Chapter 6

Medium-chain chlorinated paraffins (MCCPs) dominate in Australian sewage sludge

Sicco H. Brandsma, a, Louise M. Mourik a, b, Jake W. O’Brien b, Geoff Eaglesham b, Pim E.G. Leonards a, Jacob de Boer a, Chirstie Gallo b, Jochen F. Mueller b, Caroline Gaus b, Christian Bogdal c

a Vrije Universiteit, Dept. of Environment and Health, Amsterdam, The Netherlands
b The University of Queensland, Queensland Alliance for Environmental Health Science (QAEHS), Brisbane, Qld, Australia
c Institute for Chemical and Bioengineering, ETH Zurich, Switzerland

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Abstract
To simultaneously quantify and profile the complex mixture of short-, medium-, and long-chain CPs (SCCPs, MCCPs and LCCPs) in Australian sewage sludge, we applied and further validated a recently developed novel instrumental technique, using quadrupole time-of-flight high resolution mass spectrometry running in the negative atmospheric pressure chemical ionization mode (APCI-qToF-HRMS). Without using an analytical column, the cleaned extracts were directly injected into the qToF-HRMS followed by quantification of the CPs by a mathematical algorithm. The recoveries of the four SCCP, MCCP and LCCP-spiked sewage sludge samples ranged from 86-123%. This APCI-qToF-HRMS method is a fast and promising technique for routinely measuring SCCPs, MCCPs and LCCPs in sewage sludge. Australian sewage sludge was dominated by MCCPs with concentrations ranging from 540 to 3,600 ng/g dry weight (dw). Lower SCCP concentrations (<57-1,400 ng/g dw) were detected in the Australian sewage sludge, which were comparable with the LCCPs concentrations (120-960 ng/g dw). This is the first time that CPs were reported in Australian sewage sludge. The results of this study gives a first impression on the distribution of the SCCPs, MCCPs, and LCCPs in Australia waste water treatment plants (WWTPs).
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6.1 Introduction

Chlorinated paraffins (CPs), also known as polychlorinated \(n\)-alkanes, are complex mixtures of carbon chains of variable length and a variable number of chlorine atoms. They are currently produced in high volumes (> 1 million tonnes/yr in China alone) [1]. Concerns are rising regarding their ubiquitous occurrence and persistence in the environment [2], including detection in remote areas [3]. The so-called short-chain CPs (SCCPs), CPs with carbon chain lengths between 10 and 13, are under scrutiny as these compounds have a high bioaccumulation potential [4-6] and are chronically toxic to aquatic organisms [7, 8]. They are, therefore, classified as persistent organic pollutants (POPs) under the Stockholm Convention, as substances of very high concern and are listed as key compounds for monitoring in several legislations and guidelines. They are currently candidates to be designated as persistent organic pollutants (POPs) under the Stockholm Convention. In the case of the medium-chain CPs (MCCPs) \((C_{14}-C_{17})\) and long-chain CPs (LCCPs) \((\geq C_{18})\), relevant information on their levels, fates, and potential hazards to the environment is still insufficient to facilitate international regulations. However, the current restrictions on the use of SCCPs are expected to result mainly in a replacement by MCCPs in many applications [9].

The insufficient data about CPs is partly due to limitations regarding the reliability of analytical methods to identify and quantify these compounds. To date, the existing analytical methods for the determination of CPs suffer all from serious drawbacks that makes a reliable quantification difficult. Recently, Bogdal et al. [10] developed a promising instrumental technique to determine SCCPs, MCCPs, and LCCPs in environmental matrices in a single measurement run with a particularly fast method using high resolution mass spectrometry.

Reliable CP exposure data are needed, particularly in regions where the CP levels and fate are still unknown. In Australia, for example, limited data on CPs exist, and the capability of analysing CPs is lacking. Until today, only two non-peer reviewed studies describe the presence of CPs in the Australian environment. Gillet et al. [11] reported SCCPs in indoor and outdoor air in Melbourne with median concentrations of 52 ng/m\(^3\) and 81 ng/m\(^3\), respectively. Kemmlein et al. [12] reported SCCPs, MCCPs, and LCCPs in mussels, crabs, and sediments collected near a CP manufacturer in Australia. The levels were respectively, 2,300 \(\mu\)g/kg lipid weight (lw), 23,200 \(\mu\)g/kg lw, and 9,300 \(\mu\)g/kg lw in mussels, and 64,900 \(\mu\)g/kg lw, 30,500 \(\mu\)g/kg lw and 14,300 \(\mu\)g/kg lw in crabs. In the sediment, the concentrations ranged from 61 to 440 \(\mu\)g/kg dry weight (dw) for the SCCPs; from 1,100 to 16,400 \(\mu\)g/kg dw for the MCCPs; and from 900 to 3,100 \(\mu\)g/kg dw for the LCCPs.

Despite the lack of information, the presence of at least local CP contamination is highly likely. Australia manufactures MCCPs and LCCPs (i.e. Orica, Melbourne) with ongoing investments for expansion of CP manufacturing facilities [13]. According to a government desktop study [14], SCCPs were imported into Australia in large quantities (i.e. 360 tonnes) between 1998 and 2000 and were still imported at approximately 25
tonnes/year in 2002. MCCPs and LCCPs were imported into Australia in 2002 in even higher quantities at approximately 475 tonnes/year. NICNAS [14] reported that it is most likely that the use of MCCPs and LCCPs in Australia have increased due to their replacement of the SCCPs. While current information is lacking, to our knowledge, SCCP import or use is still permitted in Australia. In 2002, SCCPs were mainly used as lubricants in the Australian metal working industry (70%) [14]. Therefore, releases of CPs in Australia are thought to be from production, both from spills or facility washdown, and from industrial usage, either from improper disposal of used metalworking lubricants or carry-off from work pieces [14]. Such releases of CPs occur either directly or through wastewater treatments plants (WWTPs) [15]. As CPs are emerging POPs, determining the levels of CPs near suspected sources (i.e. WWTPs) would facilitate a first understanding on their release, presence, and potential risks of these compounds. Therefore, this study aims to determine CPs in sewage sludge samples (also known as biosolids) from various WWTPs in order to allow a first evaluation of SCCP, MCCP and LCCP levels in Australia.

6.2 Material and Methods

Information about the standards, chemicals and suppliers is provided in the Supporting Information (Section S4-1).

6.2.1 Sample Collection

Pooled (eight subsamples from each WWTP) sewage sludge samples were collected in 2014 at 15 different WWTPs in Australia (Figure 6-1). The WWTPs were located in five of eight states and territories of Australia, servicing populations of between 25,000 and 600,000 people, representing a combined population of approximately 2.5 million people (over 10% of the Australian population). Further details regarding sampling and sample composition are provided in the S4-3.

6.2.2 Sample pre-treatment

After freeze-drying, approximately 0.2-0.3 gram of the sewage sludge sample was extracted by pressurized liquid extraction (PLE) on an ASE350 (Dionex, Sunyvale, CA, USA). Extraction was performed with n-hexane/acetone (3:1, v/v) at 100 °C and 1500 psi with a heat- and static-time of 5 min using three extraction cycles. Clean-up was performed in two steps. Firstly with aluminum oxide (8% H₂O) and secondly with silica gel (1.5% H₂O) columns. Briefly, 15 gram of aluminum oxide (8% H₂O) was weighed into a glass column. After washing the aluminum oxide column with 25 mL of n-hexane, the extracts were brought on top of the column followed by elution of the CPs with 170 mL of n-hexane. During the second clean-up, 1.8 gram of silicagel (1.5% H₂O) was weighed into a glass column. The silicagel column was washed with 6 mL of n-hexane before the sample extract was added. Fractionation was performed in two fractions, the first one consisted of 14 mL of n-hexane (waste fraction) and the
second one of 15 mL 15% diethyl ether in n-hexane (the CPs fraction). The fraction was evaporated under nitrogen and solvent changed to 0.5 mL acetonitrile (ACN) and transferred to an LC-vial followed by addition of 100 µL of injection standard $^{13}$C$_6$-PCP (700 pg/µL in ACN).

**Figure 6-1:**
Total CP concentrations in ng/g dry weight measured in the sewage sludge samples from Australia. The LCCP values with an $R^2$ lower than 0.5 are highlighted with red stripes and are semi-quantitative values. The SCCP values lower that the LOD are highlighted with green blocks. (WA = Western Australia; NT = Northern Australia; SA = South Australia; QLD = Queensland; NSW = New South Wales; VIC = Victoria; TAS = Tasmania)

### 6.2.3 Measurement of CPs
Measurement of the CPs in the Australian sewage sludge samples was performed with a slightly adopted analytical method recently developed by Bogdal et al. [10]. Briefly, without using an analytical column, 10 µL of the cleaned sewage sludge extract was directly injected into the qToF-HRMS (Triple ToF 5600+ Sciex, Concord, Ontario, Canada) running in the negative atmospheric pressure chemical ionization (APCI) mode. The injection was performed with a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan) using ACN as eluent with an isocratic flow of 250 µL/min. To increase the sensitivity of the CP detection in negative APCI mode, dichloromethane (DCM) at a flow rate of 40 µL/min was used as a dopant and mixed with the eluent just before it entered the ion source [10]. Addition of DCM results in an excess Cl$^-$ ions in
the ion source and significantly enhances the formation of [M+Cl]-.

CPs were analysed with the APCI-qToF-HRMS using the following settings. The nebulizer temperature was optimized and set to 200 °C. The declustering potential (DP) was set at -90 V, and collision energy (CE) of -10 V was used. The mass spectrometer was operated in qToF-HRMS mode measuring the full scan range of m/z 200 to 1500. The minimal resolution was 10,000 (minimum of 7,000 is required for the separation of the m/z values of the most important [M+Cl]- ions) [10]. External mass calibration was performed with the Sciex APCI Negative Calibration solution 5600, which consist of a mix of known molecular weight polypropylene glycols (PPGs). In total, 558 m/z ratios were extracted from the full scan mass spectra using MultiQuant 3.0 software (Sciex). The 558 m/z ratios are related to the two most abundant m/z signals of the CP isotope cluster corresponding to the CP congener groups with chain lengths of C_{10}Cl_4 to C_{27}Cl_{27} (Table S4-10).

6.2.4 Quantification and deconvolution

Quantification of the SCCPs, MCCPs, and LCCPs was based on a mathematical algorithm recently applied by Bogdal et al. [10] for the quantification of CPs in various environmental matrices. A brief description of the deconvolution and quantification is given in the S4-3. An example of the deconvolution whereby the CP pattern measured in the sludge sample S2 was reconstructed into a linear combination of patterns of CPs of the technical mixtures is shown in Figure 6-2. As example for the reconstruction, the total MCCP concentration in sample S2 is 3150 ng/g dw, which is attributed to 1082 ng/g dw MCCP 42% Cl, 1582 ng/g dw MCCP 52% Cl and 486 ng/g dw MCCP 57% Cl (Table 6-1).

In our study, the variation in response factors observed for SCCPs, MCCPs, and LCCPs were considerably less depending on their chain length and chlorination degree than in the study of Bogdal et al. [10]. the quantification results are also less impacted by the goodness of fit (R^2) between the CP patterns in the technical mixtures and the sample. With their analytical set-up, Bogdal et al. [10] tested different technical CP mixtures with known concentration and reported that an R^2 of 0.50 for SCCPs results in a quantification difference by a factor of 4 from the expected concentration. For MCCPs with an R^2 > 0.60 the difference between calculated and expected concentration is a factor of 2 and for the LCCPs with R^2 > 0.50. This results in an difference of an factor less than 1.2. Because in our study the response differences for the SCCPs, MCCPs and LCCPs were comparable with those reported for MCCPs and LCCPs by Bogdal et al. [10], the differences between calculated and expected concentration here are also estimated to be below a factor of 2 for a R^2 > 0.50. Therefore, values with an R^2 < 0.50 are reported as tentative values. An example of the measured and reconstructed SCCP, MCCP, and LCCP patterns in three sewage sludge samples are provided in the Figure 6-2 and S4-5 to S4-7.
### Table 6-1 Composition profile of the SCCPs, MCCPs, and LCCPs, sample concentration attributed (ng/g dw), total SCCP, MCCP, and LCCP concentration in ng/g dw, goodness of fit ($R^2$) and the chlorination degree in the sewage sludge samples

| SCCPs percent contribution | Sample conc. (ng/g dw) attributed | Total SCCP conc. (ng/g dw) | $R^2$ | CI-degree |
|---------------------------|----------------------------------|---------------------------|-------|-----------|
| C10 6 22 21 50 | 80 64 12 | <156 | 0.65 | 60.9% |
| C11 28 45 8 19 57 73 0 | 229 738 454 | 1421 | 0.76 | 59.5% |
| C12 10 24 21 45 137 178 0 | 55 30 | <85 | 0.75 | 62.4% |
| C13 10 13 23 54 60 422 202 685 | 0.64 | 58.6% |
| Sample conc. (ng/g dw) attributed | Total SCCP conc. (ng/g dw) | $R^2$ | CI-degree |
|---------------------------|---------------------------|-------|-----------|
| MCCPs percent contribution | Sample conc. (ng/g dw) attributed | Total MCCP conc. (ng/g dw) | $R^2$ | CI-degree |
| C14 47 29 14 9 159 0 | 402 561 | 0.89 | 56.7% |
| C15 45 31 15 8 1082 1582 486 | 3150 | 0.86 | 53.5% |
| C16 32 38 19 11 114 250 345 | 710 | 0.61 | 55.6% |
| C17 51 32 11 6 1303 1656 686 | 3645 | 0.95 | 54.0% |
| Sample conc. (ng/g dw) attributed | Total MCCP conc. (ng/g dw) | $R^2$ | CI-degree |
|---------------------------|---------------------------|-------|-----------|

*Note: CI-degree represents the chlorination degree in the sewage sludge samples.*
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| S12 2 | C14 | 29 | 40 | 19 | 11 | 74 | 266 | 676 | 1016 | 0.60 | 55.2% |
|-------|-----|----|----|----|----|----|-----|-----|------|------|-------|
| S13   | 40  | 36 | 14 | 9  | 0  | 2242| 1208| 3449| 0.81 | 54.6% |
| S14   | 35  | 33 | 18 | 15 | 0  | 338 | 542 |     | 0.68 | 56.3% |
| S15   | 42  | 33 | 15 | 9  | 0  | 974 | 974 |     | 0.84 | 57.5% |
| S16   | 44  | 33 | 14 | 9  | 600| 0   | 875 | 1474| 0.86 | 56.0% |
| Bl1   | 78  | 21 | 1  | 0  | 24 **| 0 **| 0 **| 24 **| 0.93 | 49.5% |
| Bl2   | 78  | 20 | 2  | 0  | 22 **| 0 **| 0 **| 22 **| 0.90 | 49.0% |

#### LCCPs percent contribution

| S1    | C18 | 26 | 13 | 13 | 7  | 6  | 5  | 6  | 7  | 7  | 0  | 116 | 116 | 0.42 | 44.1% |
|-------|-----|----|----|----|----|----|----|----|----|----|----|-----|-----|------|-------|
| S2    | 29  | 10 | 10 | 6  | 8  | 7  | 10 | 7  | 6  | 7  | 129| 317 | 446 | 0.64 | 42.3% |
| S3    | 29  | 12 | 9  | 5  | 7  | 4  | 10 | 9  | 7  | 7  | 0  | 156 | 156 | 0.47 | 44.9% |
| S4    | 16  | 6  | 6  | 4  | 5  | 4  | 5  | 4  | 4  | 45 | 260| 554 | 814 | 0.05 | 39.9% |
| S5    | 22  | 11 | 10 | 5  | 7  | 11 | 11 | 10 | 6  | 7  | 0  | 364 | 364 | 0.29 | 45.0% |
| S6    | 34  | 12 | 11 | 5  | 8  | 6  | 7  | 6  | 6  | 6  | 0  | 349 | 349 | 0.71 | 45.1% |
| S7    | 32  | 18 | 12 | 6  | 8  | 7  | 6  | 5  | 4  | 3  | 0  | 154 | 154 | 0.50 | 48.3% |
| S9    | 18  | 8  | 8  | 6  | 9  | 12 | 13 | 11 | 9  | 6  | 172| 788 | 960 | 0.32 | 42.7% |
| S10   | 27  | 12 | 11 | 6  | 9  | 10 | 10 | 7  | 4  | 4  | 0  | 305 | 305 | 0.57 | 43.9% |
| S11   | 30  | 11 | 8  | 6  | 7  | 8  | 10 | 7  | 7  | 5  | 0  | 250 | 250 | 0.53 | 47.1% |
| S12   | 11  | 6  | 4  | 5  | 8  | 13 | 17 | 15 | 12 | 9  | 0  | 625 | 625 | 0.11 | 42.5% |
| S12 2 | 12  | 5  | 4  | 4  | 7  | 14 | 17 | 17 | 11 | 9  | 0  | 527 | 527 | 0.09 | 43.2% |
| S13   | 31  | 12 | 8  | 6  | 7  | 10 | 10 | 7  | 6  | 4  | 0  | 683 | 683 | 0.67 | 46.0% |
| S14   | 24  | 13 | 12 | 6  | 8  | 8  | 10 | 7  | 6  | 6  | 0  | 137 | 137 | 0.33 | 47.2% |
| S15   | 24  | 13 | 11 | 7  | 9  | 9  | 8  | 7  | 6  | 6  | 0  | 398 | 398 | 0.40 | 46.5% |
| S16   | 29  | 14 | 13 | 7  | 8  | 6  | 7  | 7  | 5  | 5  | 0  | 270 | 270 | 0.51 | 46.4% |
| Bl1   | 0   | 0  | 0  | 0  | 0  | 0  | 42 | 29 | 29 | 0  | 0.2**| 0.4 | 0.00 | 33.2% |
| Bl2   | 0   | 0  | 0  | 0  | 0  | 0  | 21 | 41 | 38 | 0  | 0.3**| 0.5 | 0.00 | 33.4% |

*levels for the blanks are reported in ng abs. Italic/blue = R² lower than 0.5 therefore reported as tentative values.
6.2.5 Performance characteristics of the analytical method

Three recovery standards, containing all eight commercial CP standards with known concentrations (120 to 160 ng absolute), were included in this study and underwent the same treatment as the fifteen sewage sludge samples. Acceptable recoveries were observed for all three CP categories with mean SCCPs, MCCPs, and LCCPs recoveries of 79%, 81%, and 92%, respectively (Tables S4-3, S4-5 and S4-7). The goodness of fit ($R^2$) was higher than 0.93 for all mixtures. Although acceptable recoveries were observed for the CP categories (SCCPs, MCCPs, LCCPs), the recoveries of the lowest chlorinated CP formulations within a CP category were somewhat low, with recoveries down to 56% for the SCCP 51.5% Cl, and down to 42% for the MCCP 42% Cl. These lower recoveries are related to the somewhat reduced sensitivity of the APCI-qToF-HRMS for CPs with a lower chlorination degree and shorter chain length.

Matrix interferences were investigated by spiking four of the analysed sewage sludge samples with environmentally realistic concentrations of three SCCP, three MCCP, and two LCCP mixtures in the concentration (120-160 ng added to ca. 0.2 gram sewage sludge). The mean recoveries of the four SCCP, MCCP, and LCCP-spiked sewage sludge samples ranged from 86-123%, and the $R^2$ was higher than 0.93, with the exception of the LCCP-spiked S2 sample, which had an $R^2$ of 0.54 (Tables S4-4, S4-6 and S4-8). Overall, the results of the spiked sewage sludge samples were comparable with the results of the recovery standards which indicate that the results were not influenced by matrix interferences.

The stability of the APCI-qToF-HRMS was investigated by performing an 8-fold measurement of a cleaned S9 sewage sludge sample (Table S4-9). Whereas Bogdal et al. [10] reported that multiple injections are required to obtain a good estimate of the average response factor (RF), in this study an injection standard ($^{13}$C$_6$-PCP) was applied to correct for the variability. The relative standard deviation (RSD) of the injection standard ($^{13}$C$_6$-PCP) of the 8-fold sewage sludge sample was 5%. The RSD of the sum of the CP congeners corrected for the internal standard was 5%, 2% and 4%, respectively for the SCCPs (685 ng/g dw), MCCPs (3192 ng/g dw) and LCCPs (960 ng/g dw). The calculated RSDs indicate that the variability of the APCI-qToF-HRMS was low, and by using an injection standard, multiple injections were not needed. As expected at levels close to the LOQ (peak area around 200 counts), the RSD went up to a maximum of 46%, and decreased to 20% if the peak area increased 10 times and decreased to 5% if the peak area increased 100 times (Table S4-9). One sewage sludge sample (S12) was also extracted, cleaned, and analysed in duplicate. Comparable levels were observed for this duplicate sample with SCCPs of <86 and <60 ng/g dw, MCCPs of 995 and 1016 ng/g dw, and LCCPs of 625 and 527 ng/g dw (Table 6-1).

Regardless of the effort to reduce background contamination (i.e. aluminum oxide was heated to 250 °C; silica was pre-rinsed with dichloromethane and methanol before use; and all glassware was rinsed with both n-hexane and acetone prior to use) low CP levels were observed in the two blank samples (Table 6-1). The total CP levels observed
in the two blanks were up to 16 ng absolute for the SCCPs, up to 24 ng absolute for the MCCPs, and up to 0.5 ng absolute for the LCCPs. Therefore, all CP values observed in the sewage sludge samples were blank corrected, and only the corrected levels higher than 3 times the blank were reported in this study. All MCCP and LCCP values observed in the fifteen sewage sludge samples were at least 10 times higher than the CP values in the blank. For some SCCP values, the levels were lower than 3 times the blank and therefore reported as <LOQ. The CP patterns observed in the blanks were different from the patterns observed in the sewage sludge samples, which indicates that the contamination is not related to cross-contamination during sample treatment but more likely to another source(s) (Figure 6-3 and Figure S4-1). The patterns in the blank were mainly dominated by the shorter chain CPs, C10 and C11 for the SCCPs and C14 for the MCCPs.

The chlorination degree of the eight standard technical CP formulations we measured in this study were compared with the stated chlorination degree of the manufacturer (Table S4-2). For the higher chlorinated CP formulations, the calculated chlorination degree was close to the stated chlorination degree. However, for the lower chlorinated CP formulations, the calculated values were somewhat higher (SCCP; 51.5% Cl, calculated Cl 55.9%, MCCP; 42% Cl, calculated 49.4% Cl, LCCP; 36% Cl, calculated 41.4% Cl). Congener group patterns of the eight technical CP mixtures are shown in Figure S4-2 to 4. Bogdal et al. [10] reported that the sensitivity of the APCI-qToF-HRMS method increased with chain length and chlorination degree. The authors observed a difference in response by a factor of 50 between the SCCP 49% Cl and the SCCP 70% formulations; whereas, the response of MCCP 45% Cl and MCCP 56% Cl formulations differed only by a factor 4. In our study we used the same analytical technique, but with different instrumental equipment and adapted instrumental set-up conditions. The differences in sensitivity in our study was less depending on the chain length and chlorine degree. For example, the differences in response between SCCP 51.5% Cl and SCCP 63% Cl was only a factor of 1.9, between MCCP 42% Cl and MCCP 57% a factor 3.2, and between LCCP 36% Cl and LCCP 49% a factor of 1.2. This indicates that the sensitivity of the APCI-qToF-HRMS method is influenced by the analytical set up and the instrumental equipment used.

Calibration standards were prepared in ACN from the eight CP stock solutions and ranged from 0.1 ng/μL to 10 ng/μL for the SCCPs and MCCPs and from 0.05 ng/μL to 10 ng/μL for the LCCPs. The linear range of the calibration curve were determined using the Excel linear regression tool. The linear range of the calibration curve for the SCCPs and MCCPs ranged from 0.1-10 ng/μL; whereas for the LCCP, it ranged from 0.05-5.0 ng/μL with an R² > 0.99. For the SCCPs, the lowest concentration of the calibration curve, 0.1 ng/μL, corresponds to the LOQ, (10 times the signal/noise), while for both the MCCPs and the LCCPs, the LOQ could easily be lowered by a factor of 4. However, all ΣSCCPs and ΣMCCPs concentrations detected in the sewage sludge samples were above 0.1 ng/μL and above 0.05 ng/μL for the LCCPs.
6.3 Results and discussion

6.3.1 CP concentrations in Australian sewage sludge

MCCPs were the dominant CPs and were detected in all sludge samples with concentrations ranging from 540 to 3,600 ng/g dw (Table 6-1). In eight of the fifteen samples SCCPs were detected with concentrations ranging from <57 to 1,400 ng/g dw. The LCCPs concentrations (120-960 ng/g dw) were comparable to the SCCP concentrations (Table 6-1). Nine of the fifteen LCCPs levels were reported as tentative values due to a deconvolution result with an $R^2 < 0.50$. The results of the total CPs (sum of SCCPs, MCCPs and LCCPs) detected in sewage sludge collected at the various WWTPs around Australia are illustrated in Figure 6-1. The highest total CP concentration (5,880 ng/g dw) were detected in sewage sludge from location S4 in the Northern Territory of Australia. However, elevated levels were also detected in South-East Queensland (location S2; 4,400 ng/g dw), South Australia (location S9; 4,700 ng/g dw) and Western Australia (location S13; 4,600 ng/g dw). The total CP concentrations were higher than the mean $\alpha$, $\beta$, $\gamma$-HBCDD concentrations (0.11-130 ng/g) and mean BDE209 concentrations (67-1,300 ng/g dw) detected in the same sewage sludge samples. BDE209 was the predominant PBDE and contributed to 61%-88% of the $\sum$PBDE [16].

To our knowledge, this is the first time that CPs were detected in Australian sewage sludge. The SCCP and MCCP concentrations observed in the Australian sewage sludge were comparable with the SCCP and MCCP concentrations observed in Swedish sewage sludge collected between 2004 and 2010 (median SCCPs; 1,100 ng/g dw, median MCCPs; 3,800 ng/g dw)[17], Czech sludge samples collected in 2004 near a chemical company in Usti (SCCPs ranged from 210 to 400 ng/g dw, MCCPs ranged from 740 to 2,300 ng/g dw) [18], and Swiss sewage sludge collected in 2007 around Zürich (SCCP; 140-580 ng/g dw, MCCPs; 1,100-9,000 ng/g dw) [10]. Maulshagen et al. [19] measured only SCCPs (75-860 ng/g dw) in sewage sludge from Germany collected in the early 2000s, which were also comparable with the SCCP concentrations observed in our study. Higher SCCP concentrations were found in municipal and industrial sewage sludge in China with concentrations from 800 to 53,700 ng/g dw [15]. The highest CP concentrations were found in municipal and industrial sewage sludge from the UK reported by Stevens el al. [5], with SCCP concentrations ranging from 6,900-200,000 ng/g dw and $\Sigma$MCCPs concentrations from 30-9,700 ng/g and by Nicholls et al., [20] with $\Sigma$SCCPs and $\Sigma$MCCPs concentrations ranged from 1,800 to 93,100 ng/g dw. These concentrations were up to 200-fold higher than those reported in our study.

Data about the longer chain CPs in sewage sludge are limited. Only Olofsson et al. [17] has reported LCCPs in sewage sludge samples from Sweden with median concentrations of 31,000 ng/g dw. The LCCP concentrations were 8-fold higher than the median MCCPs reported in that study and 28-fold higher than the median SCCPs in that study. These concentrations are 300-fold higher than the LCCP concentrations observed in Australian sewage sludge in this study. This may indicate a higher usage and demand.
of CPs in Sweden compared to Australia. Other reasons for the difference could be a different composition of the technical CP mixtures, or a different treatment of WWTP system.

Despite the huge challenges and uncertainties in quantification of CPs, the order of magnitude differences observed indicate that point sources of CPs can be very important. In two studies [5, 15], no correlation was observed between the CP concentration and the WWTP location, treatment capacity and serving population. In our study, the correlation between the CP concentrations in the sewage sludge samples and the WWTP size (population), geographical location (Southern Queensland versus Northern Queensland) and sewage sludge treatment (anaerobic versus aerobic/activated) was investigated by comparing the CP concentrations (multiple t test). Taken into account the relative small data set, only a significant higher mean CP concentration was observed in the anaerobic treated sewage sludge compared to the aerobic/activated sludge treatment (p < 0.05). However, other factors may have influenced this result; as for example, all anaerobic WWTPs were of a large size. A more extensive study is needed to draw more solid conclusions regarding CP concentrations and WWTP characteristics.

6.3.2 Carbon and chlorine homologue groups of CPs in sewage sludge

The carbon and chlorine homologue groups for the SCCPs, MCCPs and LCCPs in the Australian sewage sludge are given in Figure 6-3 and Figure S4-1. Among the sewage sludge samples collected at different locations in Australia, similar carbon and chlorine homologue patterns were observed for the SCCPs, MCCPs, and LCCPs. The calculated chlorination degree for the sewage sludge ranged from 57.8-63% for the SCCPs, from 53.5-57.5% for the MCCPs, and from 39.9-48.3% for the LCCPs. This finding was consistent with the study of Zeng et al. [15], who studied the SCCP composition in various sewage sludge samples from China and observed similar carbon and chlorine homologue profiles among different WWTPs. However, the SCCP homologue pattern observed in the sewage sludge from China was different than the pattern observed in the Australian sewage sludge. In the Chinese sludge, the SCCP were dominated by C_{11} (37%) followed by C_{10} (27%) and C_{12} (23%) with Cl_7 (37%) and Cl_8 (29%), whereas in our study the SCCP were dominated by C_{13} (51%) followed by C_{12} (24%) and C_{11} (18%) with Cl_7 (37%) and Cl_6 (26%). Taking into account the different instrumental techniques used, these findings may indicate that the CP composition of the commercial SCCP mixtures used in China are different than used in Australia. CPs in China are not divided in SCCPs, MCCPs and LCCPs, but into different technical products. The most common technical products are CP-42 (42% Cl), CP-52 (52% Cl) and CP-70 (70% Cl), CP-42 and CP-52 are the most widely produced in China (over 80%) in 2005 and contain apparently 3-4% and 25-40% SCCPs, respectively [21, 22]. Zeng et al. [15] only detected some MCCPs in sewage sludge from China, and these were at low concentrations, which is in contrast to our study, in which MCCPs were dominant. This may indicate that MCCPs are probably used in lesser amounts in China compared to
Australia. However, in soil and sediment of highly industrial areas in China (Pearl River Delta), higher MCCP levels were observed compared to SCCPs, and the opposite was observed across more rural areas [23, 24]. This, together with an increased MCCPs/SCCPs ratio in a sediment core in time, suggests an increased usage to MCCPs in recent years in the Pearl River Delta [24]. Overall, the carbon homologue group patterns for the SCCPs and MCCPs in the Australian sewage sludge were more comparable with the patterns found in sewage sludge from the UK and Switzerland [5, 10] which were also dominated by C_{11}-C_{13} for the SCCPs and C_{14}-C_{15} for the MCCPs.

Until today, LCCPs were only reported by Olofsson et al. [17] in Swedish sewage sludge as total LCCP concentration (median of 31,000 ng/g dw) and by Bogdal et al. [10] in sewage sludge from Zürich in Switzerland with tentative levels varying from 18 - 99 ng/g dw. The total LCCP levels reported by Bogdal et al. [10] were only based on the C_{19}-C_{27} congener groups. The C_{18} was excluded by Bogdal et al. [10] after studying the complete C_{10}-C_{27} pattern whereby the technical MCCP (52% Cl) formulation contained up to 10% of C_{18} of the LCCP congener groups. They observed that after subtracting the C_{18} congener group, the remaining pattern in the sewage sludge was more comparable with the pattern in the technical LCCP formulations (40, 49, and 70% Cl), which would improve the deconvolution process. In both standard technical LCCP mixtures (36% Cl and 49% Cl) used in this study, C_{18} was the most dominant congener group (see Figure S4-4). Also, the technical standard MCCP mixtures (42%, 52% and 57% Cl) used in our study contain only up to 1.3% LCCPs as an impurity. Therefore, LCCPs could be calculated through deconvolution using only two standard LCCP technical mixtures (36% Cl and 49% Cl) instead of three for the SCCPs and MCCPs (Figure 6-2). LCCP homologue groups in the Australian sewage sludge were dominated by C_{18} (30%) followed by C_{19} (13%) and C_{20} (10%), with Cl_{7} (22%), Cl_{8} (18%), Cl_{6} (17%) and Cl_{4}, Cl_{5}, Cl_{9} (each 11%). The only two exceptions were sewage sludge sample S4 from the Northern Territory of Australia, which had a pattern dominated by C_{23} (45%), and sewage sludge sample S12 from South Australia, which was dominated by C_{23}-C_{26}. No clear explanation could be given, but it may be related to local sources. The LCCPs technical mixtures contain mainly C_{18}-C_{20} and almost no long-chain LCCPs (C_{21}-C_{27}). Therefore, samples which are dominated by the longer chain LCCPs (C_{21}-C_{27}), cannot be entirely reconstructed by the LCCP technical mixtures. This explains the low R^2 calculated between the measured and reconstructed pattern for the sewage sludge samples S4 and S12, which were dominated with the longer chain LCCPs. For both samples the LCCP levels are reported as tentative levels.

This is the first study that applies the promising and particularly fast instrumental technique, recently developed by Bogdal et al. [10], routinely for simultaneously quantifying and profiling SCCPs, MCCPs, and LCCPs in Australian sewage sludge. Due their long-range transport, bioaccumulation and persistent potency, SCCPs are already regulated and phased-out in Europe and listed by the Stockholm Convention [25, 26]. However, LCCPs and MCCPs are hardly regulated and the knowledge about
them is poor. First studies show that the MCCP/SCCP ratios in sediment, soil, and biota increased in recent years, which may be influenced by the replacement of SCCPs by MCCPs [23, 24, 27]. However, data on MCCPs and LCCPs in the environment are rare, and more research is needed to further understand the risk and fate of CPs in the environment. Especially, because more than 59% of all the sewage sludge in Australia is reused in agriculture and through landfilling, the CPs re-enter into the environment [16].

**Figure 6-2:**
An example of chlorinated paraffins (SCCPs, MCCPs and LCCPs) patterns in sewage sludge sample S2. For each chlorinated paraffin group (SCCPs, MCCPs, and LCCPs) the measured pattern is given (A, C, E) and the reconstructed pattern based on deconvolution of the technical chlorinated paraffin mixtures (B, D, F). Deconvolution of the SCCP pattern in the sewage sample was performed with the 51.5% Cl, 55.5% Cl and 63% SCCP standards, for the deconvolution of the MCCP pattern the 42% Cl, 52% Cl and 57% Cl MCCP standards and for the deconvolution of the LCCP pattern the 36%Cl and 49% CL LCCP standards were used. The goodness of fit (R²) for the deconvolution is also given.
Figure 6-3:
Composition of MCCPs in the fifteen sewage sludge samples and the two blanks (the composition of the SCCPs and LCCPs is given in Figure S4-1). Composition based on carbon number (left) or chlorine number (right).

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Chapter 6

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