whole process.

5.1. MATERIALS AND METHODS

5.1.1. Reagents and material

- *n*-Hexane for pesticide residue analysis (Sigma Aldrich, Steinheim, Germany).
- Dichloromethane for pesticide residue analysis (Sigma Aldrich, Steinheim, Germany).
- Acetone for analysis (Sigma Aldrich, Steinheim, Germany).
- Methanol of HPLC grade (Sigma Aldrich, Steinheim, Germany).
- Cyclohexane for organic trace analysis (Merck KGaA, Darmstadt, Germany).
- Isooctane for analysis (Merck KGaA, Darmstadt, Germany).
- Sulfuric acid 96% w/w for analysis (Panreac, Montcada i Reixac, Spain).
- Sodium sulfate anhydrous for analysis (Merck KGaA, Darmstadt, Germany).
- Silica gel 60 (0.063-0.200 mm) for column chromatography (Merck KGaA, Darmstadt, Germany).
- Florisil (0.150-0.250 mm) for residue analysis (Merck KGaA, Darmstadt, Germany).

5.1.2. Standards

The following standard stock solutions were diluted by weighing to prepare the working solutions. All of them were stored in glass vials in a refrigerator at 4ºC while not being used.

- 100 ng/µL Chloroparaffin C10-C13 63% Cl in cyclohexane stock solution (Dr. Ehrenstorfer GmbH, Augsburg, Germany).
- 1 ng/µL Dechlorane Plus in isooctane stock solution (Wellington Laboratories, Guelph, ON, Canada).

- 5 ng/µL CB-209 in isooctane stock solution (Dr. Ehrenstorfer GmbH, Augsburg, Germany).
- 100 ng/µL single column analytes mix 4 in toluene/*n*-hexane containing δ-HCH (Dr. Ehrenstorfer GmbH, Augsburg, Germany).
- 2 ng/µL MXFR mixture containing PBDE-77 and PBDE-138 (Wellington Laboratories, Guelph, ON, Canada).

Six calibration solutions were prepared for the chlorinated paraffin quantification by dilution in cyclohexane of the stock standard solutions, containing SCCP concentrations of 0.5, 1, 3, 5, 10 and 15 ng/µL, CB-209 at 1 pg/µL as an injection internal standard and δ-HCH at 5 pg/µL as a surrogate internal standard.

In addition, standard solutions of 30 ng/µL of SCCPs, 100 pg/µL of δ-HCH in cyclohexane and 50 pg/µL of CB-209 in isooctane were prepared and used for assessing the quality parameters of the method, as well as for fractionation studies between the SCCPs and Dechlorane Plus (DP), which is considered to be one of the main interfering compounds. Those include 100 and 500 pg/µL DP solutions in isooctane for spiking and a 300 pg/µL PBDE-77 and PBDE-138 mixture solution (in isooctane) as a surrogate standard.

5.1.3. Samples

The present method has been established in order to perform the quantification of SCCPs on gull egg samples. These samples proceed from two different gull species (the yellow-legged gull -*Larus michahellis*- and Adouin's gull -*Larus audouinii*-) from different locations in three natural protected areas in Spain (Delta del Ebro Natural Park; Montgrí, Illes Medes i Baix Ter Natural Park; and Islas Atlánticas de Galicia Natural Park).

A total of 36 samples were collected by specialised staff from 3 different locations within each natural park (which means 12 eggs per bird settlement in each park) at the beginning of the breeding season, only from those nests that contained one single egg, since the first egg to be laid is always the one that accumulates the majority of the pollutants. Each sub-sample of 12 eggs was then lyophilised and labelled according to its source and stored and transported in adequate conditions.

In order to assess the quality parameters of the method, a lyophilised chicken egg blank sample has to be used for every run so the matrix effects of the sample can be reproduced and

taken into account during the method validation. Chicken eggs from any regular convenience store or supermarket have very similar composition and matrix characteristics to those of gull eggs, so they stand as a perfect blank sample with no detectable amounts of pollutants for spiking purposes.

5.1.4. Instrumentation

5.1.4.1. Simple instrumentation

Aside from the more complex instruments that are explained in the next sections, essential for this method, the following devices and minor instrumentation has been used:

- 1.5 mL cone-shaped vials with holed cap and Teflon-covered seal.
- 2 mL flat-bottomed vials with holed cap and Teflon-covered seal.
- Vortex mixer (Stuart, Staffordshire, United Kingdom).
- Refrigerator (Liebherr, Bulle, Switzerland).
- Labo Rota S300 rotary evaporator with water bath (Resona Technics, Zürich, Switzerland).
- Classic PB1502-L grain weighing scale (Mettler Toledo, Hospitalet de Llobregat, Spain).
- AT261 DeltaRange analytical weighing scale (Mettler Toledo, Hospitalet de Llobregat, Spain).
- 10, 25, 50 and 100 µL micro-syringes (SGE Analytical Science, Melbourne, Australia).
- Branson 5510 ultrasonic cleaner (Emerson Industrial Automation, Saint Louis, MO, USA).

5.1.4.2. Pressurised liquid extraction (PLE)

Pressurised liquid extraction is a sample extraction method that employs liquid solvents at elevated temperatures and pressures to retrieve the analytes from the sample so analysis by gas or liquid chromatography can be carried out for their adequate quantification. The improved procedure achieved by those high pressures and temperatures allows a more efficient and fast recovery of analytes than other techniques, such as Soxhlet extraction.

A pressurised liquid extractor consists of a cartridge that contains the sample (as well as other substances and adsorbents that help in the extraction or the interference-removal

processes), which is filled with a suitable solvent once inside the oven and subsequently heated and put under pressure due to a gas current for a better extraction of the analytes.

Sample extractions have been performed by an ASE 100 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA, USA). It allows the extraction with different customisable methods by varying the target temperature, cartridge capacity, number and length of cycles, etc. The particular conditions of the extractions performed in this work will be detailed in the optimised method section.

5.1.4.3. Gas chromatography-mass spectrometry (GC-MS)

GC-MS is an instrumental technique for detection and quantification of analytes in samples that combines the analyte separation capability of the gas chromatography for volatile compounds, such as organic pollutants or pesticides, and the detection and identification proficiency of the mass spectrometry. Samples are injected in the chromatograph and its compounds are separated in the chromatographic column by interactions between the analytes and the column filling. Then, they enter into the spectrometer, where they are ionised and fragmented by the ionisation source (which depends on the characteristics of the sample and the objective of the study) and finally analysed and detected separately, allowing a perfect discrimination between ions according with its *m/z* ratio. It allows to work in a wide range of conditions, adjusting each parameter to the needs of the method.

The separation and quantification of the SCCPs in the samples and the standards has been performed using a ThermoQuest Trace GC 2000 Series gas chromatograph equipped with a ThermoQuest AS 2000 autosampler and coupled to a DSQ II mass spectrometer (all from Thermo Fisher Scientific, Milan, Italy) with a quadrupole analyser. All experiments were performed in electron-capture negative ionisation mode using methane as moderate gas at 2 ml/min. Separation of the target compounds was carried out on a 15 m x 0.25 mm I.D. x 0.25 μm of film thickness DB-5MS (5% phenyl-95% dimethylpolysiloxane) fused-silica capillary column (Agilent Technologies, Santa Clara, CA, USA). The whole system was controlled by Xcalibur 1.4 computer software (Thermo Fisher Scientific, Milan, Italy).

GC-ECNI-MS allows the determination of SCCP content in samples by monitoring different negative ions of specific *m/z* value. In the present work, ions presenting a *m/z* ratio of 70 (corresponding to the ion $[Cl_2]^-$) will be monitored for their quantification since it allows a

relatively simple way of determining the SCCP content, but especial attention has to be paid to other possible chlorinated interferences that might co-elute with the analyte.

5.2. OPTIMISED METHOD

After the full optimisation process of the method, the final procedure for the validation experiments was established and can be summarised in the following paragraphs.

5.2.1. Extraction

A pressurised liquid extraction cartridge of 34 mL of capacity was filled, from bottom to top, with two 4.7 cm glass microfibre filters (Whatman International Ltd., Maidstone, United Kingdom), followed by 2 g of anhydrous sodium sulfate, 20 g of previously acidified silica gel (with 44% w/w sulfuric acid), another thin layer of sodium sulfate and a mixture of the spiked egg sample and sodium sulfate. The spiking with the standard solutions depends on the experiment, but it was always done after adding the egg sample and before mixing it with more sodium sulfate. After a slight compression, more sodium sulfate was added until the top of the cartridge was reached, and one more filter was added at the top. A scheme of the cell can be seen in section *6.1.* The cartridge, appropriately closed, is ready for the extraction.

The extracting procedure on the pressurised liquid extractor (PLE) consisted of three static cycles of 5 minutes per cycle, preceded by a pre-heat time until a temperature of 100ºC and a pressure of 1500 psi were reached and followed by a 90 seconds purge time with a 60% flush volume. The solvent used for the extraction was a 1:1 (v/v) mixture of *n*-hexane and dichloromethane, and the extracts were collected altogether in a glass bottle with a Teflon-covered seal. The resulting solution was rotary-evaporated until there were about 2 mL left in a heart-shaped flask three times, adding hexane after each step to make sure that the dichloromethane in the mixture evaporated almost completely, and then it was ready for the clean-up procedure. The extraction cartridge previously used was cleaned by ultrasounds with methanol.

5.2.2. Clean-up

A 1.5 cm diameter 25 mL glass chromatography column was hung on a support and a little ball-shaped glass wool plug was pushed into it to block its bottom. Two grams of sodium sulfate anhydrous were added into the column, and *n*-hexane was poured inside making sure not to

leave air bubbles on the bottom. Then, 15 g of Florisil (activated overnight at 550 ºC and kept in an oven at 170 ºC) were added to the column, again trying to pack it correctly without air bubbles. Once it had settled down, 30 mL of hexane were used for conditioning the column by passing through the solid phase.

The evaporated extract was carefully transferred to the column and let through the Florisil until there was no solvent on top. After that, 30 mL of *n*-hexane and 80 mL of a hexane-dichloromethane 95:5 (v/v) mixture were sequentially added to the column and collected in a heart-shaped flask, forming Fraction 1. Another flask was used to collect Fraction 2, which consisted of 30 mL of the same *n*-hexane-dichloromethane 1:1 (v/v) used for sample extraction.

Both fractions were rotary-evaporated until about 1 mL and transferred to 1.5 mL cone-shaped glass vials. The flasks were rinsed three times with 1 mL of the *n*-hexane-dichloromethane 1:1 (v/v) solution. Before GC injection, an appropriate amount of the internal standard, CB-209, was added to the vials using a micro-syringe to give a concentration in the final extract of 1 pg/µL, and the extract volume was adjusted to 100 µL by evaporating using a light stream of nitrogen. Finally the vials were covered with holey caps with a Teflon-covered seal, and it was ready for GC injection. The vials containing the samples and the calibration standard solutions were stored in a refrigerator at 4ºC until injection in a GC/MS instrument.

5.2.3. GC-MS analysis

The sample extracts and standards were analysed by GC-MS using the instrumentation indicated in section *5.1.4.3. Gas chromatography-mass spectrometry (GC-MS).* During the optimisation process the sample extracts were analysed in full-scan mode to see the evolution of the component separation through the different tests, whereas for the validation and quantification processes selected ion monitoring mode (SIM) was used. For GC separation the oven temperature was programmed from 90 ºC (held for 2 min) to 300 ºC (held for 5 min) at a rate of 15 ºC/min. Helium with a purity of 99.999% was used as carrier gas at a constant flow-rate of 1 mL/min held by electronic flow control. The injector temperature was kept at 280 ºC and 1 µL of each sample and standard was injected in splitless injection mode (closing the split vent and septum purge for 1.5 min). MS operating conditions were as follows: electron energy of 120 eV and 50 µA of electron emission working with electron capture negative ion (ECNI) mode with methane as moderate gas at 2 mL/min. Transfer line and ion source temperatures were set at 280 ºC and 160 ºC, respectively. For quantification using SIM mode, two time-

window events were defined in function of the elution time of the target compounds in the chromatogram. The first one, going from 7.00 to 9.20 min, was used for the δ-HCH surrogate standard monitoring the ions at *m/z* 255 and 257 for quantification and confirmation purposes. The second event was from 9.20 to 21.00 min and it was used for detection of the SCCPs and the CB-209 injection standard, monitoring the ions at *m/z* 70 and 72 for SCCPs and *m/z* 464, 498 and 500 for CB-209. For data acquisition, a dwell time of 100 ms and a delay time of 20 ms were used.

5.3. VALIDATION PARAMETERS

The main objective in the present work has always been the assessment of a selection of quality parameters defined with the aim to demonstrate the fitness for purpose of the studied method. These parameters have been proposed in order to characterise several aspects of any working methodology, taking into account the importance of data sharing across the scientific community and the need to establish reference points in a particular work so anyone knows its starting conditions and what to expect from it. Furthermore, a research project needs to be reliable enough as to provide trustworthy results that can be used in further investigations or applications. This vast topic has been extensively treated by many official regulations from international organisations and governments, leading to harmonised guidelines for method validation and fitness for purpose testing, among other documents.

For the quantification of SCCPs in egg samples, some easy to find technical reports, guidelines and related documents [40-43] from organisations such as the International Union of Pure and Applied Chemistry (IUPAC), the Organisation for Economic Co-operation and Development (OECD) or Eurachem have been studied in order to define the quality parameters that need to be tested and determined. Therefore, the present work aims to the validation of the proposed working methodology in accordance to these official guidelines. The next sections provide an overview on the matter as a way of introducing the steps to follow during the validation of the method.

5.3.1. Selectivity

Analytical sensitivity relates to *"the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour"*. Potentially interfering substances shall be chosen taking into account the

type of sample, and relevant blank samples and fortified blank samples (blank samples to which specific interfering compounds have been deliberately introduced) shall be analysed to detect the possible interferences and estimate its effect. After the analysis an investigation should be conducted to determine whether their presence may lead to false identification, hindering the target analyte or notably influencing its quantification. This can be carried out through visual analysis of the obtained chromatograms, and possible nonconformities should be addressed through further optimisation of the method when possible.

5.3.2. Limits of detection and quantification

The limit of detection (LOD) of an analytical procedure is *"the lowest amount of an analyte in a sample that can be detected but not necessarily quantified as an exact value"*. In other words, it represents the lowest concentration of an analyte that can be detected by the method at a specified level of confidence.

The limit of quantification (LOQ) is defined as *"the lowest concentration tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation (RSD) is obtained"*. It should then be low enough to achieve the intended purpose of the method.

For techniques such as gas chromatography that rely on detecting a peak above the noise signal, blank samples spiked with analyte concentrations above but close to the LOD are required. Those parameters can be the determined in different ways depending on their previous definition, which usually varies between studies depending on their necessities. For this study, these limits will be defined with the signal to noise ratio (S/N) of the resulting chromatograms of the mentioned spiked samples as a reference. The concentration corresponding to a S/N equal to 3 will correspond to the LOD, while a S/N of 10 will correspond to the LOQ.

5.3.3. Linearity

Linearity can be defined as *"the ability of a method to produce an acceptable linear correlation between the measured response and the concentration of the analyte in the sample"*. It relates directly to the analytical calibration of the method, which has been discussed in different ways amongst the various documents and guidelines that address this matter. Some suggest that it should extend over a range appropriate to the lowest and highest nominal

concentration of the analyte in relevant analytical matrices exceeded by a 20% on each side, while others state that it this range should encompass 0-150% or 50-150% of the concentration likely to be encountered.

Either way, a minimum of six calibration standards should conform the calibration curve with concentrations of the analyte covering the whole range with an approximately even spacing between them. The fitness of the obtained data to a simple linear regression can be checked by visual and mathematical assessment, such as examination for obvious patterns in the residuals of the regression. Anyway, the calibration chosen for this report represents a special case that will be further elaborated in the result discussion section.

5.3.4. Trueness

Trueness is *"the closeness of agreement between a test result and the accepted reference value of the property being measured"*. It is a component of the accuracy of a measurement, studied as two components: trueness and precision. Trueness is often stated quantitatively in terms of bias by comparing the response of the method to a reference material, but it can be assessed in many ways depending on the conditions of the experiment.

In the absence of suitable reference materials, as in the present work, recovery studies conducted through spiking experiments may be used to give an indication of the likely level of bias. These experiments aim to determine the proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is extracted and presented for measurement by using a surrogate compound that, if appropriate, allows the attribution of its transferring characteristics to the analyte itself. This allows to correct the loss of analyte through the entire process and to give an unbiased estimate of its concentration on the original matrix.

The recovery in this report will be calculated from nine spiking experiments at two different concentration levels (eighteen total experiments).

5.3.5. Precision

Precision is the other component of the accuracy of a measurement along with trueness. It is a measure of how close results are one to another and it is usually expressed by statistical parameters such as the relative standard deviation of a series of measurements. There are different estimates of precision, which depend on the definition of the experimental conditions.

Repeatability refers to *"the closeness of agreement between mutually independent test results obtained with the same method on identical test material, in the same laboratory by the same operator using the same equipment within short intervals of time"*, while reproducibility refers to *"the closeness of agreement between independent results obtained with the same method on identical test material but under different conditions"*. The first one is the result of the within-run variations, while the second one is related to the day-to-day variations in the analytical system (within-laboratory reproducibility, also referred to as intermediate precision) and the differences in materials, standards and instruments in different laboratories (interlaboratory reproducibility).

The scope and scale of this work only allows to focus on the repeatability of the method and its within-laboratory reproducibility (or intermediate precision). These will be assessed using the same experiments performed for the trueness determination, treating the data from a two-level multiple-day validation plan in order to determine the standard deviations between runs from the same and different days, respecting the established minimum of six samples per test.

6. RESULTS AND DISCUSSION

6.1. EXTRACTION OF SCCPS

Most commonly used techniques for the extraction of organic pollutants from the environmental samples are based on Soxhlet, pressurised liquid extraction (PLE) and, to lesser extension, microwave assisted extraction (MAE). These last two techniques have the advantage, compared with Soxhlet extraction, of an important reduction on the solvent consuming and extraction time. Nevertheless, all these extraction methods often require extensive clean-up procedures after extraction to remove matrix-interfering compounds. In order to reduce the handling time, a method based on simultaneous PLE extraction and clean-up was applied. This technique, also known as selective PLE (s-PLE), allows the in-cell extraction of the analytes and purification of the extract using an adsorbent inside of the extraction cell that acts as a fat retainer.

From previous studies in University of Barcelona's CECEM research group, s-PLE was chosen for extracting the SCCPs from lyophilised egg samples using silica modified with sulphuric acid (44% w/w) as fat retainer. Using optimal experimental conditions previously described (see section *5.2.1.*), the obtained PLE extracts were clean enough to be analysed by GC-MS. Nevertheless, for the adequate determination of SCCPs a fractionation step for removing the interfering compounds from the matrix was required.

Figure 2 shows a detailed scheme of the extraction and fractionation conditions (see section *5.2.*).

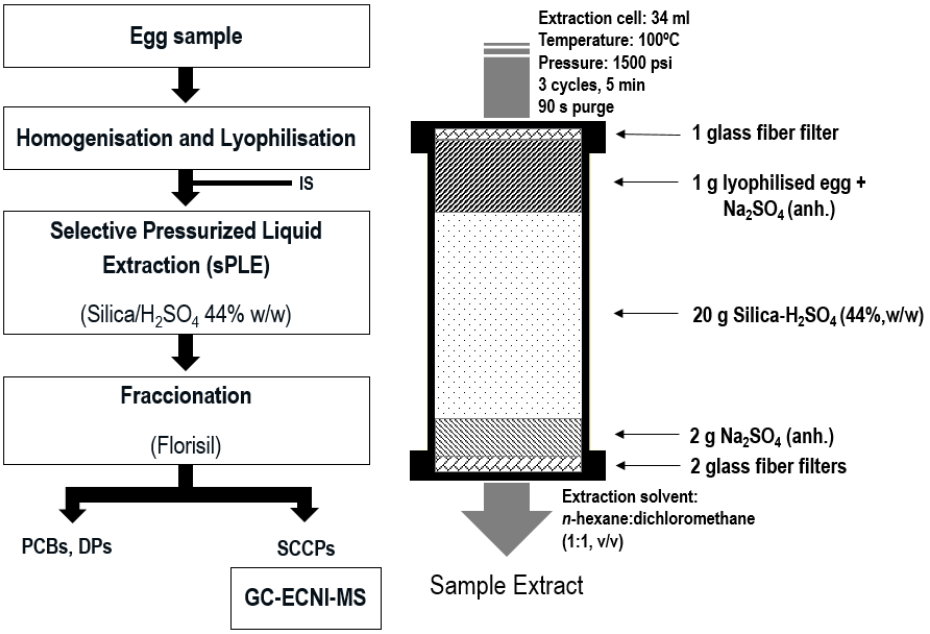

Figure 2. Scheme of the extraction and fractionation conditions.

6.2. OPTIMISATION OF THE FRACTIONATION CONDITIONS

The fractionation conditions of the analytical method tested as a starting point for the study of SCCP concentration in gull egg samples did not yield satisfying results, being unable to separate at all SCCPs from the main interference (DPs). As a result of that, an optimisation plan was designed and performed in order to achieve a better separation between compounds to allow their individual quantification.

The method involved the fractionation of PLE extracts in three fractions using Florisil as sorbent for the separation of the main matrix interferences (mainly due to the presence of polychlorinated biphenyls –PCBs– and Dechlorane Plus and analogues –DPs–). The most important parameters with a potential capability of changing the fractionation outcome (adsorbent amount in the column, polarity of the solvents and quantity of solvent per fraction) were detected and systematically changed to achieve a correct separation of the compounds. The followed strategy involved three different amounts of Florisil as an adsorbent (5, 10 and 15 g), three different polarities of the *n*-hexane:dichloromethane mixture (hex:DCM 85:15, 90:10 and 95:5) and varying amounts of these solvents according to the changes observed in each

step. The matrix effect was also taken into account by performing the optimisation on blank tests and on sample extracts for a final adjustment.

The working procedure was described at section *5.2. Optimised method*, except for the varying polarities and quantities of solvents and sorbent. Several extracts and blank samples were spiked with the standard solutions mentioned in *5.1.2. Standards* at 25 pg/µL for DPs and 3 ng/µL for SCCPs, and the quantification was carried out by GC-ECNI-MS working in full-scan mode according to the conditions in *5.2.3. Quantification*. The next paragraphs contain a summary of the optimisation process. The most relevant results of the described process are shown in Table 3.

The initial conditions for fractionation (fraction 1: 20 mL of hexane, fraction 2: 30 mL of *n*-hex:DCM 85:15 (v/v), fraction 3: 30 mL of *n*-hex:DCM 1:1) with 5 g of Florisil lead to a very poor separation of the SCCPs from interfering compounds in a blank test, and to an even worse (actually null) separation in extracts containing the matrix of the sample (over 95% of the analytes and potential interferences appearing in fraction 2) (Table 3, exp.1A, 1B).

In order to increase the retention of the analytes in the Florisil column, the Florisil amount was increased from 5 g to 10 g, and the influence of the solvent polarity on the fractionation outcome was studied by performing three experiments with 85:15, 90:10 and 95:5 (v/v) *n*-hex:DCM mixtures (Table 3, exp. 2A, 2B, 2C). A decrease of the polarity of the elution solvent avoided the elution of the SCCPs in this fraction rather than in fraction 3. Experiment 2A showed again poor results, so the solvent amount in fraction 2 was increased to 60 mL. Better results were obtained in both cases because of the increase on the Florisil amount and because of the decrease on the solvent's polarity, but still being insufficient for a full separation of SCCPs from other interfering compounds. Therefore, 80 mL of the 95:5 (v/v) solvent were used for two other experiments (Table 3, exp. 3A, 3B), leading to acceptable results in a blank test, but the matrix effect on the Florisil column induced a decrease in the separation in exp. 3B, probably by saturating part of the Florisil with other interferences and matrix components and leaving it unable to adsorb as much SCCPs as before, resulting in part of those eluting again in fraction 2.

Given those results, a third amount of Florisil (15 g) was used to perform a last test using a sample extract (with fraction 1: 30 mL of hexane, fraction 2: 100 mL of *n*-hex:DCM 95:5 (v/v), fraction 3: 30 mL of *n*-hex:DCM 1:1 (v/v)). These conditions allowed the adequate separation of the compounds, setting a splitting point at 80 mL of the 95:5 (v/v) n-hex:DCM solvent mixture to

maintain an equilibrium between the residual amount of DPs still eluted in fraction 3 and the residual amount of SCCPs eluted in fraction 2. This lead to the optimised fractionation conditions, consisting of two fractions (fraction 1: 30 mL of hexane + 80 mL of *n*-hex:DCM 95:5 (v/v) –joining fraction 1 and fraction 2 in one unique fraction– and fraction 2: 30 mL of *n*-hex:DCM 1:1 (v/v)) (Table 3, exp. 4A).

Exp. (Sample)	Florisil amount	Fractionation	Recovery (%) of SCCPs	Recovery (%) of DP
1A (blank)	5 g	F1: 20mL *n*-hex	0 %	15 %
		F2: 30mL *n*-hex:DCM 85:15	88 %	82 %
		F3: 30mL *n*-hex:DCM 1:1	12 %	3 %
1B (matrix)	5 g	F1: 20mL *n*-hex	0 %	4 %
		F2: 30mL n-hex:DCM 85:15	97 %	95 %
		F3: 30mL *n*-hex:DCM 1:1	3 %	1 %
2A (blank)	10 g	F1: 20mL *n*-hex	0 %	0 %
		F2: 30mL *n*-hex:DCM 85:15	64 %	96 %
		F3: 30mL *n*-hex:DCM 1:1	36 %	4 %
2B (blank)	10 g	F1: 20mL *n*-hex	0 %	0 %
		F2: 60mL *n*-hex:DCM 90:10	32 %	100 %
		F3: 30mL *n*-hex:DCM 1:1	68 %	0 %
2C (blank)	10 g	F1: 20mL *n*-hex	0 %	0 %
		F2: 60mL *n*-hex:DCM 95:5	12 %	87 %
		F3: 30mL *n*-hex:DCM 1:1	88 %	13 %
3A (blank)	10 g	F1: 20mL *n*-hex	0 %	0 %
		F2: 80mL *n*-hex:DCM 95:5	27 % (a)	98 %
		F3: 30mL *n*-hex:DCM 1:1	73 %	2 %
3B (matrix)	10 g	F1: 20mL *n*-hex	0 %	0 %
		F2: 80mL *n*-hex:DCM 95:5	65 %	99 %
		F3: 30mL *n*-hex:DCM 1:1	35 %	1 %
4A (matrix)	15 g	F1: 30mL *n*-hex + 80mL *n*-hex:DCM 95:5	10 % (a)	85 %
		F2: 30mL *n*-hex:DCM 1:1	90 %	15 %

(a) Obtained by integrating the whole area where SCCPs appear, but a SCCP characteristic area is not fully present.

Table 3. Recoveries (%) of SCCPs and the main interference in the different fractions obtained during the optimisation of the fractionation.

A few comments should be done regarding the results in Table 3. As can be seen, there are some results marked with (a) that have been obtained by integrating the area of the peaks eluted in the retention time range of SCCPs as a way of systematically performing the

quantification. However, they do not add up to a whole SCCP characteristic area and might actually be considered in its majority as noise, which means that an overestimation of the results in the first fractions may have occurred. The actual recoveries for experiments 3A and 4A for the SCCPs in the third fraction were expected to be higher, leading to a better separation of the compounds. In fact, a better separation was observed in the validation chromatograms, which reinforces this idea. On the other hand, the recoveries of DPs in the third fraction of each experiment were mainly due to a specific compound of the DP mixture (Cl-11-DP). All the other compounds eluted mainly in the previous fractions, so the aim of the optimisation experiments was to recover as much Cl-11-DP in fraction 2 as possible without getting too much SCCPs in there, but a splitting point had to be established at 80 mL of *n*-hex:DCM 95:5 (v/v) for fraction 2, losing some of it instead of losing more SCCPs.

6.3. VALIDATION OF THE ANALYTICAL METHOD

The validation was performed throughout seven working days and in accordance to the harmonised guidelines for method validation and fitness for purpose testing proposed by Eurachem in 2014 [40]. Aside from the required tests for the LOD and LOQ determination and those performed during the optimisation process that can also be used for the selectivity assessment, a blank chicken egg spiked at two concentration levels, 0.7 ng/g and 2.3 ng/g wet weight (ww), were used for the study of recovery and precision. A total of 9 experiments at each level were carried out in two batches (6 on the first one for the inter-day precision and 3 more on the second one some days later for the intra-day precision), adding up to a total of 18 experiments, performed as exposed in section *5.2. Optimised method*. The definitions of the following parameters can be found in section *5.3. Validation parameters*.

6.3.1. Selectivity

The assessment of the selectivity can be visually approximated from the chromatograms obtained through the optimisation process as well as from those obtained from the analysed samples. Since it is a qualitative parameter, a sufficiently good separation of the analyte from its interferences is enough to prove an acceptable selectivity. In this case, the DPs were considered to be the main interference for the SCCP quantification. As can be seen in Figure 3, where the GC-ECNI-MS chromatograms obtained working in SIM mode for fraction 1 and 2 are given, there are no important peaks that hinder the elution profile of the SCCPs or that preclude

their quantification in any way. Those compounds that appear in the retention time range of the SCCPs can be distinguished by their different *m/z* ratios. These findings demonstrated enough selectivity for the quantification of SCCPs in egg samples.

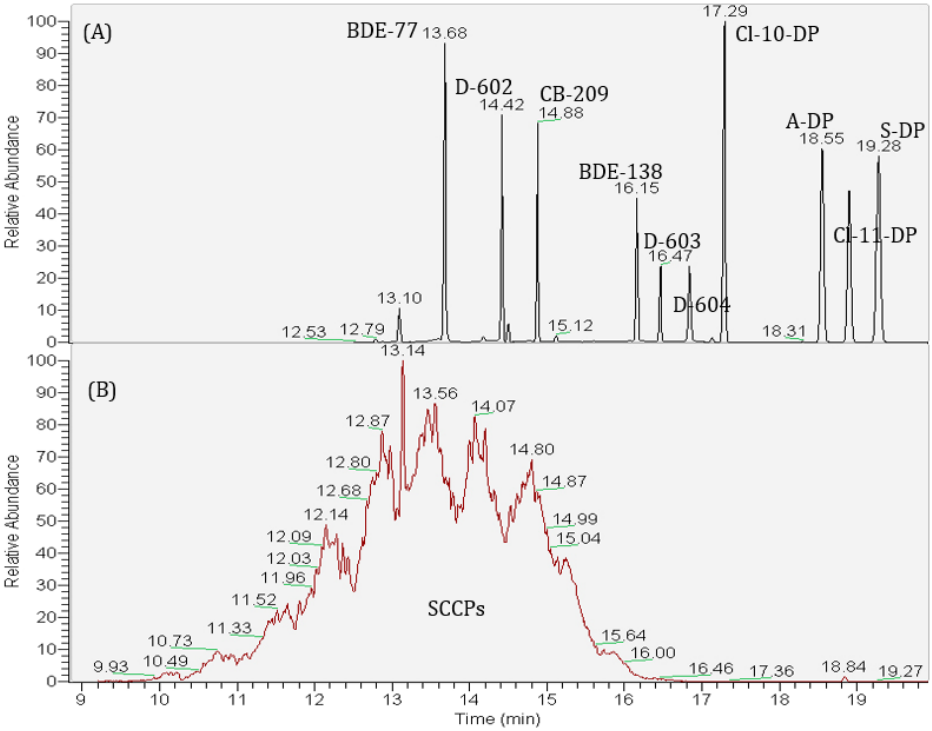

Figure 3. GC-ECNI-MS chromatograms in SIM mode of fraction 1 (A) and 2 (B) from a spiked extract at high level (2.3 ng/g ww).

6.3.2. Linearity

Linearity was tested by repeatedly injecting six calibration curve standards containing SCCP concentrations of 0.5, 1, 3, 5, 10 and 15 ng/µL, PCB209 at 1 pg/µL as an injection internal standard and δ-HCH at 5 pg/µL as a surrogate internal standard. Figure 4 shows as an example the GC-ECNI-MS chromatogram of the 10 ng/µL calibration standard and Figure 5 shows one calibration curve relative to the surrogate standard δ-HCH (IS).

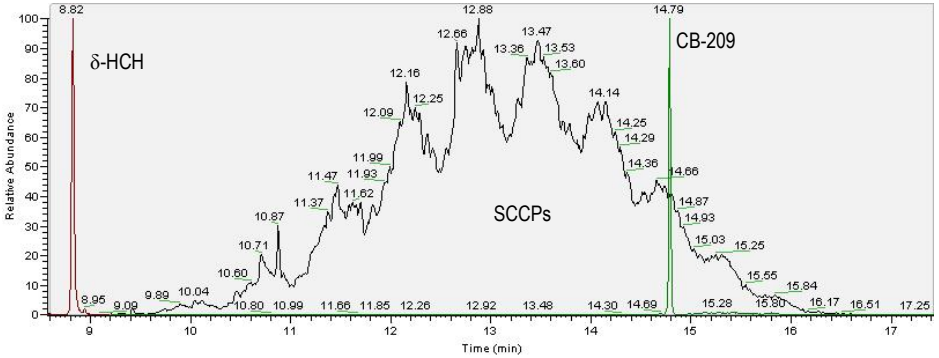

Figure 4. GC-ECNI-MS chromatogram in SIM mode of the 10 ng/µL calibration standard containing 1 pg/µL of CB-209 (IS) and the surrogate standard δ-HCH at 5 pg/µL.

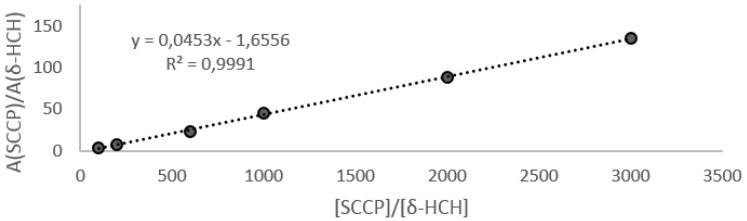

Figure 5. Linear regression of the SCCP calibration standards relative to δ-HCH used as internal standard.

As can be seen, good correlation of the calibration curve was obtained, with R^2 factor always higher than 0.99. Even better fitness could be achieved with a polynomial regression, especially taking into account that at higher concentrations the slope described by the data is expected to grow, losing the linearity. Even so, given that the concentrations of SCCPs in the samples are expected to be lower than that, the linear regression is good enough to generate reliable results through a much easier calculating process.

6.3.3. Limits of detection and quantification

A sample extract spiked with CB-209 at 1 pg/µL and SCCPs at 0.05 ng/µL will suffice for the signal to noise ratio (S/N) estimation using the Signal to Noise Calculator software (Thermo Fisher Scientific, Milan, Italy). The concentration of SCCPs that gave a S/N equal to 3 corresponds to the method's LOD, while a S/N of 10 corresponds to the method's LOQ. The obtained results are shown in Table 4, both calculated in ng/g of dry weight and wet weight sample.

Measure	Dry weight (dw)	Wet weight (ww)
mLOD	0.17 ng/g	0.04 ng/g
mLOQ	0.55 ng/g	0.13 ng/g

Table 4. Method's LOD and LOQ.

The mLOD and mLOQ obtained were low enough for the adequate detection and quantification of SCCPs in egg samples and are better than those reported in the literature for biota samples.

6.3.4. Recovery and trueness

Given the lack of certified reference materials for SCCPs, the trueness of the method must be assessed by means of recovery. The recovery (R, in %) in this report was calculated from eighteen spiking experiments at two different concentration levels (nine experiments per level) using a blank chicken egg sample. Trueness was determined considering the relative error (ER%) between the theoretical SCCP concentration expected according to the added amount during the spiking of the sample and the SCCP concentration determined after applying the analytical methodology (both in ng/g egg ww).

The low and high concentration levels studied were 0.7 ng/g ww and 2.3 ng/g ww of SCCPs. Both spiking levels contained the surrogate (δ-HCH) and injection (CB-209) internal standards to give a concentration in the final extract of 5 µg/L and 1 µg/L, respectively. The standard solution used for the spiking experiments contained DPs as potential interfering compounds at a concentration of 10 pg/µL. The amount of δ-HCH that contains the sample during the injection step allows the quantification of SCCPs referenced to the surrogate standard, which means an automatic correction of the recovery values for the determined SCCP concentrations. These results are summarised in Table 5.

Level	Recovery (%) ± sd [(a)]	[SCCP] added	[SCCP] found ± sd [(a)]	RE% [(a)]
Low level	94 ± 6	0.7 ng/g ww	0.65 ± 0.04 ng/g ww	- 6.4
High level	97 ± 7	2.3 ng/g ww	2.2 ± 0.2 ng/g ww	- 5.7

(a) Mean of 9 independent determinations.

Table 5. Method recovery (%) (± standard deviation) and relative error (%) for each studied spiking level.

The recoveries of the method were between 94±6% and 97±7% for low and high level respectively, and the trueness, expressing the bias as relative error (%), was always lower than

7%, demonstrating the validity and good performance of the method for the determination of SCCPs.

6.3.5. Intra-day and inter-day precision

The intra-day or within-day precision, which is a way of expressing the repeatability of the method, was studied analysing (6 independent analysis) a blank chicken egg sample spiked at two concentration levels, 0.7 ng/g ww and 2.3 ng/g ww of SCCPs. The inter-day precision (also called within-day precision) is a way of expressing the intermediate precision of the method or within-laboratory reproducibility. This parameter was obtained by analysing blank chicken egg samples spiked at the above mentioned levels in three different days (3 independent analysis per day) and using different calibration solutions and conditions. The relative standard deviation (RSD%) of all measured samples from the different batches was calculated, showing an estimate of how well does the method work in different days with different operating conditions. The results are shown in Table 6.

Parameter	Low level (RSD%)	High level (RSD%)
Intra-day precision [(a)]	6.5 %	8.5 %
Inter-day precision [(b)]	7.5 %	8.9 %

(a) n= 6 independent analysis at two concentration levels
(b) n= 3 independent analysis x 3 days at two concentration levels

Table 6. Intra- and inter-day precisions of the method at two SCCP spiking levels (0.7 and at 2.3 ng/g ww).

Higher variations in results from different batches were expected, since variations in working conditions from day to day are supposed to be higher than within the same day, resulting in higher inter-day relative standard deviations of the results. Even so, these results lead to a good enough repeatability (under 9% both for within-day and day-to-day experiments), which means that the method is suitable for sample analysis in terms of repeatability and within-laboratory reproducibility.

6.4. APPLICATION OF THE METHOD TO GULL EGG SAMPLES

The present method was applied to gull egg samples collected and lyophilised in 2013 and properly stored until the moment of the analysis. They were collected from three natural parks in Spain as described in section *5.1.3. Samples*.

Yellow-legged gull (*Larus michahellis*) is a common species in the Iberian Peninsula, widespread in Europe, Middle East and North Africa. These gulls are opportunistic omnivores,

meaning that they are general feeders that lack specialisation for food. Their diet consists of fish, insects, molluscs, crabs, reptiles, small mammals and ground squirrels, bird eggs and chicks, and they have scavenger habits based on rubbish tips. They live up to 20-30 years and are a monogamous species, usually laying 3 eggs per year and breeding always in the same colony.

Audouin's gull (*Larus audouinii*) is a protected gull species only present in the Mediterranean and in the western coast of Saharan Africa. It lays 2-3 eggs per breeding season, colonially or alone. They are omnivores with a diet mainly consisting of fish, molluscs and crabs.

Samples were analysed as described in *5.2. Optimised method*, and the obtained results are shown in Table 7.

		Concentration (ng/g ww)		
Sampling area	**Gull species**	**Mean [a]**	**Minimum**	**Maximum**
Medes Islands	*Larus michahellis*	2.26	1.78	2.66
Atlantic Islands of Galicia	*Larus michahellis*	1.61	1.20	1.98
Ebro Delta	*Larus michahellis*	2.68	2.11	3.70
Ebro Delta	*Larus audouinii*	4.73	4.40	5.08

(a) Mean of 3 sampling sub-areas.

Table 7. SCCP concentrations found in gull species in different areas across Spain in 2013.

Results for *Larus michahellis*, ranging from 1.78 to 3.70 ng/g of wet weight egg sample, are notably lower than those of *Larus audouinii*, ranging from 4.40 to 5.08 ng/g ww. This indicates a presumable dependence on the diet, specially comparing samples from the same natural park (Ebro Delta Natural Park in Catalonia, Spain), meaning that SCCPs tend to accumulate more in organisms in water ecosystems such as fishes or molluscs rather than in other places, which leads to higher bioaccumulation in egg samples from species feeding from those ecosystems and not in opportunistic omnivores with a more varied diet.

Results from Atlantic Islands of Galicia Natural Park in 2013 were compared with levels found in previous years by CECEM research group in University of Barcelona, belonging to a monitoring project started in 2010 and focused on the determination of SCCPs in eggs from several natural and protected areas in Spain. These results are reported in Table 8.

Year	Mean [a]	Concentration range (ng/g ww)	
		Minimum	Maximum
2013	1.61	1.20	1.98
2012	10.79	9.47	12.32
2011	8.03	7.05	8.97
2010	4.53	1.72	6.95

(a) Mean of 3 sampling sub-areas.

Table 8. SCCP concentrations found in *Larus michahellis* gull species in Atlantic Islands of Galicia Natural Park in Spain from 2010 to 2013.

There is significant trend in SCCP concentrations found in *Larus michahellis* in the Atlantic Islands of Galicia Natural Park over the first three years of the study, growing from mean values of 4.53 to 10.79 ng/g ww. These increasing amounts (Figure 6) suggest an increase on the SCCP accumulation in the bird's ecosystem and organism throughout the years, but data from samples collected in 2013 contrast with this hypothesis with the mean concentration value dropping to 1.61 ng/g ww.

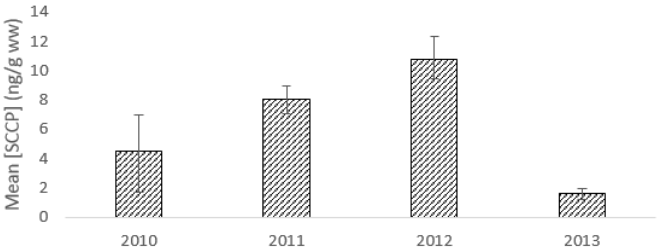

Figure 6. Mean values (with range of values from the lowest to the highest results) of SCCP concentrations found in *Larus michahellis* in Atlantic Islands of Galicia Natural Park in Spain from 2010 to 2013.

In addition, the low SCCP levels found in 2013 are correlated with a similar descent in the concentrations of other persistent organic pollutants, such as PCBs, PBDEs and organochlorine pesticides, suggesting a global reduction of the contamination of this area as a result of the regulations and actions applied for removing and controlling the possible sources of these and other pollutants in the environment.

7. CONCLUSIONS

This report summarises the work performed in order to develop and validate an analytical method for the determination of SCCPs in gull egg samples. The main conclusions of the research work are the following:

- A simple analytical method has been successfully developed, optimised and validated according to international guidance on fitness for purpose testing.
- The method optimisation lead to a sample treatment step capable of separating SCCPs from what was considered to be one of the main interferences (Dechlorane Plus).
- Validation of this method showed positive results, indicating good recovery values (from 94±6% to 97±7%), suitable precision values (method repeatability and within-laboratory reproducibility expressed as RSD% lower than 9%) and adequate limits of detection and quantification (LOD of 0.04 ng/g ww and LOQ of 0.13 ng/g ww) to perform determinations in gull egg samples. Although these parameters are enough to state the method suitability in a within-laboratory validation, further testing could be done in the future to ensure a full validation regarding ruggedness characteristics (to determine critical parameters that can affect the method performance) or inter-laboratory precision for actual reproducibility.
- Regarding the applicability of the method to the analysis of gull egg samples, the obtained values lie within the linear range of the method, allowing a correct quantification. Results for *Larus michahellis* from different natural parks in Spain have proved to be very similar, but significant differences were observed for *Larus Audouinii* due to different diets and feeding habits (the first one is an opportunistic omnivore with scavenger habits while the second one's diet consists of fish and molluscs). Moreover, data obtained in the present work have demonstrated a significant decrease on the SCCP levels compared to those found in eggs collected from 2010 to 2012 in Atlantic Islands of Galicia Natural Park that breaks the growing trend of previous years.

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9. ACRONYMS

ASE	Accelerated Solvent Extraction
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
CPs	Chlorinated Paraffins
DCM	Dichloromethane
DDT	Dichlorodiphenyl trichloroethane
DPs	Dechlorane Plus
ECNI	Electron-Capture Negative Ionisation
EPA	Environmental Protection Agency
GC	Gas Chromatography
HLC	Henry's Law Constant
HRMS	High Resolution Mass Spectrometry
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
K_{ow}	Octanol-Water Partition Coefficient
LCCPs	Long-Chain Chlorinated Paraffins
LOD	Limit of Detection
LOQ	Limit of Quantification
HRMS	High Resolution Mass Spectrometry
LRMS	Low Resolution Mass Spectrometry
MAB	Metastable Atom Bombardment
MCCP	Medium-Chain Chlorinated Paraffins
MS	Mass Spectrometry
OECD	Organisation for Economic Co-operation and Development
PBDEs	Polybrominated Diphenyls
PCAs	Polychlorinated n-Alkanes
PCBs	Polychlorinated Biphenyls
PLE	Pressurised Liquid Extraction
POPs	Persistent Organic Pollutants
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RSD	Relative Standard Deviation
SCCP	Short-Chain Chlorinated Paraffins
SIM	Selected Ion Monitoring
s-PLE	Selective Pressurised Liquid Extraction
SVHC	Substances of Very high Concern
TSCA	Toxic Substances Control Act
UAE	Ultrasonic Assisted Extraction
UNEP	United Nations Environment Programme
δ-HCH	delta-hexachlorocyclohexane