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Thomas J. McGrath[a], Franck Limonier[b], Giulia Poma[a], Jasper Bombeke[a], Raf Winand[c], Kevin Vanneste[c], Mirjana Andjelkovic[b], Els Van Hoeck[b], Laure Joly[b], Adrian Covaci[a,*]

[a]Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium

[b]Chemical and Physical Health Risks Department, Sciensano, Rue Juliette Wytsman 14, 1050, Ixelles, Belgium

[c]Transversal activities in Applied Genomics, Sciensano, Rue Juliette Wytsman 14, 1050, Ixelles, Belgium

*corresponding author

Abstract

This study reports on concentrations of short- and medium-chain chlorinated paraffins (SCCPs and MCCPs, respectively) in a wide range of food samples (n=211) purchased in Belgium during 2020. Samples were analysed by gas chromatography-mass spectrometry (GC-MS) and quantified using chlorine content calibration. ∑SCCPs were present above LOQ in 25% of samples with an overall range of <LOQ to 58 ng/g wet weight (ww), while ∑MCCPs were identified in 66% of samples ranging from <LOQ to 250 ng/g ww. ∑MCCP concentrations were greater than those of ∑SCCPs in all 48 samples in which both groups were detected with an average ∑MCCP/∑SCCP ratio of 5.8 (ranging from 1.3 to 81). In general, the greatest CP concentrations were observed in foods classified as animal and vegetable fats and oils and sugar and confectionary for both SCCPs and MCCPs. Significant correlations between lipid content in food samples and CP levels illustrated the role of lipids in accumulating CPs within foodstuffs, while industrial processing, food packaging and environmental conditions are each likely to contribute to overall CP loads. Selected samples (n=20) were further analysed by liquid chromatography-high resolution MS (LC-HRMS) to investigate homologue profiles and the occurrence of long-chain CPs (LCCPs). LCCPs were detected in 35% of the 20 subset samples while the HRMS results for SCCPs and MCCPs matched closely with those obtained by GC-MS. This study reveals the widespread occurrence of SCCPs and MCCPs in Belgian food and indicates that LCCPs may represent a substantial contribution to overall CP levels in foodstuffs.

1 Introduction

Chlorinated paraffins (CPs) are chlorinated linear chain alkanes with the general formula $C_xH_{(2x+2-y)}Cl_y$. They are typically classified by carbon chain length into three groups, short-chain CPs – SCCPs (C_{10-13}), medium-chain CPs – MCCPs (C_{14-17}) and long-chain CPs – LCCPs ($C_{>17}$), and by degree of chlorination varying between 30% and 70% by weight. CPs have been produced since the 1930s for a wide range of applications, including metalworking fluids, lubricants and additives, and consumer products (van Mourik *et al.*, 2016). Information on production, import and use of CPs is still limited, especially on a group level. Based on available data and reported emission factors, the global production of total CPs in 2016 was estimated to exceed 1 million t/year while production of SCCPs was 165,000 t/year (Glüge *et al.*, 2016).

As a consequence of their high production volumes, application rates and unintentional release, CPs have been detected worldwide in environmental media (including air, water, sediment and soil, biota) (van Mourik et al., 2016) and humans (serum and milk) (Li *et al.*, 2017; Hu *et al.*, 2021). Due to their environmental persistence, long-range transport, bioaccumulative and toxic properties (Geng *et al.*, 2019; Ren *et al.*, 2019), SCCPs were globally regulated and classified in 2017 as persistent organic pollutants (POPs) by the United Nations Environment Stockholm Convention (UNEP, 2017). Such legal restrictions have thus opened the way to increasing application of MCCPs and LCCPs (Glüge *et al.*, 2018).

Human exposure pathways to CPs primarily include food intake and inhalation of indoor air (Li *et al.*, 2020), but also dermal absorption and dust ingestion (Fridén *et al.*, 2011; Gao *et al.*, 2018). Although toxicological studies are still scarce, long-term exposure to SCCPs has been associated with potential adverse effects on human and animal health, including carcinogenicity and neurotoxicity (Bucher *et al.*, 1987; Yang *et al.*, 2021), sub-chronic nephro- and hepatotoxicity (Geng *et al.*, 2019), and impairments of metabolism (Gong *et al.*, 2019). SCCPs are also considered as thyroid-disrupting chemicals (Zhang *et al.*, 2016; Gong *et al.*, 2018).

Foodstuffs in particular can be contaminated with CPs via bioaccumulation in the environment (Huang *et al.*, 2018; Dong *et al.*, 2021), food processing equipment (Yuan *et al.*, 2017b) and migration from food contact materials (FCM) (Wang *et al.*, 2019a). A recent opinion released by the European Food Safety Authority (EFSA) pointed out the lack of data on occurrence and toxicity of CPs in food, feed and domestic animals (EFSA CONTAM Panel *et al.*, 2020). In addition, due to their similar physico-chemical properties and toxicity profiles (Ren *et al.*, 2019), simultaneous exposure to SCCPs and MCCPs may increase the risk of adverse effects in humans through dietary intake (Huang et al., 2018). For these reasons, broad-ranging dietary studies are required to fully evaluate the contamination status of CPs in food (McGrath *et al.*, 2021a).

To date, one of the biggest obstacles hampering the estimation of consumers' exposure to CPs through food is represented by the unprecedented analytical challenges due to the high number of possible congeners. Commercial CP formulations consist in fact of very complex mixtures of thousands of isomers with different carbon chain lengths and chlorination degrees (van Mourik et al., 2015). The most common approach to CP analysis globally has been gas chromatography coupled to quadrupole mass spectrometry (GC-MS) using electron capture negative ionization (ECNI). Although full separation of CP congener groups with defined $C_xH_{(2x+2-y)}Cl_y$ molecular formula, also referred to as homologues, cannot be achieved and analysis is generally limited to chain lengths of $C_{<18}$, this technique provides ∑SCCP and ∑MCCP concentrations via chlorine-content calibration (Reth *et al.*, 2005), as well as tentative congener group abundance data (Tomy *et al.*, 1997). Recent advances have applied liquid chromatography (LC) with high resolution MS (LC-HRMS) using chlorine enhanced soft ionisation to achieve simultaneous analysis of SCCPs, MCCPs and LCCPs. Indeed, a MS resolution of >50,000 is required to resolve individual homologues (Yuan *et al.*, 2019) in order to investigate

congener group distribution without interferences between groups. However, even with the application of advanced technologies, a lack of available single compound analytical standards prevents true congener group specific quantification.

To the best of the authors' knowledge, there are no studies concerning the presence of SCCPs and MCCPs in food in Belgium and only limited data for these compounds are available at the European level. The present study intends to respond to the recommendations of EFSA for more data on the occurrence of CPs in food by assessing the levels and patterns of SCCPs and MCCPs in over 200 foods belonging to multiple food categories representative of the Belgian diet. Congener group distribution and the contribution of LCCPs to total CP loads was also investigated to provide a comprehensive assessment of the CP contamination status of Belgian foodstuffs.

2 Materials and methods

2.1 Sampling strategy

A total of 211 food samples were purchased from Belgian retailers in 2020 to represent the consumption habits of the local population. Food sample priorities were determined according to the probability of CP contamination based on previous reports (Iino *et al.*, 2005; COT, 2009; Cao *et al.*, 2015; NFA, 2017; Gao *et al.*, 2018; Huang *et al.*, 2018; Perkons *et al.*, 2019; Krätschmer *et al.*, 2021) and contribution to exposure within the total diet (Bel *et al.*, 2016; Bel & De Ridder, 2018). This was further refined by taking into account the variability of food classification and probability of food coming from international market (import). Products with the highest mean daily consumption were purchased from each of 13 selected categories based on the EFSA FoodEx food classification system (EFSA, 2015) including grains and grain-based products (GRA, n=26), vegetables and vegetable products (VEG, n=18), starchy roots and tubers (STA, n=10), fruit and fruit products (FRU, n=10), meat and meat products (MEA, n=30), fish and other seafood (FIS, n=27), milk and dairy products (MIL, n=25), egg and egg products (EGG, n=10), sugar and confectionary (SUG, n=16), animal and vegetable fats and oils (LIP, n=26), composite dishes (COM, n=5), seasoning, sauces and condiments (SSC, n=5) and food supplements (OTH, n=3) (Table S4). The water content and lipid-fraction of samples were determined gravimetrically according to Xu *et al.* (2015).

2.2 Sample preparation and extraction

Samples were cut into pieces with a knife, lyophilized in covered aluminium trays and then homogenized to a fine powder using a Retsch Grindomix GM 200 mixer (Verder Scientific, GmbH & Co., Aartselaar, Belgium). For 26 samples (including dry cereals, sugars, food supplements and chocolate), freeze drying was not necessary and for 33 samples (oils, butters, chocolate spread and honey) neither freeze-drying nor motorized mixing were required. Samples were extracted for GC-MS analysis as described by the recently validated method published by McGrath *et al.* (2021a) and extracted separately for LC-HRMS analysis with only the internal standard (IS), external standard (ES) and final solvent modified. Briefly, 5 g of sample homogenate (10 g for GRA samples) was spiked with 5 ng of 1,5,5,6,6,10-hexachloro[$^{13}C_{10}$]decane (^{13}C-HCD) IS for GC-MS analysis or 20 ng of γ-1,2,5,6,9,10-hexabromo[$^{13}C_{12}$]cyclododecane (^{13}C-γ-HBCD) for LC-HRMS analysis. Extraction was carried out in 20 mL of a 3:1 mixture of *n*-hexane:dichloromethane (DCM) by 1 min of vortexing and 10 min of ultrasonication. The extraction was repeated with fresh solvents and the supernatants combined.

Extracts were purified using 6 mL of concentrated sulphuric acid (98%) followed by 6 g of acidified silica (44% w/w) and then concentrated to 0.5 mL by evaporation under nitrogen stream. The concentrated extracts were then loaded to Agilent Bond Elut silica cartridges (500 mg) after pre-conditioning with 6 mL of dichloromethane and 6 mL *n*-hexane. Silica cartridges were eluted with 6 mL of *n*-hexane (fraction 1, for disposal) and 12 mL of DCM (fraction 2, for analysis). The analysis fraction was evaporated to near dryness and reconstituted in 100 µL of *iso*-octane containing 5 ng each of ESs ε-hexachlorocyclohexane (ε-HCH) and 6-methoxy-2,3,3',4,4',5'-hexabromodiphenyl ether (6-MeO-BDE-157) for GC-MS analysis. Samples selected for LC-HRMS analysis were reconstituted in 100 µL acetonitrile (ACN) containing 20 ng of d18-β-1,2,5,6,9,10-hexabromocyclododecane (d-β-HBCD) ES. Chemicals and reagents are described in Section S1 of the Supplementary Information and quality assurance and quality controls (QAQC) relating to the extraction procedure are provided in Section S2.1.

2.3 Instrumental analysis and quantification

2.3.1 GC-MS

The GC-MS instrumental acquisition and quantification procedures underwent full validation as described by McGrath *et al.* (2021a). All samples were analysed using an Agilent 7000D GC-MS operated in ECNI mode. A pulsed splitless injection of 2 µL was delivered to a multimode inlet at a temperature of 92˚C (0.04 min hold), which then ramped at 700˚C/min to 300˚C. The instrument was equipped with an Agilent DB5-MS capillary column (15 m, 0.25 mm internal diameter, 0.1 µm film thickness) and the oven temperature programmed to hold at 90˚C (1.25 min), ramp at 25˚C/min to 180˚C and then 10˚C/min to 325˚C (6 min hold). The helium carrier gas flow rate was 1 mL/min for 17.85 min and then increased to 2.5 mL for an overall run time of 25.85 min. The temperature of the MS ion source was 150˚C, the transfer line 300˚C and the quadrupoles each set to 150˚C.

Acquisition was conducted in four separate injections using single ion monitoring (SIM) mode and two m/z values from characteristic $[M\text{-}Cl]^-$ or $[M\text{-}HCl]^-$ monitored for each of 24 SCCPs ($C_{10\text{-}13}$, $Cl_{5\text{-}10}$) and 24 MCCPs ($C_{14\text{-}17}$, $Cl_{5\text{-}10}$) (Table S1). For each CP congener group the m/z of the theoretically most abundant isotope was used for quantification and another isotope from the same ion cluster used for qualitative purposes. Integration of congener groups was performed using Agilent Mass Hunter software by careful comparison of retention time region and quantifier/qualifier ratios in respective CP standards when signal-to-noise ratio exceeded 10. Quantification of ∑SCCPs and ∑MCCPs was achieved using a chlorine-content calibration method modified from Reth *et al.* (2005). Exponential regression curves were established between total response factor and calculated chlorine-content in each of five technical standard mixtures of varying chlorine-content, prepared separately for ∑SCCPs and ∑MCCPs (Section S1). The chlorine-content and relative response measured in sample extracts was then used to determine the concentrations of ∑SCCPs and ∑MCCPs in samples via the calibration regression equations. The instrument response of the 6-MeO-BDE-157 ES was used to derive relative responses for quantification and the responses of ^{13}C-HCD and ε-HCH were used to monitor extraction efficiency and general method performance, respectively. Full details of the calibration and quantification are described by McGrath *et al.* (2021a) and quality assurance and quality control (QA/QC) measures are detailed in Section S2.2.

2.3.2 LC-HRMS

LC-HRMS analysis was conducted using a Vanquish UHPLC+ pumping system coupled to a Q-Exactive Focus Orbitrap MS with electrospray ionization in negative mode (ESI^-) (Thermo Fischer Scientific). 5 µL of each sample was injected onto a reversed-phase C_{18} liquid chromatography (LC) column (Hypersil Gold analytical column, 50 mm x 2.1 mm, 1.9 µm, Thermo Fischer Scientific). The chromatographic separation was performed at a flow rate of 0.4 mL/min with an elution gradient starting with 70% of ACN in water and linearly evolving until 100% ACN in 5 min and maintained during 3 min. The column was re-equilibrated for the next injection during 2 min with 70% of ACN in water. Importantly, a 1:1 mixture of DCM:ACN was added post-column using a T-connection, at a constant flow rate of 0.08 mL/min to ensure the formation of $[M+Cl]^-$ ions in the ESI- source. HRMS data were acquired in full scan mode (m/z 150 to 1900) with a resolution of 70,000 full width at half maximum (FWHM) at m/z 200. No MS/MS fragmentation was performed, and no lock mass was used during the analysis. Optimized acquisition parameters are summarized in Table S2.

Raw LC-HRMS data were first converted to the open mzXML format using the open-source software MSConvert v3.0 (ProteoWizard) and applying the Peak Picking filter. The theoretical centroid m/z values (with resolution R = 70,000 FWHM at m/z 200) and relative abundance of the two most abundant isotopes of each CP homologue (C_nCl_m with $8 \leq n \leq 36$ and $1 \leq m \leq n+2$), as well as the selected IS and ES, were obtained from the enviPat calculation tool, queried using the *isopattern*, *envelope* and *vdetect* functions in R (Loos *et al.*, 2015; Mézière *et al.*, 2020a). Considered ions included $[M+Cl]^-$ ions as adducts of interest, but also possible interfering ions $[M-H]^-$, $[M+NO_3]^-$, $[M+HCOO]^-$ and $[M+C_2H_3O_2]^-$.

All further bioinformatic analyses were performed with in-house developed Python (v3.7) scripts, calling R scripts when needed. For each of the considered theoretical m/z values, extracted ion chromatograms (EIC) and their corresponding intensities were computed in the open-source R software environment v3.6.1 using the *rawEIC* function from the *xcms* package v3.6.0 (Smith *et al.*, 2006; Tautenhahn *et al.*, 2008; Benton *et al.*, 2010) with the following parameters; *peakwidth* (10,70), ppm 10, and *snthresh* 20. Because of the very limited separation of CPs using LC, only minimal retention time filtering was applied for data retrieved after >500 s. The ratios of the observed intensities (*into* value from the *xcms* results) of the most abundant isotope (the quantifier) and the second most abundant isotope (the qualifier) were automatically calculated and compared to their theoretical abundance ratio. To increase the robustness of the calculated intensities, triplicate injections were performed. The intensities were corrected with the fractional abundance of the considered isotope and the IS, and the relative standard deviations (RSD) were calculated. Details of the QA/QC measures applied for LC-HRMS analysis are provided in Section S2.3.

2.4 Statistical analysis

Statistical analyses were performed in Excel 16 and SPSS 26. Statistics were calculated using a value of half LOQ for measurements <LOQ and included concentration data for samples with Cl% above the calibration range as indicated in Table S4 (three ∑SCCP and three ∑MCCP values). One-way ANOVA with a post-hoc Tukey's test was used to compare CP concentrations between food categories with >50% of samples >LOQ and at least 3 measurements >LOQ, which excluded all ∑SCCP data from the tests. ∑MCCP concentrations in the categories which met these criteria were confirmed to approximate log-normal distribution by Shapiro-Wilks tests and were thus log-transformed prior to ANOVA analysis. The ∑MCCP concentration in sample GRA-12 exceeded 1.5 times the overall interquartile range and was excluded from the ANOVA as an outlier. Spearman correlation calculations included all samples, but measurements <LOQ were replaced with zero to

avoid the influence of inherent correlations between respective SCCP and MCCP LOQs. A level of $p<0.05$ was considered statistically significant for all applied tests.

3 Results and discussion

3.1 SCCP concentrations

SCCPs were present above LOQ in 25% of samples with an overall range of <LOQ to 58 ng/g ww (Table 1, Table S4). The highest detection frequency among food categories with more than 3 samples was observed in GRA samples (58%), followed by SUG (44%), LIP (31%) and FIS (30%). Although low detection rates precluded statistical assessment of the differences between categories, SCCPs were most prevalent in the GRA, SUG and LIP categories. The highest ∑SCCP concentration measured, 58 ng/g ww in white, wheat-flour dürüm (sample GRA-12), was approximately an order of magnitude higher than all other measurements in the GRA category and more than twice the levels seen in the next highest sample overall. The ∑SCCP concentrations among GRA samples without the GRA-12 outlier, ranging from <LOQ to 24 ng/g ww, were similar to those reported in grain-based baked goods from Latvia (n=53), ranging from 0.3 to 23 ng/g ww (mean; 6.3 ng/g ww) (Perkons *et al.*, 2019). The Latvian samples comprised similar products to those of the present study, but included also some items with fillings (meat, cheese, etc). Comparable overall concentrations of ∑SCCPs were also observed in grain-based products from Germany (Krätschmer *et al.*, 2021), Sweden (NFA, 2017), the United Kingdom (UK) (COT, 2009) and Japan (Iino *et al.*, 2005), with a maximum of 14 ng/g ww reported among the studies.

CP contamination of food items contained within the GRA category may derive from a wide variety sources. Mechanical mixers have been shown to release CPs from lubricants and internal components during use (Yuan *et al.*, 2017b) and high levels of CPs measured in fat residues in ovens implies that baking may contribute to CP contamination. Potentially contaminated oil-based additives could also constitute a proportion of the CP load in specific samples, such as croissants or waffles (>20% lipids). The combination of these potential sources may account for the higher SCCP detection frequency among GRA samples than other food categories in Belgium. Given that no correlations between lipid content and ∑SCCP concentrations were identified within the GRA category in the present study, it appears likely that the elevated level observed in sample GRA-12 derives from the specific processing techniques used for this item. Indeed, such findings are not unprecedented, with concentrations of ∑SCCPs ranging from 52 to 980 ng/g ww in store-bought cereal products from a wide-ranging study of Chinese foodstuffs indicating that grain-based foods have the capacity to accumulate high levels of CPs (Wang *et al.*, 2019b).

Some of the highest ∑SCCP levels were determined in samples of the LIP category, with overall concentrations ranging from <LOQ to 19 ng/g ww. The most contaminated samples were a peanut and palm oil blend for frying (LIP-16, 19 ng/g ww) and walnut oil (LIP-24, 12 ng/g ww), while SCCPs were not detected above LOQs in any of the four animal-based fat products. Concentrations of ∑SCCP reported in fats and oils were similar in the UK (1.23 and 25 ng/g in butter and cod liver oil, respectively) (COT, 2009), but generally higher in Germany (n=56), where levels ranged from <LOQ to 290 ng/g ww, although SCCPs were only present above LOQs in approximately 20% of samples (Krätschmer *et al.*, 2021). Substantially higher ∑SCCP concentrations have been observed in cooking oils and fats from Korea (310 to 1930 ng/g ww, n=28) (Lee *et al.*, 2020) and China (<9 to 7500 ng/g, n= 49) (Cao *et al.*, 2015), and in palm and marine oil-based dietary supplements purchased in Germany (<LOD to 61,100 ng/g lipid weight, n=25) (Sprengel *et al.*, 2019). Sprengel

et al. (2019) suggested that processes employed to enrich the nutrient content of oil-based supplements may have also concentrated CPs in these products to cause the extremely high levels observed. The lipophilic characteristics of CPs lead to the assumption that lipid-rich foods have a greater potential to accumulate CPs from environmental or manufacturing origins, as evidenced by significant, although weak, correlations between ∑SCCP concentrations and lipid content (Spearman coefficient = 0.178, p = 0.010, Figure 1). Most of the samples in the LIP group of this study have lipid contents close to 100%.

Cao *et al.* (2015) determined differing SCCP homologue patterns in several cooking oil types and the raw seeds from which they were processed, indicating that CPs in plant oils are unlikely to derive from the raw ingredients. Food packaging was also identified as a potential source of CP food contamination by Wang *et al.* (2019a), who reported concentrations of ∑SCCPs ranging from 970 to 4700 ng/g in foil-lined polypropylene commercial food containers purchased in China. Subsequent experiments demonstrated migration of SCCPs into several food simulants, with the greatest transfer observed in the *n*-hexane oil substitute (Wang *et al.*, 2019a). Lubricants in mechanical pressing and mixing components or polymeric materials inherent to the manufacturing of animal and plant oils may also be considered as potential sources of CPs to oil products (Yuan *et al.*, 2017b).

A number of samples in the SUG category contained elevated ∑SCCPs, including the second highest measurement among the Belgian samples, in dark chocolate fondant (SUG-6, 28 ng/g ww). Products within this category may be prone to accumulate CPs due to the same reasons outlined for both the GRA and LIP groups. Approximately half of the food items in the SUG category are chocolate-based products with high lipid proportions ranging 27-38% and most could be considered as highly processed foods. ∑SCCPs were reported as <LOQ in a single analysis of pooled sweets samples (including chocolate, honey and ice cream) from Sweden (NFA, 2017) but no other data on such products is available for comparison.

Only low levels of SCCPs were observed in samples from the MEA, FIS and MIL categories, with maximum ∑SCCP levels of 2.3, 2.7 and 5.1 ng/g ww determined, respectively. This is somewhat unexpected, as foods in these categories have been considered higher risk due to generally greater lipid contents and heightened potential for environmental CP accumulation (Huang *et al.*, 2018; Wang *et al.*, 2018). SCCPs were reported above LOQs with similarly low frequency in meat and dairy samples purchased in Germany, with ∑SCCP concentration ranging <LOQ to 72 ng/g ww and <LOQ to 9.9 ng/g ww, respectively, while SCCPs were more prevalent in fish samples ranging <LOQ to 180 ng/g ww (Krätschmer *et al.*, 2021). ∑SCCP levels in fish from France were close to those of the present study (n=22, 0.3 to 10.6 ng/g ww) (Labadie *et al.*, 2019), but generally higher levels were reported in aquatic foods from Spain (n=22, 3.1 to 141 ng/g ww) (Parera *et al.*, 2013) and another study of salmon purchased in Germany (n=133, 0.97 to 170 ng/g ww) (Krätschmer *et al.*, 2019). A cause for the discrepancy between ∑SCCP levels in fish purchased in Belgium and other European countries could not be determined, although differences in the source location of fish could be responsible (this data was not collected in the present study). ∑SCCP concentrations in samples of meat, fish and dairy purchased in Korea and China were approximately two- to three- orders of magnitude above those from Belgium (Huang *et al.*, 2018; Huang *et al.*, 2019; Dong *et al.*, 2020; Lee *et al.*, 2020). The elevated ∑SCCP levels determined in these regions are likely related to China's role as the world's largest producer of CPs (Glüge *et al.*, 2016), and indeed extreme CP contamination (>1,000 ng/g ww) reported in foods from highly polluted e-waste sites in China illustrates the link between environmental contamination and CP accumulation into foodstuffs (Chen *et al.*, 2018; Zeng *et al.*, 2018).

3.2 MCCP Concentrations

MCCPs were present above LOQs in 66% of the food samples with an overall ∑MCCP range of <LOQ to 250 ng/g ww and median level of 6.0 ng/g ww. MCCPs were more prevalent than SCCPs in all food categories, with the highest detection frequencies observed in EGG (100%), MEA (87%), LIP (85%) and FIS (81%) (Table 1, Table S4, Figure 2). ANOVA tests showed the ∑MCCP levels in LIP and SUG samples to be higher than each of the GRA, MEA, FIS and MIL categories by a statistically significant margin ($p<0.05$), while the LIP samples also contained significantly higher ∑MCCP concentrations than EGG. The remaining categories were excluded from ANOVA tests due to low detection frequency and no other significant differences were determined between the categories. As with SCCPs, MCCP concentrations have typically been elevated in fats and oil samples with respect to other food types in market basket studies (COT, 2009; NFA, 2017; Krätschmer *et al.*, 2021). Concentrations of ∑MCCPs in fats and oils purchased in Germany ranged from <LOQ to 1800 ng/g ww with a mean level (120 ng/g ww) approximately an order of magnitude higher than those of all other food groups (Krätschmer *et al.*, 2021). Fats and oils were, likewise, the most contaminated food group in the limited market basket studies from Sweden (NFA, 2017) and the UK (COT, 2009). Very high levels of ∑MCCPs have also been reported in oil-based dietary supplements in Germany (mean 15,200 ng/g lw, n=25) (Sprengel *et al.*, 2019) and cooking oils from China (median 1420 ng/g lw, n=5) (Chen *et al.*, 2018). A significant correlation was determined between ∑MCCP concentrations and lipid content across the total Belgian sample set (Spearman coefficient 0.678, $p<0.001$, Figure 1), which illustrates the role of lipids in accumulating CPs within foodstuffs. The extensive degree of processing inherent to most of the LIP samples also creates a high potential for contamination from manufacturing sources.

Similar factors may have contributed to the high levels of MCCPs observed in SUG samples, as most confectionary foodstuffs are highly processed and many of the samples in this group have lipid contents of around 30%. Indeed, MCCP contamination in the SUG samples could be differentiated by lipid content with most low-lipid (<1%) samples, like refined sugar, honey and syrup having concentrations <LOQ, while ∑MCCPs ranged from 23 to 140 ng/g ww in chocolate-based confectionary (lipid content >25%). CPs in sugar and chocolate samples have not been widely studied, though a concentration of 6.3 ng/g ww ∑MCCPs was reported in a pooled sample of sweets from Sweden (NFA, 2017), and CPs have been routinely identified in dairy and oil products which often form the basis of many confectionary goods (Cao *et al.*, 2015; Dong *et al.*, 2020; Lee *et al.*, 2020; Krätschmer *et al.*, 2021).

MCCP levels were similarly distributed in each of the MEA, FIS and MIL and EGG categories which represent the major classes of animal-based foods, with median ∑MCCP levels of 7.7, 6.0, 5.1 and 10 ng/g ww, respectively. ∑MCCP levels were approximately similar in several fish species purchased from markets in Germany (<LOQ to 48 ng/g ww) (Krätschmer *et al.*, 2021) and the UK (<1.0 to 31.9 ng/g ww) (COT, 2009) as well as wild fish from France (1.3 to 72.7 ng/g ww) (Labadie *et al.*, 2019). Another study of salmon purchased in Germany from a range of sources reported slightly higher ∑MCCP concentrations and suggested greater CP accumulation to occur in farmed conditions than in the wild (Krätschmer *et al.*, 2019). ∑MCCPs in aquatic foods (mostly fish) from China were about an order of magnitude higher than the European samples in one study (Wang *et al.*, 2018) and two- to three- orders of magnitude higher from a Chinese e-waste recycling region (Chen *et al.*, 2018), further illustrating the influence of environmental conditions on CP bioaccumulation. However, details on source location and farming type were not collected with the present samples for comparison. The levels of ∑MCCPs in the MEA and MIL categories were also broadly similar to those of other

European food samples from Germany (Krätschmer *et al.*, 2021) and the UK (COT, 2009), while MCCPs were below detection limits in pooled Swedish samples of these food groups (NFA, 2017).

∑MCCP concentrations were significantly correlated ($p<0.001$) with lipid content in each of the MEA, FIS and MIL sample categories, while the degree of sample processing or specific packaging is also likely to have affected CP loads. MCCPs measured in whole egg samples, however, could be considered to derive entirely from bioaccumulation of CPs, as the contents of eggs are minimally influenced by other contamination sources. Experiments involving oral dosing of chickens via CP-spiked feed demonstrated C-chain-length and Cl% specific transfer of SCCPs and MCCPs into eggs (Mézière *et al.*, 2021). In this respect, ∑MCCPs detected in each of the whole egg samples and the lack of SCCPs in the same samples provide a unique insight as to the respective contamination pathways of animal-based foods.

Among the plant-based food categories, GRA contained ∑MCCP levels similar to those of the animal-based foods (median 7.4 ng/g ww), while low detection frequencies were observed in the VEG, STA and FRU categories at 39, 20 and 20%, respectively. The elevated MCCP levels in GRA samples are most likely related to the combined effects of contaminated oil-based additives and accumulation of CPs during processing and baking processes. In particular, the overall maximum ∑MCCP concentration of 250 ng/g ww was determined in the same sample which contained the highest SCCP level, the wheat-flour dürüm (GRA-12). The low levels observed in the other classes of vegetable-based foods is expected due to the low overall lipid contents and lower degrees of processing. These food groups are rarely studied for these reasons and ∑MCCPs have not exceeded 1.0 ng/g ww in fruits and vegetables analysed in limited studies from the UK (COT, 2009) and Sweden (NFA, 2017). In contrast to other samples in the VEG, STA and FRU groups, however, two samples of lentils (VEG-16 and VEG-17) contained ∑MCCP concentrations of 23 and 20 ng/g ww, respectively, approximately four to five times higher than the next highest levels in these groups. This may indicate that lentils have a higher capacity to accumulate CPs from the environment or that particular processing techniques have contributed to MCCP contamination. Indeed, ∑MCCPs were present at high levels in Chinese legume samples ranging from 40.1 to 598 ng/g (mean 184 ng/g) purchased from a broad range of regions and sources (Wang *et al.*, 2019b).

3.3 Congener group patterns

3.3.1 GC-MS data

Overall, MCCPs were more prevalent in the Belgian food samples than SCCPs. ∑MCCP concentrations were greater than those of ∑SCCPs in all 48 samples in which both groups were detected with an average ∑MCCP/∑SCCP ratio of 5.8 (ranging from 1.3 to 81). There were another 91 samples (43% of all samples) in which only MCCPs were present >LOQ, 5 samples (2.4%) in which only SCCPs were present and 67 samples (32%) in which neither group were identified. MCCPs were likewise dominant over SCCPs in fish samples from Germany (Krätschmer *et al.*, 2019) and France (Labadie *et al.*, 2019), oil-based supplements from Germany (Sprengel *et al.*, 2019) and in general in the UK (COT, 2009). SCCPs were determined as dominant, however, in a comprehensive market basket study from Germany in all food categories except for fats and oils (Krätschmer *et al.*, 2021), which may in-part reflect differences in the sample types which populate respective food categories. SCCPs have also been reported at levels one- to two- orders of magnitude above MCCPs in meat, aquatic foods and raw milk from China (Huang *et al.*, 2018; Wang *et al.*, 2018; Dong *et al.*, 2020), while proportions of the two groups were similar in a Chinese whole meal assessment (Gao *et al.*, 2018).

Although GC-MS analysis is unable to provide reliable congener group specific concentration data, responses recorded for individual congener groups were corrected for fractional isotopic abundance of monitored m/z species and chlorine number according to Tomy *et al.* (1997) to gain tentative C and Cl distribution information in samples. Among the total sample set, C_{10} congeners accounted for an average of 21% of total ∑SCCPs, while C_{11}, C_{12} and C_{13} contributed mean proportions of 23, 29 and 36% of the SCCP load respectively. C_{14} congener groups made up an average of 45% of ∑MCCPs, overall, with the C_{15}, C_{16}, and C_{17} groups accounting for 28, 22 and 3% on average, respectively. The average distribution of Cl_5 to C_{10} congener groups in samples was approximately Gaussian for both SCCPs and MCCPs, with Cl_7 and Cl_8 groups dominating, followed by Cl_6 and Cl_9, and lesser amounts of Cl_5 and Cl_{10}. There was little difference in C or Cl distribution observed between food categories, while low detection rates in some instances prevented thorough comparisons.

The distributions of SCCP and MCCP homologues observed in the present study are broadly similar to those of industrial and technical CP mixtures (Bogdal *et al.*, 2015; Yuan *et al.*, 2017a; Li *et al.*, 2018), though highly variable CP patterns have been reported in food processing equipment (Yuan *et al.*, 2017b) and general consumer goods from Belgium (McGrath *et al.*, 2021b). Significant correlations between ∑SCCP and ∑MCCP concentrations in the Belgian food samples (Spearman coefficient = 0.441, $p < 0.001$, Figure 1) also suggest that the two homologue groups might share similar sources to food. Complex interactions between the respective bioaccumulation properties of individual congener groups, environmental contamination load and uptake by plants and animals during farming may also be important factors effecting CP distribution patterns.

3.3.2 LC-HRMS data

A subset of 20 food samples with some of the highest concentrations of SCCPs and MCCPs measured by GC-ECNI/MS were selected for LC-HRMS analysis in order to conduct a more robust homologue profiling and additionally, to investigate the contribution of LCCPs in Belgian food (indicated in Table S4). Despite the ability of the HRMS method applied in this study to resolve individual congener groups (R>50,000), true homologue level quantification remains impossible due to the influence of carbon-chain length and chlorination-degree on the MS instrumental response (Krätschmer & Schächtele, 2019; Yuan *et al.*, 2019), and a lack of well-characterized single homologue reference standards. For these reasons, congener group patterns are presented here as heatmaps, where the colour code is applied per row, that is, per homologue (assuming an underlying fixed response factor for each). Reported signals were corrected for isotopic abundance and IS response to account for variation between injections.

Results of the relative SCCP and MCCP content by LC-HRMS in the selected samples were very consistent with the GC-ECNI/MS results (Figure 3 and Figure 4). Both SCCP and MCCP contents in the dürüm bread sample (GRA-12) exceeded all other samples substantially, with a predominance of C_{10} and C_{13} to C_{15}. Next to this sample, the goat cheese with chives (MIL-24) showed the highest levels in C_{10} & C_{11} SCCPs, but almost no MCCPs. The dark chocolate (SUG-6), wok oil mix (LIP-16) and mayonnaise (SSC-1) also contained higher levels of SCCPs. Another bread (GRA-15), oil (LIP-17), fish (FIS-9) and lentils (VEG-16) samples also exhibited measurable SCCP content, but to a lesser extent. MCCPs were also detected in each of these samples by LC-HRMS and were present in 18 of the 20 samples (Figure 4). High MCCP levels were widespread among the LIP samples, as three out of the four profiled samples in this category (LIP-16, LIP-17, LIP-13) exhibited the highest levels in C_{15} to C_{17}. Some meat and fish samples (ham, MEA-23; filet Américain, MEA-9; tarama, FIS-25; mackerel fillets in olive oil FIS-9) with almost no SCCPs had however relatively high

MCCP content. This could be explained because these samples correspond to highly processed and/or oil mixed products and manufacturers may have switched to longer chain CPs with the SCCP legal restriction.

Finally, the CP congener groups profiling was extended to LCCPs. LCCPs were detected in 35% of the selected samples (Figure 5). The low-level C_{18} LCCPs detected in the fondue oil (LIP-13) and dark chocolate (SUG-6) can be seen as a consequence of their high MCCPs content. Nevertheless, it is interesting to see that some samples containing almost no SCCPs and only few MCCPs, namely the mackerel fillets in olive oil (FIS-9) and the egg yolk (EGG-8), showed very high levels of LCCPs, including very long C-chain CPs (C >30) for FIS-9. Next to these two samples, the oily samples (LIP-17, SSC-1 and to a lesser extent LIP-16) also contained LCCPs. This confirms the potential market shift towards the use of longer chain CPs resulting in possible food contamination, as also recently evidenced in Germany (Krätschmer *et al.*, 2021).

Of note, the C8-C9 very short-chain CPs (vSCCPs) were not detected, suggesting that the vSCCPs do not represent a major contamination in the investigated samples. vSCCPs with C<8, however, could not be included in the LC-MS screening because of their high volatility.

Regarding chlorination degree profiles, an overall trend was observed towards CPs with chlorination around 50%. Very low (number Cl <5) and very high (Cl degree >75%, corresponding respectively to maximum observed number Cl >9 for SCCPs, >10 for MCCPs and >19 for LCCPs) chlorination degrees were not detected. It had been previously reported that LC-MS with atmospheric pressure source, in opposition to GC-ECNI, enables the detection of lower chlorinated compounds (Krätschmer & Schächtele, 2019; Yuan *et al.*, 2019; Matsukami *et al.*, 2020; Krätschmer *et al.*, 2021). On the other hand, highly chlorinated homologues might suffer from decreased ionization efficiency, as recently observed with a similar LC-ESI-MS set-up (Mézière *et al.*, 2020b). The comparison of obtained CP homologue patterns to other European studies on food contamination with CPs available (Parera *et al.*, 2013; Labadie *et al.*, 2019; Krätschmer *et al.*, 2021) is currently not advisable. Indeed, reported CP patterns are still strongly dependent on the instrument and quantification method (Krätschmer & Schächtele, 2019; Mézière *et al.*, 2020b; Krätschmer *et al.*, 2021). Further harmonization efforts and robust conclusions on the CP congener group profiles contaminating the European food market will require the availability of adequate (labelled) individual homologue CP reference standards to validate homologue-level quantification.

4 Conclusions

This study provides evidence of widespread CP contamination of a wide variety of foodstuffs purchased in Belgium. MCCPs were detected with greater frequency and in higher concentrations in all food types, suggesting that recent global restriction of SCCPs has influenced usage towards longer-chained CPs. The application of LC-HRMS profiling on a selection of samples strengthened the results obtained by GC-MS, as consistent relative abundances of SCCPs and MCCPs were observed between samples. The LC-HRMS analysis also revealed LCCPs to be present among Belgian foods to further indicate shifts in CP application due to the regulation of SCCPs. While this study provides insights on the congener group profiles of CPs in food, true homologue level quantification will require the further availability of single compound and mass-labelled CP reference standards.

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Tables

Table 1. Detection frequency and descriptive statistics for concentrations (ng/g wet weight) of ∑SCCPs and ∑MCCPS in food samples.

		∑SCCPs				∑MCCPs			
Category	n	Det Freq (%)	Min	Median	Max	Det Freq (%)	Min	Median	Max
GRA	26	58	<LOQ	1.0	58	69	<LOQ	7.4	250
VEG	18	22	<LOQ	<LOQ	6.9	39	<LOQ	<LOQ	23
STA	10	10	<LOQ	<LOQ	0.7	20	<LOQ	<LOQ	5.2
FRU	10	10	<LOQ	<LOQ	1.3	20	<LOQ	<LOQ	5.2
MEA	30	20	<LOQ	<LOQ	2.3	87	<LOQ	7.7	27
FIS	27	30	<LOQ	<LOQ	2.7	81	<LOQ	6.0	73
MIL	25	4	<LOQ	<LOQ	5.1	52	<LOQ	5.1	22
EGG	10	0	<LOQ	<LOQ	<LOQ	100	3.3	10	16
SUG	16	44	<LOQ	<LOQ	28	69	<LOQ	26	140
LIP	26	31	<LOQ	<LOQ	19	85	<LOQ	40	190
COM	5	0	<LOQ	<LOQ	<LOQ	40	<LOQ	<LOQ	16
SSC	5	20	<LOQ	<LOQ	9.5	40	<LOQ	<LOQ	51
OTH	3	33	<LOQ	<LOQ	5.2	67	<LOQ	18	20
Total	211	25	<LOQ	<LOQ	58	66	<LOQ	6.0	250

GRA= grains and grain-based products, VEG= vegetables and vegetable products, STA= starchy roots and tubers, FRU= fruit and fruit products, MEA= meat and meat products, FIS= fish and other seafood, MIL= milk and dairy products, EGG= eggs and egg products, SUG= sugar and confectionary, LIP= animal and vegetable fats and oils, COM= composite dishes, SSC= seasoning, sauces and condiments and OTH= food supplements.

Figures

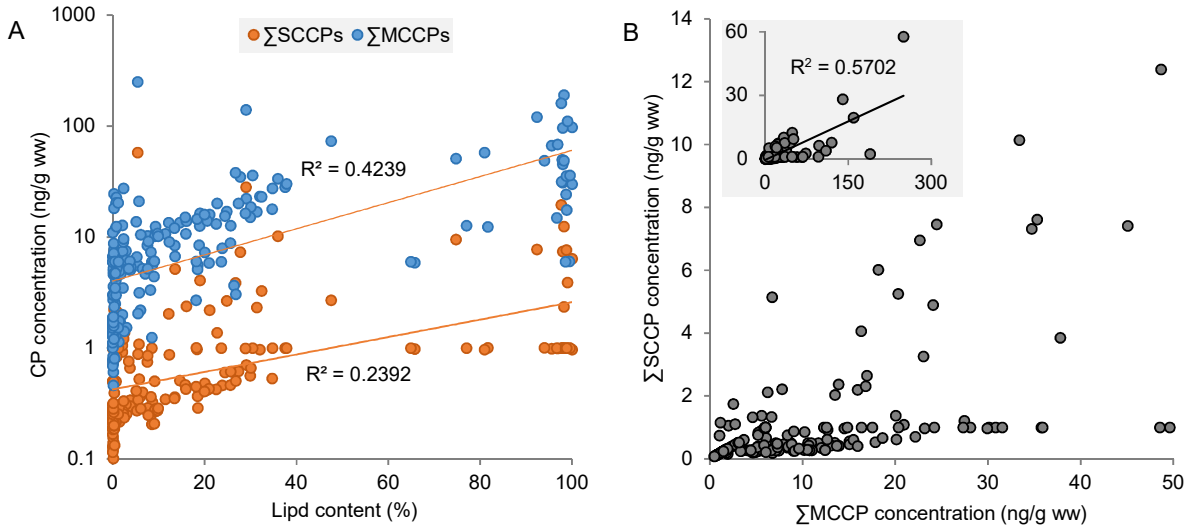


Figure 1. A) Correlations between concentrations (ng/g ww) and lipid contents (%) for ∑SCCPs (Spearman coefficient = 0.178, p = 0.010) and ∑MCCPs (Spearman coefficient = 0.678, p < 0.001). B) Correlations between ∑SCCP and ∑MCCP (ng/g ww) concentrations (Spearman coefficient = 0.441, p < 0.001). Values <LOQ were replaced with 1/2 * LOQ in figures and with 0 for Spearman correlation analysis.

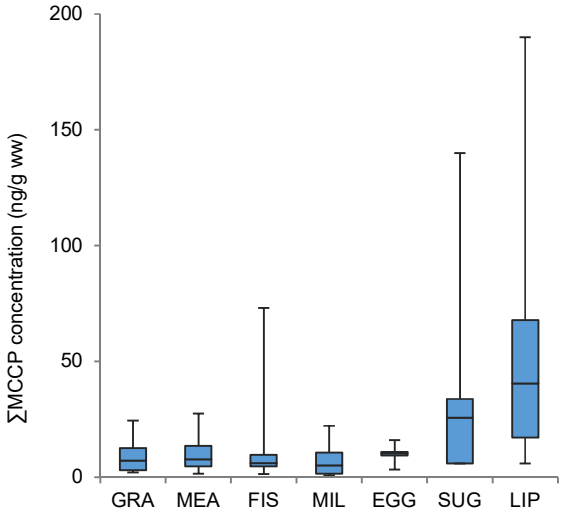


Figure 2. ∑MCCP concentrations in selected food categories. Boxes indicate 25th, 50th and 75th percentiles and whiskers (error bars) represent minimum and maximum. Only food categories with detection frequency >50% and with more than 3 samples per category >LOQ are shown. Values <LOQ have been replaced with 1/2 * LOQ. Statistical outlier GRA-12 (250 ng/g ww) is not shown. GRA= grains and grain-based products, MEA= meat and meat products, FIS= fish and other seafood, MIL= milk and dairy products, EGG= eggs and egg products, SUG= sugar and confectionary, LIP= animal and vegetable fats and oils.

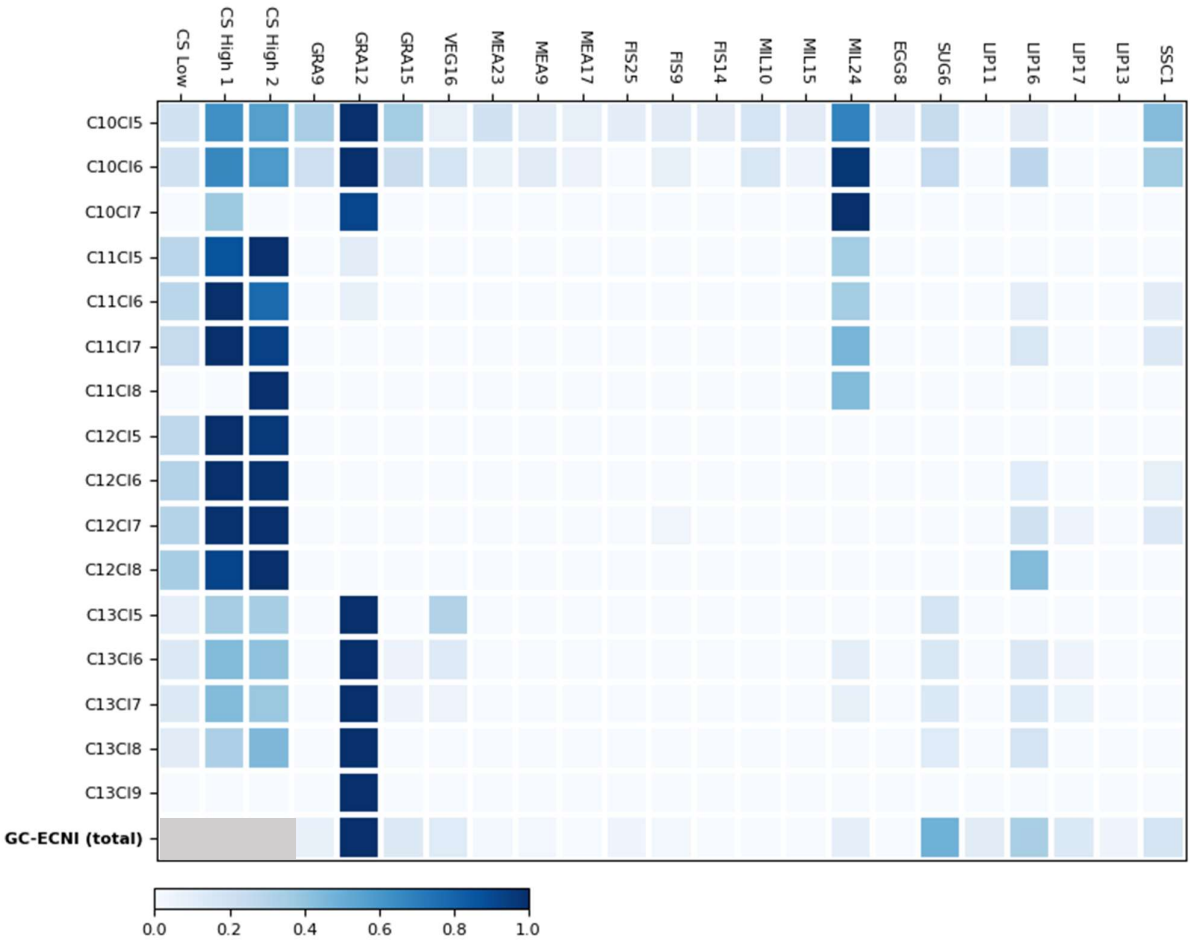
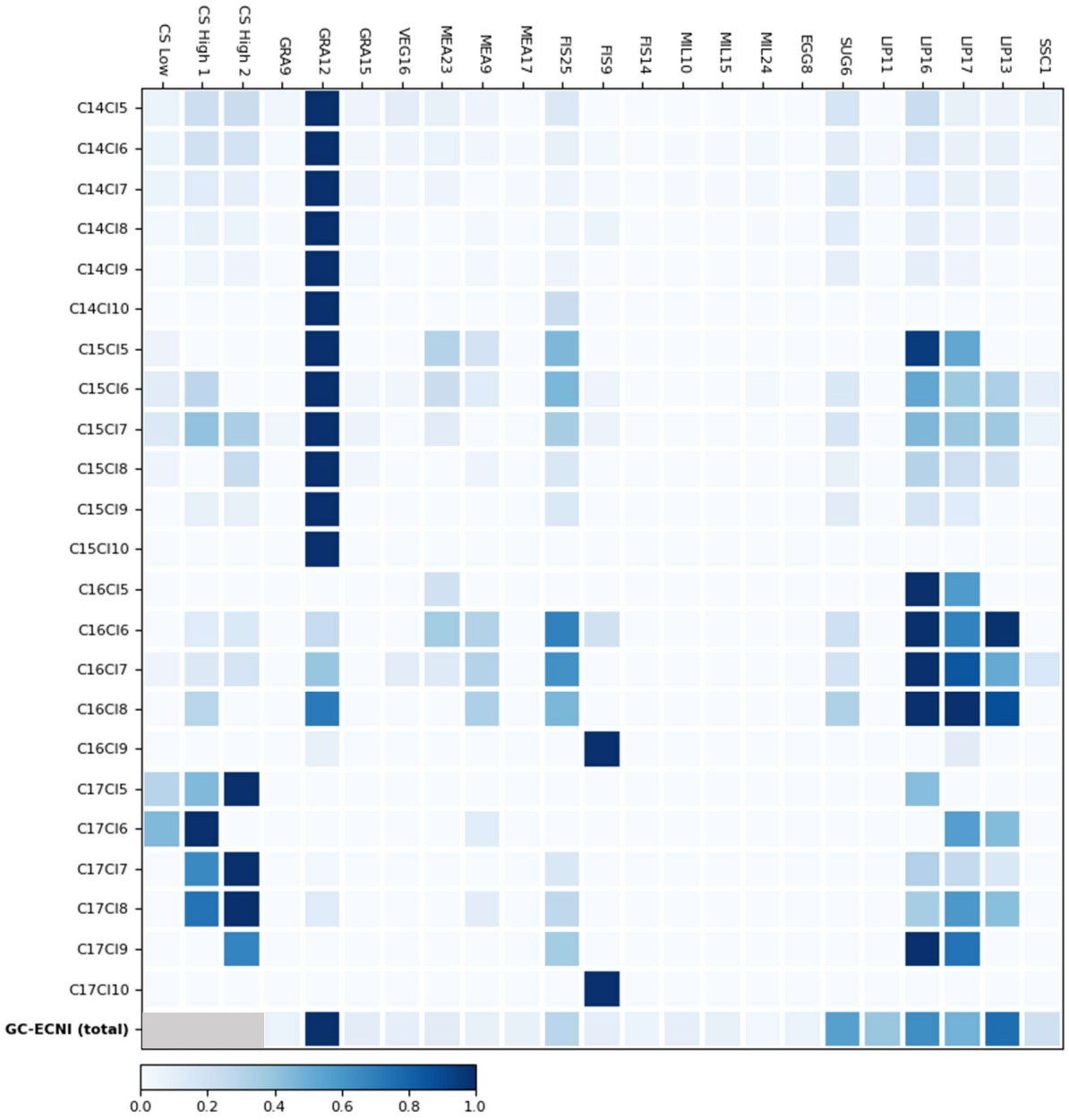


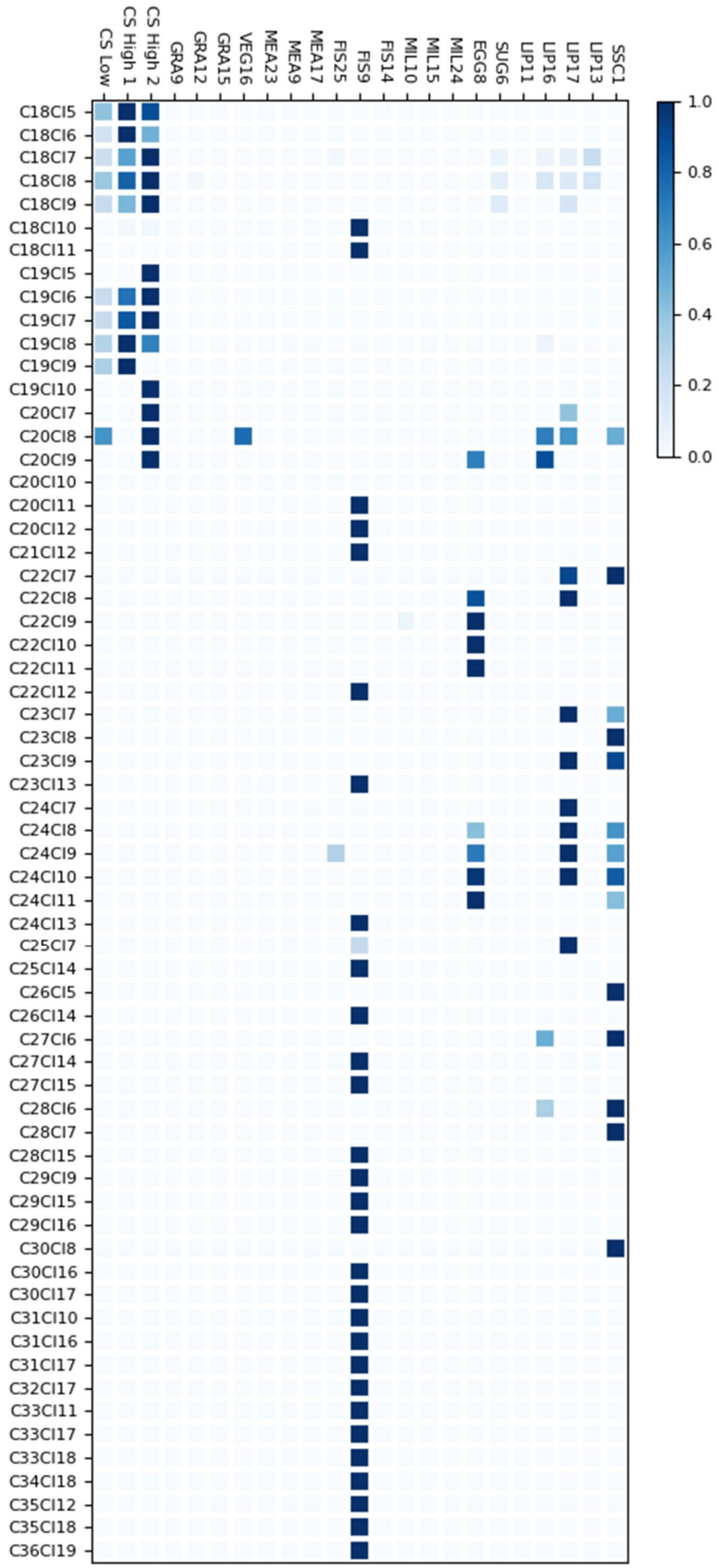
Figure 3. SCCP congener groups profile in selected samples by LC-HRMS. Full sample details for sample codes shown on the horizontal access are provided in Table S4. Heatmap colour coding is applied per row/homologue, using averaged MS signals from triplicates analyses, corrected for isotope abundance and IS signal. Reported signal values can be found in Figure S1. CS Low, High 1 and 2 are control samples consisting of procedural blanks spiked (pre-extraction) with 100 µL of a mixed standard containing SCCP 55%Cl, MCCP 52%Cl and LCCP 49%Cl at concentrations of 3 µg/mL each (low spike) or 10 µg/mL each (high spike). The last line applies the same heatmap colour coding to the total SCCP concentration quantified with the GC-MS method (not applied to CS).



672

673 Figure 4. MCCP congener groups profile in selected samples by LC-HRMS. Full sample details for sample
674 codes shown on the horizontal access are provided in Table S4. Heatmap colour coding is applied per
675 row/homologue, using averaged MS signals from triplicate analyses, corrected for isotope abundance and IS
676 signal. Reported signal values can be found in Figure S2. CS Low, High 1 and 2 are control samples consisting
677 of procedural blanks spiked (pre-extraction) with 100 µL of a mixed standard containing SCCP 55%Cl, MCCP
678 52%Cl and LCCP 49%Cl at concentrations of 3 µg/mL each (low spike) or 10 µg/mL each (high spike). The
679 last line applies the same heatmap colour coding to the total MCCP content quantified with the GC-MS method
680 (not applied to CS).

681


CS Low
CS High 1
CS High 2
GRA9
GRA12
GRA15
VEG16
MEA23
MEA9
MEA17
FIS25
FIS9
FIS14
MIL10
MIL15
MIL24
EGG8
SUG6
LIP11
LIP16
LIP17
LIP13
SSC1
C18CI5
C18CI6
C18CI7
C18CI8
C18CI9
C18CI10
C18CI11
C19CI5
C19CI6
C19CI7
C19CI8
C19CI9
C19CI10
C20CI7
C20CI8
C20CI9
C20CI10
C20CI11
C20CI12
C21CI12
C22CI7
C22CI8
C22CI9
C22CI10
C22CI11
C22CI12
C23CI7
C23CI8
C23CI9
C23CI13
C24CI7
C24CI8
C24CI9
C24CI10
C24CI11
C24CI13
C25CI7
C25CI14
C26CI5
C26CI14
C27CI6
C27CI14
C27CI15
C28CI6
C28CI7
C28CI15
C29CI9
C29CI15
C29CI16
C30CI8
C30CI16
C30CI17
C31CI10
C31CI16
C31CI17
C32CI17
C33CI11
C33CI17
C33CI18
C34CI18
C35CI12
C35CI18
C36CI19
1.0
0.8
0.6
0.4
0.2
0.0

682

Figure 5. LCCP congener groups profile in selected samples by LC-HRMS. Full sample details for sample codes shown on the horizontal access are provided in Table S4. Heatmap colour coding is applied per row/homologue, using averaged MS signals from triplicate analyses, corrected for isotope abundance and IS signal. Reported signal values can be found in Figure S3. CS Low, High 1 and 2 are control samples consisting of procedural blanks spiked (pre-extraction) with 100 µL of a mixed standard containing SCCP 55%Cl, MCCP 52%Cl and LCCP 49%Cl at concentrations of 3 µg/mL each (low spike) or 10 µg/mL each (high spike).