Determination of chlorinated paraffins (CPs): Analytical conundrums and the pressing need for reliable and relevant standards

Alwyn R. Fernandes [a,*], Walter Vetter [b], Caroline Dirks [c], Louise van Mourik [d], Ronan Cariou [e], Jannik Sprengel [b], Norbert Heeb [f], Anouk Lentjes [d], Kerstin Kratschmer¨ [g]

[a] School of Environmental Sciences, University of East Anglia, Norwich, NR4 7TJ, UK
[b] Institute of Food Chemistry, (170b), University of Hohenheim, Garbenstraße 28, 70599, Stuttgart, Germany
[c] Wageningen Food Safety Research, Akkermaalsbos 2, 6708 WB, Wageningen, the Netherlands
[d] Department of Environment and Health, Faculty of Sciences, Vrije Universiteit, De Boelelaan 1087, 1081 HV, Amsterdam, the Netherlands [e] LABERCA, Oniris, INRAE, 44307, Nantes, France
[f] Laboratory for Advanced Analytical Technologies, Swiss Federal Institute for Materials Science and Technology Empa, Überlandstrasse 129, 8600, Dübendorf, Switzerland
[g] European Union Reference Laboratory (EURL) for Halogenated POPs in Feed and Food, Bissierstraße 5, 79114, Freiburg, Germany

Abstract

The determination of chlorinated paraffins (CPs) has posed an intractable challenge in analytical chemistry for over three decades. The combination of an as yet unspecifiable number (tens - hundreds of thousands) of individual congeners in mass produced commercial CP mixtures and the steric interactions between them, contrive to defy efforts to characterise their residual occurrences in environmental compartments, food and human tissues. However, recent advances in instrumentation (mass spectrometric detectors and nuclear magnetic resonance), combined with interlaboratory studies, have allowed a better insight into the nature of the conundrums. These include the variability of results, even between experienced laboratories when there is insufficient matching between analytical standards and occurrence profiles, the poor (or no) response of some instrumentation to some CP congener configurations (multiple terminal chlorines or < four chlorines) and the occurrence of chlorinated olefins in commercial mixtures. The findings illustrate some limitations in the existing set of commercially available standards. These include cross-contamination of some standards (complex CP mixtures), an insufficient number of single chain standards (existing ones do not fully reflect food/biota occurrences), lack of homologue group standards and unsuitability of some configurationally defined CP congeners/labelled standards (poor instrument response and a smaller likelihood of occurrence in commercial mixtures). They also indicate an underestimation in reported occurrences arising from those CPs that are unresponsive during measurement. A more extensive set of standards is suggested and while this might not be a panacea for accurate CP determination, it would reduce the layers of complexity inherent in the analysis.

1. Introduction

Chlorinated paraffins (CPs) are one of the largest volume produced industrial chemicals of present times, although large-scale production has been known since the 1930s. As versatile products, they are used in several applications, e.g. as plasticizers, temperature moderators during machining, high-pressure lubricants, flame retardants, etc. Research laboratories and risk assessment studies have conveniently characterised these products on the basis of the chlorinated alkyl chain lengths, as short-, medium- and long-chain chlorinated paraffins (SCCPs C_{10} to C_{13}, MCCPs C_{14} to C_{17}, and LCCPs C ≥ 18), but there is a wide variation in the degree of chlorination (%Cl) as well as chain length in many technical products. In reality, the technical products often have only an indication of the overall %Cl, and are complex mixtures of several thousands of individual congeners with varying numbers of chlorine atoms and different chain lengths (Tomy et al., 1997).

As a significant proportion of CP utilisation is open-ended, they are unsurprisingly detected in a wide range of environmental compartments, as well as in human and animal tissues. Although much of the research on hazard investigation has focussed on SCCPs - characterising these mixtures as bio-accumulative, persistent and toxic, there is little to suggest that the other technical mixtures do not also show these properties. However, there has been little investigation on the toxicology of MCCPs (Zellmer et al., 2020) and particularly LCCPs (Ren et al., 2019), even though emerging occurrence data (Glu¨ge et al., 2018; Yuan et al., 2019A; van Mourik et al., 2020; Kratschmer et al., 2021a,b¨) has demonstrated that levels of these mixtures in the environment and humans may exceed those of SCCPs. The Stockholm Convention lists SCCPs in Annex A (Elimination of production), but this retrospective view is inadequate, as manufacturing has simply been extended to the other technical categories. Similarly, the European Food Safety Authority (EFSA) was also unable to characterise the risk to humans or animals from dietary exposure to CPs because of the lack of adequate supporting information on occurrence and toxicology (EFSA, 2020). Thus, in order to assess risk and subsequently substantiate regulation, more, and current data is required, both on occurrence in the environment, food and animal feed, as well as on the nature of toxicological endpoints, which would help to clarify and better characterise the risk.

Although CPs of all chain lengths have been produced and used for decades, the lack of data characterising their occurrence, environmental behaviour, toxicity, etc., is striking. This sparsity of information is driven by a number of factors of which the complexity of the technical mixtures, perhaps contributes the most. The complex isomerism that is present in these may be seen for example in a technical short chain chlorinated paraffin (SCCP) mixture containing 60% chlorine by weight. If only configurational isomers are considered (without the inclusion of stereoisomers), the theoretical number can be calculated (Tomy, 2010). This calculation assumes that the mixture contains only straight chain chloroalkanes with the empirical formula $C_nH_{2n+2-z}Cl_z$. It also assumes that no more than one chlorine atom is attached to any carbon atom since its presence induces deactivation to further substitution. Obviously, for z = 1 there are seven positional isomers and for z = 13 there is only one. For a single chain length - chlorotridecanes (C_{13}-CPs) in this mixture a theoretical, 4160 configurational isomers are possible (Tomy, 2010). The main contributors would be $C_{13}H_7Cl_6$ and $C_{13}H_6Cl_7$, the major congeners present in SCCP mixtures containing 50–60% chlorine. The theoretical number of each of them is 868 which gives a total of 1736 configurational isomers. Since, in the industrial synthesis, attack of methylene groups is preferred over attack of methyl groups, most of these positional isomers can be expected to contain largely chiral -CHCl- moieties, each having either an R or an S configuration. Considering 2,5, 6,8,9,11-hexachlorotridecane as an example, the six stereogenic centres could theoretically yield up to 64 (2^6) stereoisomers of this compound alone. This example provides an indication of the numerical complexity for a single chain length, and this level of complexity rises as the chain length increases (i.e. the

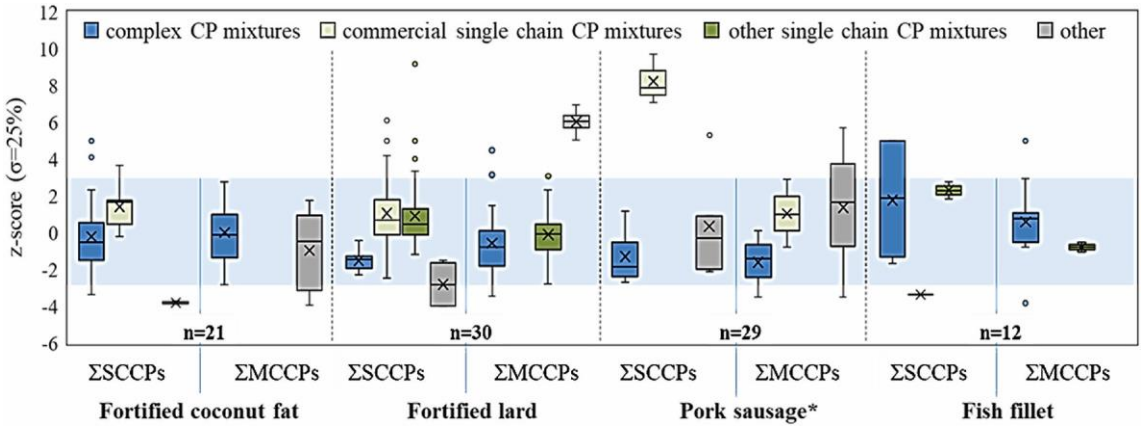


Fig. 1. Performance range (as Z-scores) of participating laboratories (n = 12 to 30) in interlaboratory exercises on CPs, characterised by the type of standard used.

numbers associated with a C_{16} chain length for example, will be considerably higher than for C_{14}). Logically, it follows that in terms of numerical complexity, LCCPs > MCCPs > SCCPs. As the technical products are mixtures of different chain lengths, they are inherently more complex. The possibility of multiple chlorination of some carbons (particularly in highly chlorinated technical products) and branched, rather than straight chains (also possible in commercial products) are additional factors that may also be considered. Collectively, these considerations indicate the complexity involved even in theoretical calculations of absolute numbers. However, in reality, fewer isomers are likely to be formed in commercial products as seen from the examples of other chlorinated contaminants such as polychlorinated biphenyls (PCBs).

In order to characterise and quantify the occurrences, different types of analytical standards have been used by CP researchers, ranging from complex technical products to individual compounds and these are described below and used to refer to analytical standards throughout the remainder of this work:

- Complex CP mixtures, which are produced and behave like technical products (e.g. SCCP 55.5% Cl);
- Single-chain CP mixtures, which only include homologues of a specified carbon chain length (e.g. C_{10} 65% Cl). These may be commercially available or synthesised by research laboratories (e.g. Sprengel and Vetter, 2019);
- Configurationally defined individual CP congeners with a specified number of carbon and chlorine atoms in addition to a defined position of the chlorine atoms (e.g. 1,5,5,6,6,10-hexachlorodecane), which might also be isotopically labelled.

Currently, no technique is capable of separating the several thousands of individual congeners within a technical mixture. Instead, the most recent research (Mezi´ ˋere et al., 2020A; Kratschmer et al., 2021a¨ , 2021b; van Mourik et al., 2021) has focussed on trying to collectively characterise congeners within a single chain length (e.g. $C_{12}Cl_x$) and from single homologue groups (e.g. $C_{12}Cl_5$) or as part of a technical mixture (e.g. sum of SCCPs) (van Mourik et al., 2019, Yuan et al., 2019A). This level of characterisation when applied, for example, to food analysis, would provide an indication of which CPs humans might be exposed to, through dietary intake. However, this ability is only available within a few specialist laboratories worldwide, and proficiency testing (Kratschmer and Sch¨ achtele, 2019b¨) has shown that consensus on determined amounts in test samples is far from universal between participating laboratories. While inter-laboratory variation has improved since 2017 in general, particularly among laboratories experienced in CP analysis (Mezi´ ˋere et al., 2020B), comparison of the tentatively derived z-scores with the type of calibration standards used, shows the dependency of that improvement on the choice of standards, or rather the compatibility of the standards with the sample (Fig. 1).

During the studies in 2017 and 2018, when fortified lipid samples were analysed, results for complex CP mixture standards, commercially available and other (synthesised in-house) single chain CP mixtures (Sprengel and Vetter, 2019) that were provided by the exercise co-ordinator were generally in good agreement. However, in later exercises when naturally contaminated sample material was used, considerably higher levels of variation were seen in the reported results, depending on the standards used for quantitation. This indicates a dependency on the ability to match standards with the occurrence patterns in naturally contaminated test materials or samples. Although there may be other lateral causes for the observed variability in results, such as different quantitative approaches, different instrumental techniques, etc., the most important reason for this discrepancy was identified as the limitations of the currently (commercially) available CP reference standards (Kratschmer and Schachele, 2019B¨). In particular, for the 2020 interlaboratory comparison, no participant was able to use commercially available MCCP single chain CP mixtures as these have only recently become available.

The following sections of this work describe some of the experiences of using currently available standards, in terms of cross contamination, definition and lack of adequate specification, etc. Some of the complex mixture standards are derived from CP technical products with ambiguity of content (as discussed later) and thus prove unsuitable to support the advancements in this field. The lack of some types of standards, e.g. specific chain-length mixtures, isotopically labelled individual CP compounds for use as internal standards, homologue group standards etc., hinders a more reliable determination of occurrence levels in environmental matrices, food and animal feed and human tissues. Based on recent observations in a number of laboratories, standards which may be more suitable have been proposed, and these may vary depending on the measurement technique used by a laboratory. Additionally, considering future research requirements, consideration has also been given to individual compound standards, based on the premise that particular molecular configurations are more likely to occur in technical mixtures. This approach, although indicative (as characterisation of individual compounds is currently not feasible), is likely to better reflect the composition of occurrence in environmental and food residues. It may also aid toxicological studies where the effects observed may depend on the dominance of particular chemical configurations.

Table 1

Producer	Type	Description		
AccuStandard	technical product	Chlorafin 40 (Chlorinated Paraffin), Chlorowax 500C (Chlorinated Hydrocarbon 59% Cl) Diablo 700× (Chlorinated Hydrocarbon 70% Cl) Unichlor 40–90 (Chlorinated Hydrocarbons 38.5% Cl) Unichlor 502-50 (Chlorinated Hydrocarbons 52% Cl) Unichlor 70AX (Chlorinated Hydrocarbons 70% Cl)		
Cambridge Isotope Laboratories	single compound (labelled)	**1,5,5,6,6,10-Hexachlorodecane ($^{13}C_{10}$, 99%/95% + pure/unlabelled)** 1,1,1,3,10,12,12,12-Octachlorododecane ($^{13}C_{12}$, 99%/unlabelled)		
Chiron	technical product	Chlorinated paraffins (70% Cl), technical mix		
	single compound	$7C_{10}$ CPs: Cl_4, Cl_6, Cl_8	$6C_{14}$-CPs: Cl_4, Cl_6, Cl_8	$4C_{18}$-CPs: Cl_6, Cl_7, Cl_8
		$9C_{11}$-CPs: Cl_4, Cl_6, Cl_8	$2C_{15}$-CPs: Cl_6	$1C_{19}$-CPs: Cl_8
		$8C_{12}$-CPs: Cl_4, Cl_6, Cl_8	$2C_{16}$-CPs: Cl_6, Cl_8	$1C_{20}$-CPs: Cl_8
		$5C_{13}$-CPs: Cl_4, Cl_6, Cl_8	$2C_{17}$-CPs: Cl_6, Cl_8	
	reconstructed technical product	$C_{10\text{-}13}$ mix 49.0% Cl (“Hordalub 17¨”)	$C_{10\text{-}13}$ mix 56.0% Cl (“Hordalub 80¨”)	$C_{10\text{-}13}$ mix 62.3% Cl (“Hordalub 500¨”)
		$C_{10\text{-}13}$ mix 60.0% Cl (“Cereclor 60¨”)	$C_{10\text{-}13}$ mix 64.7% Cl (“Cereclor 70¨”)	
	reconstructed mixed standard	$C_{10\text{-}13}$ mix 51.5% Cl	$C_{14\text{-}17}$ mix 42% Cl	$C_{18\text{-}20}$ mix 36% Cl
			$C_{14\text{-}17}$ mix 52% Cl $C_{14\text{-}17}$ mix 57% Cl	$C_{18\text{-}20}$ mix 49% Cl
LGC/Dr. Ehrenstorfer	single chain standard	Chloroparaffin C_{22} 72.1% Cl C_{10} mixes 44.82% Cl - 65.02% Cl C_{11} mixes standards 45.50% Cl - 65.25% Cl Chloroparaffin C_{14} standards 45% Cl–65% Cl Chloroparaffin C_{16} standards 45% Cl–65% Cl Chloroparaffin C_{18} 40, 50 and 60% Cl Chloroparaffin C_{22} 36 and 50% Cl	Chloroparaffin C_{12} standards 45.32% Cl-69.98% Cl Chloroparaffin C_{13} standards 44.90% Cl-65.18% Cl Chloroparaffin C_{15} standards 45% Cl–65% Cl Chloroparaffin C_{17} standards 45% Cl–65% Cl Chloroparaffin C_{20} 40 and 50% Cl Chloroparaffin C_{24} 37 and 46% Cl	
	Complex mixed standard	**Chloroparaffin $C_{10\text{-}13}$ 51,5% Cl**	**Chloroparaffin $C_{10\text{-}13}$ 55,5% Cl**	Chloroparaffin $C_{10\text{-}13}$ 63% Cl
		Chloroparaffin $C_{14\text{-}17}$ 42% Cl	**Chloroparaffin $C_{14\text{-}17}$ 52% Cl**	Chloroparaffin $C_{14\text{-}17}$ 57% Cl
		Chloroparaffin $C_{18\text{-}20}$ 36% Cl	**Chloroparaffin $C_{18\text{-}20}$ 49% Cl**	

Indicative (non-exhaustive) listing of currently available CP standards. The most commonly used standards reported in the literature are marked in **bold** text. (A more extensive listing is given in the SI).

2. The limitations of currently available CP standards for current and future research and monitoring studies

A broad listing of the most commonly used standards is summarised in Table 1. This represents a non-exhaustive listing as other products (including those from other producers) may also be commercially available, but it serves to illustrate the range of CP standards that laboratories can currently purchase for analytical determination. A more extensive listing is given in the supplementary information –Tables SI–1.

The list in Table 1 (and Tables SI–1) generally represents standards that are available in Europe, but additional or different products may be available in other regions, i.e. Asia, North America, etc. Many of these are derived from technical products, but there are also a number of individual compounds, single chain mixtures and currently, two ^{13}C labelled individual CP compounds.

Primary standards are characterised by a number of requirements, the most important of which are purity and stability. It is therefore vitally important that the calibration standards (of any type) used for CP analysis are free of impurities. In particular, where low (unit) resolution MS or non-mass spectrometric detection methods are used, such impurities can compromise the quantitation results, depending on the proportion to which they occur in the calibration standards. Of the eight most commonly used complex CP mixture standards in Europe, five showed a range of impurities across production batches spanning decades, when analysed for homologue groups by GC-ECNI-HRMS at 60,000 resolution (FWHM at *m/z* 200) (Kratschmer et al., 2019C¨). In general, these impurities often arise from other CP mixtures (Fig. 2) that are outside the specification of the standards, and the total response of these impurities was as high as 53% in some mixtures that were sold as LCCP standards. Perhaps reflecting the longer period of study that followed the recognition of SCCPs as environmental and food contaminants (relative to other CPs), these standards showed lower levels of impurities followed by similar proportions of impurities in MCCP standards. The moderately chlorinated (52–55.5% Cl) SCCP and MCCP standards appear to be more affected (Fig. 2), although the limited number of mixtures examined does not exclude higher contamination in standards of other chlorination ranges. Clearly the relative proportion of impurities seen in some LCCP standards would result in a very poor quantitative assessment as seen by the relative proportion of impurities.

Even though it is currently not possible to characterise CP mixtures to the individual congener level, single CP congeners may be useful as surrogate standards for monitoring losses during sample processing or for monitoring instrument variations from run to run. In addition, they could be used in laboratory studies to provide an insight into the environmental and biological fate of these contaminants which in turn would enable direction for future occurrence and toxicological studies (Fernandes et al., 2020). There are a number of currently available single compound CP standards, but many of these, including the only two ^{13}C labelled compounds on sale, are chlorinated at the terminal carbon, in some cases to saturation (e.g. 1,1,1,3,9,11,11,11-$C_{11}Cl_8$). Although as discussed in section 3, this configuration may be expected to be present in the more highly chlorinated mixtures, it is considered to be less likely in lower and moderately chlorinated (<50%) commercial formulations that have a higher frequency of application and use (van Mourik et al., 2015; Sprengel et al., 2019). Emerging evidence indicates that chlorination is likely to occur centrally on carbon chains, including a projection of the highest likelihood of chlorination occurring at the third carbon (Yuan et al., 2020).

There is also a lack of specification on some commercial CP standards. Many complex CP mixture standards have levels of chlorination that are too low for some mass spectrometric ionisation techniques such as NCI/ECNI, even if the overall %Cl of the standard appears to be reasonable. This is related to both, the manner in which instruments respond as well as to the differences in %Cl based on different CP chain lengths and is seen most acutely for LCCP standards as discussed in the next section.

3. Discussion

The ability to provide a reliable quantitative estimate for CP content in environmental or biota samples is influenced by a number of factors such as the analytical standards used, the measurement technique and the quantitation method. Extraction and purification procedures for biotic samples at least, are seen as less of an issue, as most of the matrix is usually acid hydrolysed, followed by chromatographic purification to exclude other interferants (van Mourik et al., 2020; Kratschmer et al., ¨ 2021A, 2021B; Mezi´ `ere et al., 2021A, 2021B). Many of the conundrums in the determination of CPs relate to the limitations of currently available standards and are discussed in terms of:

- the variability in the results of inter-laboratory exercises (using complex mixtures and single chain standards),
- the purity of existing CP standards,
- occurrence data on homologue group totals (where further work is impacted by the lack of homologue standards),
- the variability in instrument response for single congener standards (using different measurement techniques),

Standard	Expiry date Range	n	&Chain length identified	% of total response by CP groups		
				SCCPs	MCCPs	LCCPs
SCCP 51.5 % Cl	2013-2018	4	C_{10} - C_{16}	98-99%	1.2-1.8%	n.a.
SCCP 55.5 % Cl	2008-2022	8	C_{10} - C_{17}	95-97%	2.9-4.8%	n.a.
SCCP 63 % Cl	2013-2022	3	C_{10} - C_{16}	99-100%	0.3-0.6%	n.a.
MCCP 42 % Cl	2013-2020	3	C_{12}, C_{14} - C_{16}	0.2-0.4%	99-100%	n.a.
MCCP 52 % Cl	2008-2021	7	C_{11} - C_{17}	0.9-10%	89-99%	n.a.
MCCP 57 % Cl	2013-2019	3	C_{13} - C_{17}	0.5-0.6%	99-100%	n.a.
LCCP 36 % Cl	2006-2018	4	C_{11} - C_{12}, C_{14} - C_{20}	6.6-7.5%	25-37%	55-75%
LCCP 49 % Cl	2003-2019	5	C_{11} - C_{12}, C_{14} - C_{20}	2.6-2.8%	46-53%	47-52%

n.a. = not analysed, n = number of batches analysed, & identified in the standard

All standards originally purchased from Dr. Ehrenstorfer, Augsburg, Germany.

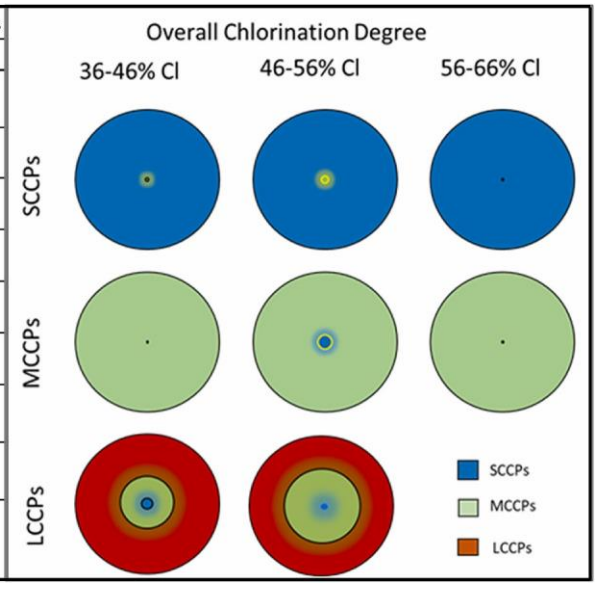


Fig. 2. Indication of the level of impurity (as homologue groups from other mixtures) in complex mixture standards, as seen by the relative response during measurement of commercially available standards.

- the presence of potentially interfering by-products such as chlorinated olefins (in complex mixtures) which could affect the determination.

As mentioned earlier, the variation in quantitative CP estimates provided by different laboratories for the same test samples can be seen in the results of inter-laboratory comparisons where analytical standards were provided or specified as an aid to reduce variability.

3.1. Complex and single-chain CP mixtures

These inter-laboratory studies indicated that while the use of mixed standards (e.g. SCCP or MCCP) provides better results with a fortified test material, the uncertainties increase substantially when real food samples (naturally contaminated) are used as the test material (Fig. 1). This is an expected outcome as the profile of the fortified sample is likely to provide a better match to the standard. However, some comparative outcomes (e.g. interlaboratory exercise 2019, Fig. 1) also indicate that simply providing a pre-designed CP mixture as a standard, to the participants, does not improve quantitation results, as most commonly used quantitation strategies have different requirements for their standards (Fig. S2, Supplementary information).

For example, the spectral deconvolution approach (Bogdal et al., 2015) relies on the ability to match the obtained sample profile to a variety of standards or technical mixtures. A single CP standard is thus unlikely to provide adequate matching. Similarly, linear calibration aligned to the chlorination degree of the sample (Reth et al., 2006) requires calibration standards with different overall chlorination degrees. Other methods (Tomy et al., 1999; Yuan et al., 2017) also often rely on a range of standards for determination of response factors and quantitation. The studies however, do show that increasing the availability of a broad range of single chain standards and congener standards are likely to improve quantitation for many of these methods (Yuan et al., 2017; Schinkel et al., 2018A; Hanari and Nakano, 2020).

In reality, the perfect matching of a standard or a mix of standards to the CP profile in a biotic or even a weathered abiotic medium is not possible. This is because CP residues in real foods/animal tissues/sediments/soils etc. are modified (due to changes during utilisation, environmental degradation, metabolism, etc.) integrals of the original CP mixtures that were produced and used (Perkons et al., 2019; Fernandes et al., 2020). But homologue groups that are dominant in real samples could at least partly be matched to the most appropriate standard compositions. With the comparability of CP homologue patterns steadily increasing (Mezi´ `ere et al., 2020B), focus is likely to shift towards chain length specific concentrations or even quantitation of specific congener groups as a longer term objective. Such a development is however only possible if appropriate standards are available with adequate purity.

Lower resolution detection methods (non-MS or MS techniques that use unit resolution) are particularly compromised by lower levels of purity of the quantitation standards, as the objective here is to provide a total CP estimate and/or additionally, indicate dominant CP groups by comparison with quantitation standards (van Mourik et al., 2018; Kratschmer and Schachele, 2019B¨). Although commercially available complex CP mixtures showed only minor impurities for SCCP and MCCP standards, markedly higher impurities were evident in the two available types of LCCP standards (Fig. 2). The high proportion of MCCPs (in particular) would make it impossible to discern the LCCP portion, based on the overall response of these standards, thus making them inadequate for such quantitation methods. Even when used with high resolution instruments, the high proportion of MCCPs (or other CPs) impedes metrological traceability of the standard concentration as a sum of LCCPs.

The other issue with CP mixture standards is the differences in composition that arise from the percentage of chlorine in the mixture, often expressed as %Cl. Unlike other polyhalogenated classes of contaminants such as PCBs which share a single structure i.e. biphenyl that is chlorinated to different extents, CPs have a range of progressively varying structures i.e. alkanes of different chain lengths. During the commercial process of chlorination, this difference affects the extent to which each chain length is chlorinated and therefore has a strong impact on %Cl of the resulting mixture. For instance, the shortest SCCP chain length (C_{10}) and the longest MCCP chain length (C_{17}) differ by almost 2-fold in mass. Hence, at a given %Cl, the mean degree of chlorination of these two chain length is strikingly different. E.g. hexachlorodecanes ($C_{10}Cl_6$-CPs) correspond to a %Cl of 61 whereas $C_{17}Cl_6$-CPs would correspond to a %Cl of 48 (Fig. 3). This difference has implications for the commercial applications of CPs. For instance, the suitability of CP mixtures in plasticizer applications decreases as %Cl increases, with an optimum range of 40–50%, which on average would correspond to tetra- and penta-chlorinated CPs for this application (Fig. 3).

The difference also has considerable implications during analysis because the response factors of most MS detectors – and especially GC- ECNI-MS and LC-ESI-MS are directly affected by the level of chlorination on each molecule. On most currently used instrumentation, MS response is only seen for molecules with at least 4–5 Cl atoms (van Mourik et al., 2015). Thus, di- and trichlorinated CPs, which are likely present in technical CP products with low %Cl, are usually not considered by calibration methods. Therefore, quantitation methods should not only consider %Cl but also the (mean) chain length of CPs to be expected in samples. Several of these requirements are currently not covered by commercially available SCCP and MCCP standards. This lack of standards becomes even more acute for LCCPs. As an example, for a mean chain length of C_{30}, 62 %Cl corresponds to an average of 12.6 Cl substituents per molecule. Technical LCCPs are often available as 70% Cl (Li et al., 2018; Sprengel and Vetter, 2020), which corresponds to a mean chain length of C_{25}, with an average of 22 Cl substituents. Thus for LCCPs, a considerably larger number of reference standards than are currently available, will be required for calibration.

Despite being reduced to a single carbon number, single chain length CP standards are still complex mixtures of many thousands of theoretically possible congeners. This results in an inherent variation in the composition of the homologue group during the synthesis of these by different laboratories (commercial or research). The variation may arise

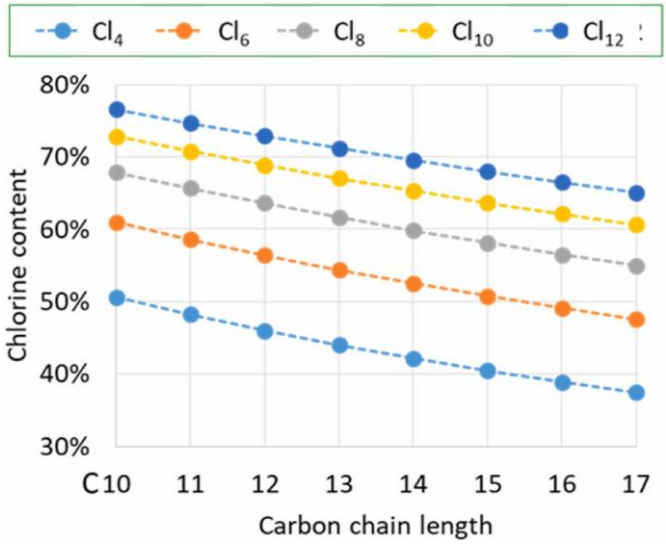


Fig. 3. The effect of increasing chlorine content during synthesis on the degree of chlorination of CPs at different carbon chain lengths.

from subtle changes in the conditions of synthesis, the purity of the alkane feedstock and the thermodynamic conditions (e.g. temperature, pressure) that prevail during synthesis. An example of this variation may be seen in Fig. 4 which shows the differences in response at different chlorine numbers for C_{10} to C_{13} single chain length standards that were obtained from two different sources (analysed simultaneously by GC- ECNI-HRMS at 60,000 resolution). This variation is an indication of the complex mixing that is to be expected in abiotic and biotic matrices – the results of the subtle (or larger) differences in manufacturing conditions, by the numerous CP producers worldwide.

3.2. Homologue group standards

As mentioned earlier, the increasing comparability of homologue patterns may see a shift in focus from the measurement of group totals (e.g. SCCPs) to the separation and quantitation of homologue groups. In addition to the potential differences between the compositions of these groups that arises during synthesis, conditions during measurement also affect the determination (Mezi´ `ere et al., 2020B). This is illustrated by Fig. 5 which shows overlapping of mass fragment signals for the Cl_8 homologue series from C_{10}–C_{13}, when GC-ECNI is used (Fig. 5A), or from charge competition during adduct formation during LC-ESI measurements (Fig. 5B). Recently, Matsukami et al. (2020) reported a separation of SCCPs on a cyanopropylsilane LC stationary phase that greatly sharpened the peak shape. This improved separation by LC (Fig. 5C), would help the analysis of CPs by minimising homologue interferences as well as increasing apex intensities which in turn, improves the method sensitivity. In addition, the peak shapes appeared Gaussian, unlike with GC. This feature provides additional advantages, e.g. the more regular peak shape facilitates automation of the data processing, which proves useful considering the high number of homologue groups that require measurement. Also, while the CP peak shapes obtained with GC or C18-type stationary LC phases are barely detected by conventional tools because of peak broadening and must be extracted by mass-to-charge ratio signals (increasing the potential of integrating noise), current deconvolution tools (e.g. xcms or MZmine 2 - Pluskal et al., 2010) work more effectively with Gaussian shaped peaks (Myers et al., 2017). This type of analysis would benefit from a range of homologue group standards. In order to direct the effort of CP analysis in food within the European network of national reference laboratories, a database that collates the homologue-specific occurrence of CPs in different food and human tissues as identified by different network laboratories is currently being compiled.

indication of the unknown composition of weathered or metabolised CP profiles. This information would be key to the understanding of which CPs humans and animal were exposed to and would also allow more targeted toxicological studies. Some of the limitations of currently available individual CPs (see Table 1 and SI) arise from the disposition of the chlorine atoms and the manner in which these structures respond during measurement by mass spectrometric methods. The variability of response to these individual standards is related to the MS ionisation conditions coupled with chlorine number and chemical configuration, and results in poor ion yields for specific types of substitution patterns. Both GC- and LC-based MS systems are affected by these conditions. So, for example, of the investigated compounds (Cl_4 to Cl_8), hexa- chlorinated CPs provided the most intense responses when either GC-ECNI-Orbitrap-MS, LC-APCI-TOF-MS or LC-ESI-Orbitrap-MS was used (MS resolution typically from 70,000 to 120,000). On the other hand, for the Orbitrap-based HRMS measurements, most octa-chlorinated CPs gave a good response in GC-ECNI mode, but when ionised by ESI using the LC platform, the only response observed was for the octachloro- tetradecane ($C_{14}H_{22}Cl_8$) configuration with adjacent chlorines (Fig. 6).

This lack of signal may arise from the chlorine adduct formation mechanism during ESI. These are preferably formed by binding to a positively charged carbon that is adjacent to two carbons substituted

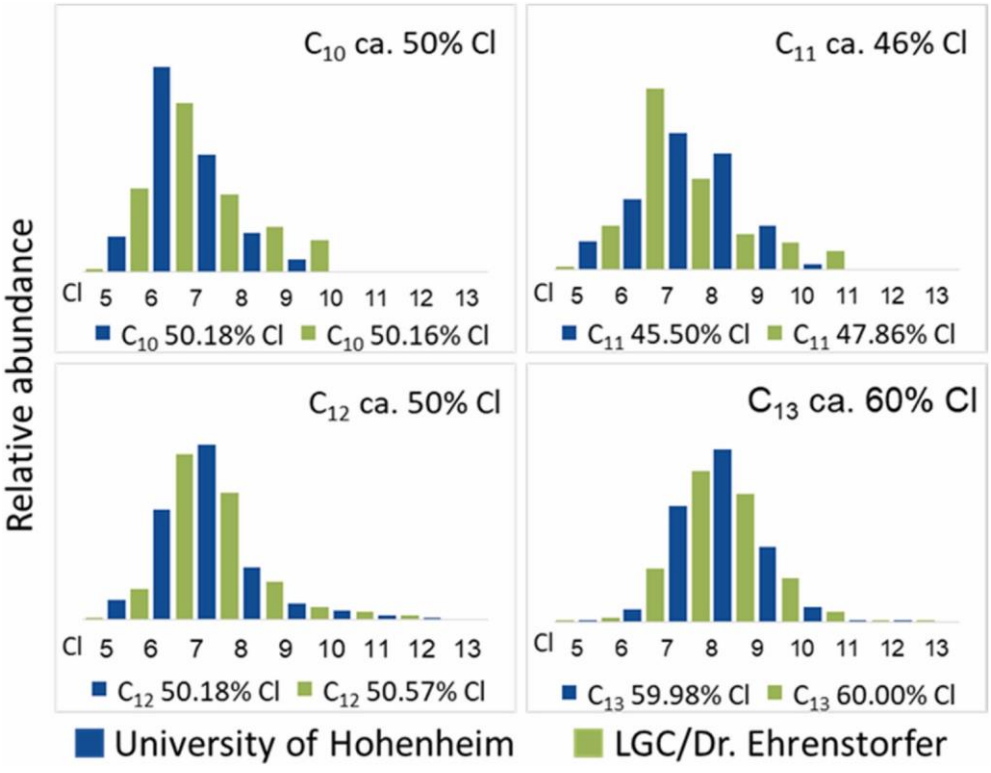


Fig. 4. Compositional differences observed in single chain CP standards (measured simultaneously) obtained from two different sources.

3.3. Individual compound CP standards

Standards of individual CP compounds represent perhaps the most interesting tools for future research as they could be used to provide an

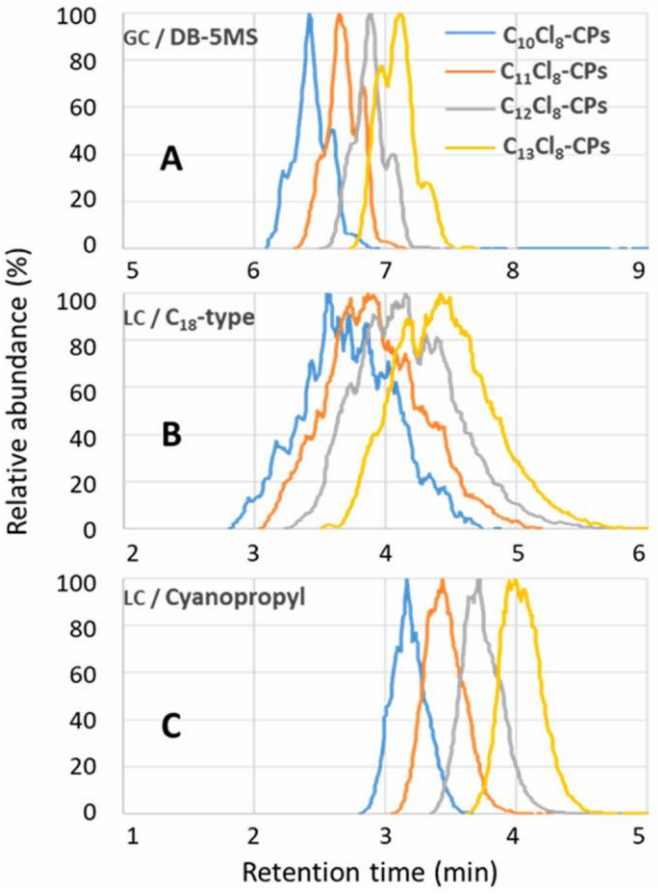


Fig. 5. Chromatographic overlap of C_{10}–C_{13} homologue groups as seen for A: GC-based separation, B: conventional LC-C18 phase-type separation and C: LC- cyanopropylsilane phase separation which shows a more deconvolution- recognisable Gaussian shape.

with electronegative chlorine atoms. This, together with the lack of steric hindrance from other proximate chlorine atoms promotes the formation of more responsive adducts. In general, this configuration appears to lead to preferable adduct formation in chlorine enhanced LC- ESI-MS, since CPs with a number of adjacent chlorine substitutions along the chain gave the best response. On the other hand, configurations with multiple chlorine substitutions on the terminal carbons (or geminal chlorines on secondary carbons) are likely to suffer a greater degree of steric hindrance, which is further exacerbated by the presence of proximate adjacent chlorines. These standards show poor, or in some cases, no response, making them unsuitable for chlorine enhanced LC-ESI-MS. Increasing the supply of chlorine in the ion source by using mobile phase modifiers such as ammonium chloride and dichloromethane did not appear to improve the response. The number of chlorines per CP molecule also influences the intensity of response and, as expected, tetra-chlorinated CPs measured by this technique showed very low response due to the low chlorination degree.

It would be helpful to know the frequency of occurrence of these multiple chlorinated (geminal chlorines) carbons and carbon chains in commercial mixtures and ultimately in real samples, and to this end, the use of nuclear magnetic resonance (NMR) analysis may provide some useful indication. This analysis is based on the strong deshielding effect (on the nuclei) that is exerted by electron withdrawing Cl substituents (instead of protons) which moves signals of the affected carbons downfield to higher ppm values in the NMR spectra. Although seen most strongly on carbons with geminal chlorines, the effect is also seen on vicinal carbons and their protons although it reduces with distance. The resulting NMR spectra (both ^{1}H and ^{13}C) for CPs can be characterised by peaks in the “chlorine-bearing” and “non-chlorinated” ranges (Gusev et al., 1968; Panzel and Ballschmiter, 1974). The information that is present, but not resolvable in these conventional NMR spectra of CP mixtures, can be substantially visualised through two dimensional heteronuclear spectral quantum coherence (HSQC, ^{1}H–^{13}C) experiments (Sprengel et al., 2019; Yuan et al., 2020; van Mourik et al., 2021) which provide information on the correlations between protons and the carbons to which they are attached. In these spectra, the wider dispersion of signals in the two dimensions of the HSQC plots helps to define several structural elements such as the extent of chlorination (Sprengel et al., 2019; Yuan et al., 2020). This can be seen in the analysis of C_{14}-CP single chain standards of different Cl% (Fig. 7). At 37 %Cl, the cluster around 4 ppm that corresponds to chlorination of terminal carbons is just visible (Fig. 7A, cluster VII), but steadily increases with increasing Cl% (Fig. 7B–D). Increasing %Cl to 45% leads mainly to the more intense clusters, V, VI and VII (Fig. 7A and B), which are further enhanced at 53 Cl% (Fig. 7C) along with a downfield shift of clusters III and V. At this chlorination degree, each carbon bears on average 0.44 Cl. In the last frame which corresponds to 67 Cl% (0.78 Cl/carbon), clusters IX and X can be seen which indicate the presence of geminal Cl on secondary (-CCl_2-) carbons but also on terminal primary carbons ($CHCl_2$-) (Fig. 7D). Chemically, transformation is favoured at positions with geminal chlorine atoms, as seen e.g. in toxaphene, a class of compounds that is structurally related to CPs (Vetter and Oehme, 2000). Hence, in the case of MCCPs, technical products with 52 %Cl and less, may be more stable than technical MCCPs with 70% Cl, although in general, biodegradability usually decreases with high levels of chlorination. In real samples, the results of weathering and metabolism could potentially enhance this effect, but unfortunately the HSQC technique was not (currently) sensitive enough to be applied to contaminated environmental or biota samples.

In terms of purity, the individual CP congener standards were specified at between 63 and 99% pure, and on an absolute basis, this was more or less confirmed by both, GC- and LC-based MS techniques. However, the nature of the impurities led to different results between Orbitrap measurements using both, LC-ESI and GC-ECNI ionisation, for almost half (14 out of 31) of these standards. In seven cases, a penta- chlorinated CP impurity was detected by LC-ESI-Orbitrap, but GC- ECNI-Orbitrap measurements showed a hexa-chlorinated CP instead and this was observed for all the available chain lengths. Similarly, in two cases, both for tetra-chlorinated configurational standards, the GC- ECNI-MS technique detected a hepta-chlorinated impurity rather than a penta-chlorinated CP impurity that was detected when measured by LC- ESI-MS. It would appear, based on the limited number of standards and the experiences using these two techniques that during ECNI, the chlorination degree and position of the chlorine seems to be the determinant whereas during chlorine enhanced LC-ESI-MS, only the positioning of the chlorine appears to determine the efficacy of ionisation.

3.4. Other influences on CP quantitation outcomes

Apart from the limitations of existing standards and the variable responses of some ionisation techniques, other factors may influence the quantitative assessment of CP content. Analytical issues such as the signal baseline during measurement, particularly when levels are near the limit of quantitation, and the quantitative approach itself, are clearly important factors, but these are a matter of judicious choice and within the control of the analyst. Other factors arise from more mundane considerations, such as the purity of the paraffin feedstock or the chlorination conditions, which can give rise to other, closely related impurities during production – namely chlorinated olefins. These have been reported in technical mixtures (Schinkel et al., 2018B) but were also detected during the laboratory synthesis (Sprengel and Vetter, 2019; Heeb et al., 2020) of CPs as seen in Fig. 8. Three C_{14}-CP standards, synthesised through a sulfuryl chloride-mediated process (Sprengel and Vetter, 2019; Heeb et al., 2020) were purified by LC and measured by high-resolution MS (R > 20,000) followed by mathematical deconvolution (Schinkel et al., 2018A), to reveal varying proportions of chlorinated olefins (P_{CO}, in Fig. 8). In each of the synthesised standards, olefin proportions decreased from lower- to higher-chlorinated homologues, indicating that higher-chlorinated paraffins were possible precursors for lower-chlorinated olefins. The formation of chlorinated olefins has also been reported during abiotic and biotic transformations or during some MS ionisation processes (Schinkel et al., 2018A, 2018B, 2018C; Heeb et al., 2019; Knobloch et al., 2021), and their presence adds a further layer of complexity to CP analysis. However, as most CP occurrence in food and animal tissue originates through environmental contamination, mechanisms such as photo-degradation in the atmospheric phase and microbial and enzymatic action in soil and sedimentary phases are likely to reduce or remove these unsaturated compounds. Additionally, most CP analysis of food and animal tissue uses sulphuric acid treatment to hydrolyse the co-extracted lipid material, and this process may also help to degrade the unsaturated bonding in olefins.

Notwithstanding the difficulties described in the above discussions which may arise through limitations of the standards, limitations in ionisation during mass spectrometric measurement, or the presence of interferants, a more reliable set of targeted standards would help quantitation, contribute to a reduction in the discrepancies seen in reports/PTs and ultimately enable a better estimate of CP distribution and occurrence. An improved set of standards would also help in the establishment of quality targets for CP analytical methods, such as detection limits, precision and accuracy of measurement. Such a set of quality targets is currently being compiled by the working group on CPs within the European network of national reference laboratories.

3.5. Types of standards that would aid the progress of current and future studies

The volume of data and information on occurrence of CPs in food, human tissues and the environment is currently small, but continues to grow, as does the level of characterisation of this occurrence. It is therefore difficult to be specific about the range of CP standards that would be immediately useful, but there are increasing indications from the more recent literature and from the discussions above. The following suggestions would help to progress some of the existing and future CP studies, mostly through facilitating better quantitation and characterisation of observed contamination:

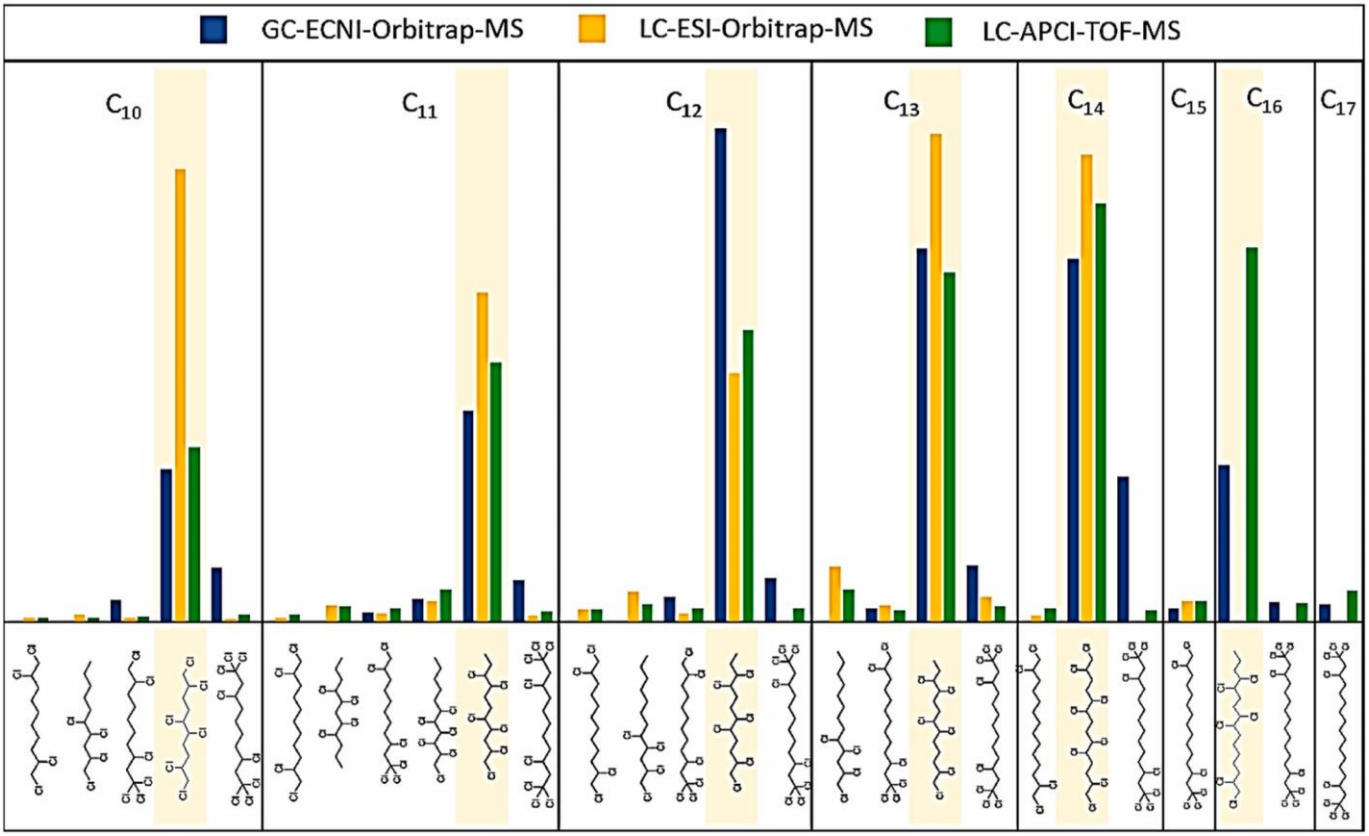


Fig. 6. Configurationally defined CP congeners measured on three types of MS-based instrumentation. Generally better response is observed for CPs with chlorine distribution along the chain length (rather than terminal) while multiple terminal Cl-substitution and low chlorine number are associated with poorer response.

- Better quality of mixed standards for SCCPs, MCCPs and LCCPs, i.e. free from impurities (or with very low, <1%, levels). These standards require analytical specification such as the purity, the range of chain lengths to be expected, any known impurities, etc. Mixed standards would be universally useful for CP studies with both low and high resolution MS methods.
- Single chain standards across the various chain lengths, including a fuller range of C_{10}–C_{13} single chain standards to complement the existing range - this would help characterise the current occurrences in food, human tissues and environmental compartments. • Similarly, a fuller range of C_{14}–

C_{17} single chain standards, chlorination range <40 %Cl to >70 %Cl, for each single chain standard set

- A selection of single chain standards for LCCPs (none are available at the moment), with the same chlorination range as SCCPs and MCCPs
- Additional labelled CP congeners for use as internal standards which ideally would provide a response, both, in chlorine enhanced LC-ESI- MS or LC-APCI/APPI-MS systems as well as GC-ECNI-MS and GC-EI- MS with at least two (most abundant) ions distinguishable from their native counterparts. Standards covering the full range of SCCPs, MCCPs, and LCCPs have been proposed earlier (Schinkel et al., 2018C) – $^{13}C_{12}$-hexachlorododecane, $^{13}C_{12}$-decachlorod odecane, $^{13}C_{16}$-hexachlorohexadecane, $^{13}C_{16}$-decachlorohexadecane, $^{13}C_{20}$-hexachloroeicosane, $^{13}C_{20}$-decachloroeicosane, $^{13}C_{26}$-hexachlorohexacosane, $^{13}C_{26}$-decachlorohexacosane and $^{13}C_{26}$-eicosachlorohexacosane. However, following the observations on occurrences in food and human tissues labelled C_{11}, C_{13}, C_{14} and C_{18} congeners with $Cl_{4\text{-}8}$ substitution may be more useful for monitoring studies. Terminal chlorination is not desirable so that a more universal instrument response is seen.
- In the absence of individual congener information, data on homologue group occurrence in foods (and in environmental samples) would yield a higher level of characterisation and allow more targeted toxicological and risk assessment studies. Early indications from the database on homologue-specific occurrence, based on a limited number (n > 200) of collated data from different sources, suggest that CP occurrence in different foods covers the range of SCCP, MCCP and LCCP chain lengths between C_9–C_{20}, with greater frequency for C_{11}–C_{14} and C_{18}. For most foods studied thus far, and human blood, chain lengths with Cl_5–Cl_8 substitutions appear to be the most predominant homologue groups of the overall occurrences.
- A range of individual CP congeners is currently available but as discussed, the terminal chlorine-rich configurations show poor response on some instruments and perhaps more importantly, may not reflect the resulting environmental and biotic distribution. Chlorine distribution along the chain length may be a more representative feature and would help in future research studies.

4. Conclusion

The availability of new standards will not in itself be a panacea for accurate determination of CPs, but it will aid the ongoing efforts to reduce the different layers of complexity inherent in the analysis of CPs. A more extensive range of single chain standards as well as homologue group standards would allow a higher degree of matching to the profiles seen in environmental and food/other biota samples – a necessary step to better and more consistent quantitation and characterisation of the CP content, and the continuing work on a database of profiles in different types of food. These standards would be most useful if they were reflective of the bulk of CPs produced, both in terms of %Cl as well as the commercial characterisation of mixtures as SCCPs, MCCPs and LCCPs. Given the inability to separate individual CPs, the use of these standards is currently more directed towards research that may provide an indication of the unknown composition of environmental and food samples. An understanding of these profiles in food, human and animal tissues would help to qualitatively characterise human exposure and allow more targeted toxicological studies. The variability in MS response observed, both from GC and LC based instruments appears to be related to configurational differences but it does raise the possibility that CPs with some configurations may not be captured during quantitation due to poor response. This could result in underreporting of concentrations in those samples with low CP content. This effect is also likely to be exacerbated as many quantitation methods do not consider, or are unable to detect the lower chlorinated (di- and tri-chloro) CPs. For low (unit) resolution MS applications, mixed CP standards with low levels of impurities (other CP mixtures) would allow better quantitation. These

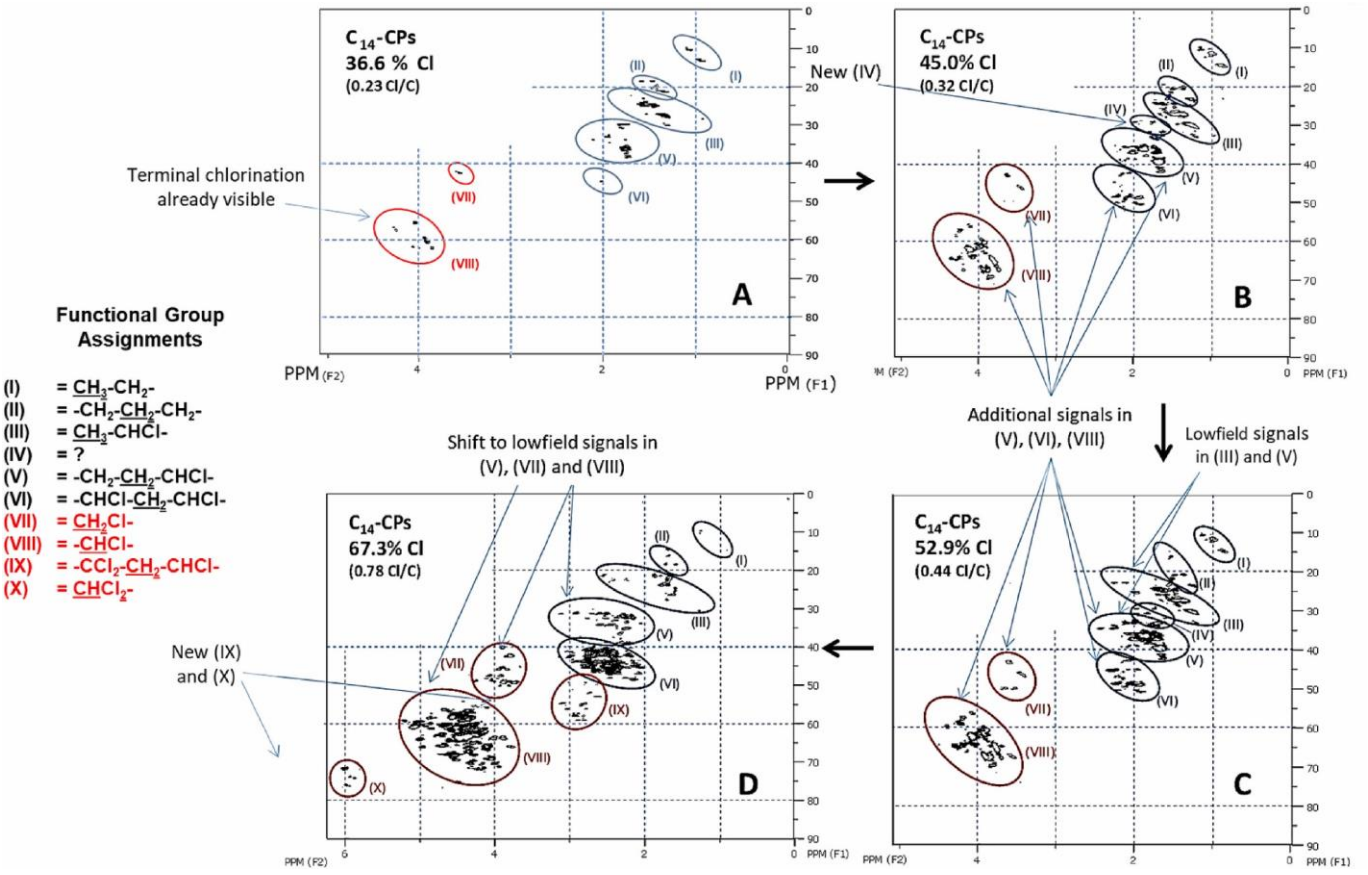
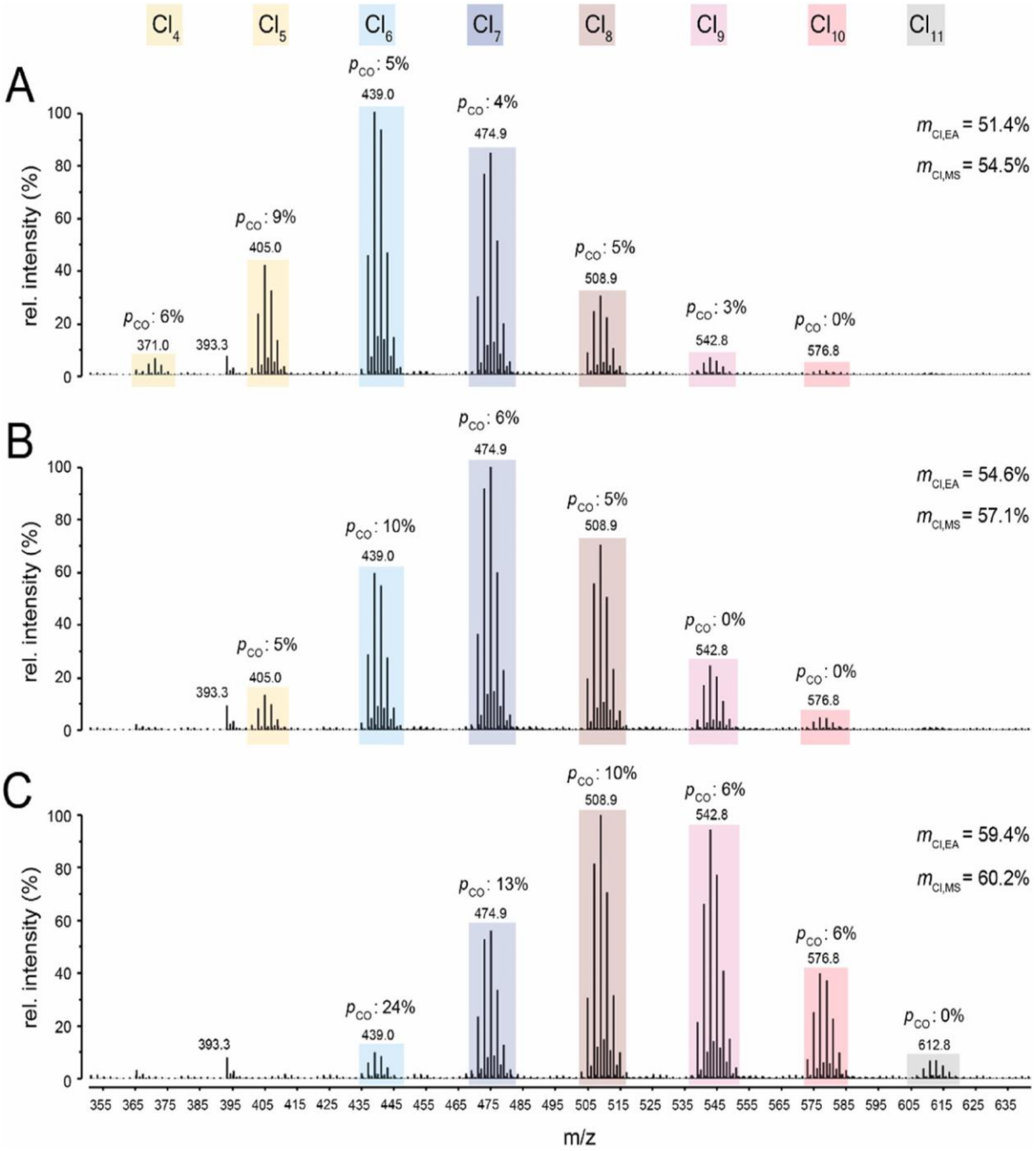


Fig. 7. Two dimensional heteronuclear (^1H, ^{13}C) single quantum coherence (HSQC) NMR spectra of C_{14} single chain CP standards showing the increased prevalence of geminal- and terminal-carbon chlorines with increasing levels of chlorination in the standards.

Fig. 8. Mass spectra of three laboratory synthesised C_{14}-CP standards showing the proportion of chlorinated olefins (P_{CO}) formed. $M_{Cl,EA}$ and $M_{Cl,MS}$ are the chlorine



contents as determined by elemental analysis and MS respectively.

are still fairly important as the majority of laboratories worldwide do not have access to the higher (>10,000) resolution MS instruments that are required for e.g. homologue specific, or single compound research. Additionally, almost all of the current toxicological insights on CPs are based on the use of mixed standards.

At a practical level, there would be multiple beneficiaries of an extended range of CP standards such as those suggested here. The field of CP analysis has grown rapidly in recent years, particularly with respect to estimation of environmental levels in different compartments and more recently in foods and human tissues. Establishing baseline levels in different matrices would allow assessment of products or media with elevated levels. However, other researchers working on structural elucidation, environmental fate and of course toxicologists would also benefit from individual compound standards. Indirectly, the better quality of the resulting data would allow or refine risk assessments and help to facilitate control strategies for CP dispersion to the environment and eventually to food and humans.

Credit author listing

Alwyn Fernandes: Conceptualisation, Writing – original draft, Review and Editing, Supervision. Walter Vetter: Conceptualisation, Visualisation, Writing – original draft. Caroline Dirks: Investigation, Resources, Writing – original draft. Louise van Mourik: Investigation, Resources. Ronan Cariou: Visualisation, Writing – original draft. Jannik Sprengel: Investigation, Visualisation, Writing – original draft Norbert Heeb: Investigation, Visualisation, Writing – original draft. Anouk Lentjes: Investigation, Resources. Kerstin Kratschmer¨ : Investigation,
Data curation, Project administration, Writing – original draft

Declaration of competing interest

The authors assert that they have no conflict of interest (financial or non-financial) in the subject matter discussed in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.131878.

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