

ANALYSIS OF SHORT-CHAIN CHLORINATED PARAFFINS (SCCPs) BY GC-HRMS (NCI) WITH GC-HRTOF-MS APPLIED FOOD SAMPLE

Takasuga T¹, Matsukami H¹, Nouda C¹, Harada K² and Koizumi A²

¹ Shimadzu Techno Research, INC., 2-13, Nishinokyo-Sanjo-Bocho, Nakagyo-ku, Kyoto 604-8435, Japan

² Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan

Introduction

Chlorinated paraffins (CPs) including short chain CPs (SCCPs, C10–13) are industrial products used as metal-working fluids and flame retardants for plastic materials (1). SCCPs seem to persist in the environment and bioaccumulate in biota, and are under review by the Stockholm Convention on persistent organic pollutants. SCCPs have been produced in the USA, Europe, Japan, India, China and other countries (2). The production volumes of SCCPs were 1,500–2,500 tonnes in the European Union (EU) in 2006, 8,800 tonnes in the USA in 2005 and 502 tonnes in Japan in 2001 (2,3). In Japan, since 2005 and metal-working industries voluntarily phased out the use of SCCPs by 2007. In contrast, the production of total CPs in China has continued to increase, reaching 600,000 tonnes in 2007 (4). Although the huge production and use of CPs in China could imply potential contamination of various media, there is little information on exposure to SCCPs (5). Dietary intake is considered to be the major route of exposure to hydrophobic pollutants, such as polychlorinated biphenyls and brominated diphenyl ethers (6,7). However, there was limited information on dietary intake of SCCPs. A previous survey in Japan was conducted only in 2003 (8). In the present study, the analytical methods for SCCP were investigated in detail and applied for dietary samples.

Materials and methods

Chemicals. Polychlorinated decanes (44.82%, 55.00% and 65.02% Cl contents), undecanes (45.50%, 55.20% and 65.25% Cl contents), dodecanes (45.32%, 55.00% and 65.08% Cl contents) and tridecanes (44.90%, 55.03% and 65.18% Cl contents) were obtained from Dr. Ehrenstorfer GmbH, Germany and used as reference solution for quantification. 13 individual isomer standards of polychlorinated decanes, i.e., 1,1,1,3-tetrachlorodecane, 2,5,6,9-tetrachlorodecane, 1,2,9,10-tetrachlorodecane, 1,2,5,6,9-pentachlorodecane, 1,1,1,3,9,10-hexachlorodecane, 1,2,4,5,9,10-hexachlorodecane, 1,2,5,6,9,10-hexachlorodecane, 1,2,5,6,9,10-hexachlorodecane, 1,2,4,5,6,9,10-heptachlorodecane, 1,2,5,5,6,9,10-heptachlorodecane, 2,3,4,5,6,7,8,9-octachlorodecane, 1,1,1,3,8,10,10,10-octachlorodecane and 1,2,3,4,5,6,7,8,9-Nonachlorodecane, were obtained from Dr. Ehrenstorfer GmbH and Chiron AS (Norway) and used for analysis of response factor. ¹³C₁₂-2,3,3',5,5'-pentachlorobiphenyl (CB-111; CIL) was used as the internal standard for the SCCPs.

Extraction, clean-up procedure and instrumental analysis.

The chemical analysis was conducted as previously reported (8,9) with several modifications.

Crude extracts were concentrated up to 20 mL by rotary evaporator. 2 mL of each extracts was subjected to 8 g of activated florisil column chromatography and eluted with 90 mL of 20% of dichloromethane in hexane.

Eluate was concentrated to 0.1 mL, spiked ¹³C₁₂-PeCB-#111, and measured by GC/HRMS-NCI.

An GC-HRMS system was used, which comprised a Hewlett-Packard 6890 Series Gas Chromatograph connected to a Thermo Fisher Scientific Finnigan MAT 95 XL (Thermo Fisher Scientific Inc.). A short and thin capillary column (DB-5MS; 15 m × 0.25 mm i.d., 0.1-μm film thickness; Agilent Technologies) was employed. The GC-HRMS conditions are Reaction gas: Methane (2.5 × 10⁻⁴ mbar), Ion source temperature: 130°C Interface temperature: 300°C. All analytes were quantified by comparing the peak area of the particular compound in sample extracts with that of the internal standard (¹³C₁₂-labeled CB-111). The highest peak in the [M-Cl]⁺ ion group was employed as the quantification ion as previously described (10).

Quantification. Previous report by Zencak et al. (11) showed that quantification using reference SCCPs solution with different chlorine content caused errors greater than 100% in ECNI/MS because response factors of each

congeners highly depends on chlorine numbers. In this study, firstly, 13 isomer standards of polychlorinated decanes were analyzed and relationship between chlorine number and response factor were evaluated. Peak areas of each chlorinated decane isomers were compared between time-of-flight mass spectrometry with electron impact ionization (EI/MS) and ECNI/MS modes. Total ion chromatograms (TIC) monitored ions of m/z 50-550, $[M-2H_3Cl]^+$ and $[M-Cl]^-$ were chosen as relatively specific ion fragments in EI/MS and ECNI/MS modes, respectively.

45%, 55%, 65% Cl and their 1:1:1 mixtures were prepared for each carbon chain length (C10–13). Composition of congeners with different chlorine number was investigated in four different reference solutions. Chlorine content was calculated based on peak areas of TIC peaks (m/z 50-550) in EI/MS and then compared with contents certified by a manufacturer.

A 1:1:1 mixtures of SCCPs with 45%, 55% and 65% Cl contents was diluted and used for calibration curves of each congener based on composition determined by EI/MS.

Quality control. Instrumental detection limits (IDL) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of 3. Since procedural blank samples contain no detectable concentrations of SCCPs, the method detection limits (MDL) value was considered to be equal to the IDL.

The recoveries were evaluated by seven replicate fortifications (fortified by 100 ng of polychlorinated decanes and polychlorinated undecanes or 200 ng of polychlorinated dodecanes and polychlorinated tridecanes) of a food composite sample with low contamination.

Procedural blanks were processed in parallel with every batch of seven samples to check for interference or contamination by solvents and glassware.

Results and discussion

Quantification and QA/QC. In EI/MS, difference in TIC peak areas of isomers with different chlorine number and position was within 4-fold whereas ionization efficiency significantly differed in ECNI/MS. TIC in EI/MS was considered to provide relatively comparable response to different chlorine numbers of SCCPs and thus employed to quantify SCCPs with different chlorine contents in the reference solutions.

To validate the above principle, we planned to determine compositions of congeners with different chlorine numbers. Good agreements of calculated chlorine contents with nominal chlorine contents, was assumed to indicate accuracy in analysis of composition. In EI/MS, the congener composition of SCCPs was assumed to be proportional to the peak area percent of TIC. Reference solution with chlorine content of 45% containing 2 and 3 chlorinated congeners showed deviations between calculated content and nominal content (56.04% vs 44.82% in polychlorinated decanes, respectively), which indicated 2 or 3 chlorinated SCCPs might have low responses in EI/MS mode. On the other hand, reference solutions with 55% and 65% Cl provided good agreement between calculated content and nominal content (56.67% vs 55.00% for 55% solution; 65.62% vs 65.02% for 65% solution in polychlorinated decanes, respectively). Furthermore, 1:1:1 mixtures of SCCPs with 45%, 55% and 65% Cl contents were analyzed in EI/MS. Although calculated chlorine contents in 1:1:1 mixtures of SCCPs indicated slightly higher than nominally estimated content possibly due to low response in 2 and 3 chlorinated congeners, its deviation was within 5% (60.03% vs 54.95% in polychlorinated decanes). Polychlorinated undecanes, dodecanes and tridecanes also showed similar results to polychlorinated decanes. Therefore, composition of 1:1:1 mixtures of SCCPs with 45%, 55% and 65% Cl contents was considered to be optimum for calibration curves because it contained fair amounts of 5 to 9 chlorinated congeners without losing agreement between calculated and nominal chlorine contents.

The calibration curves for quantification in ECNI/MS were made using peak areas of $[M-Cl]^-$ ion chromatogram of each congener. It was assumed that congener concentrations in the 1:1:1 mixtures of SCCPs were proportional to percentage of peak areas determined in EI/MS: e.g. a 100 ng/mL of 1:1:1 mixtures for decans was assumed to contain C10H17Cl5 at 28.93 ng/mL because its composition was calculated to be 28.93% based on the area of TIC. The 1:1:1 mixtures of SCCPs with five difference dilutions were analyzed and values were plotted using a linear fit (Table 1). Linearity (r) was higher than 0.998 through the congeners.

The method detection limits (MDLs) in ECNI/MS are listed in Table 2. Nona-chlorinated congeners were the most sensitive while penta-chlorinated congeners showed the highest MDLs for each carbon chain length. Recoveries ranged from 81% to 134% (Table 2). Since there was no available isotope labeled standard for SCCPs, internal standard ($^{13}C_{12}$ -labeled CB-111) was applied in this analysis. There were no detectable residues

in any of the procedural blanks (n=9). In addition, analysis of wash out with hexane from a control vacant container revealed SCCPs at less than detection limits.

For comparisons with other laboratories, composition of technical mixture, Chlorowax 500C was determined. Its calculated chlorine content was 59.80% which was comparable to product specification (58%).

Table 1. Calibration curves and detection limits for SCCPs in 1:1:1 mixture of each carbon chain solution in NCI/MS

SCCPs congeners	Range ^a (ng mL ⁻¹)	Calibration curves (Y=ax+b)			Instrument detection limit ^b (pg μL ⁻¹)	Reproducibility RSD (%) ^c (n=7)
		Slope (a)	Intercept (b)	Linearity (r)		
C ₁₀ H ₁₇ Cl ₅	29-579	0.00131	-0.000791	0.9999	7.9	5.9
C ₁₀ H ₁₆ Cl ₆	4.8-477	0.00947	-0.0138	0.9996	2.2	12.8
C ₁₀ H ₁₅ Cl ₇	3.6-357	0.0369	-0.0563	0.9997	0.5	4.8
C ₁₀ H ₁₄ Cl ₈	1.9-192	0.0464	-0.0399	0.9997	0.4	6.3
C ₁₀ H ₁₃ Cl ₉	0.44-44	0.0612	-0.0123	0.9994	0.15	9.8
C ₁₁ H ₁₉ Cl ₅	23-458	0.000854	0.00314	0.9996	8.1	8.1
C ₁₁ H ₁₈ Cl ₆	23-453	0.00666	0.00122	0.9999	4.2	4.5
C ₁₁ H ₁₇ Cl ₇	3.0-303	0.0187	-0.0146	0.9998	0.9	8.6
C ₁₁ H ₁₆ Cl ₈	2.6-261	0.0302	-0.0169	0.9996	0.7	7.0
C ₁₁ H ₁₅ Cl ₉	0.7-70	0.0695	-0.0047	0.9998	0.2	6.5
C ₁₂ H ₂₁ Cl ₅	40-399	0.000667	-0.00149	0.9995	11.1	7.0
C ₁₂ H ₂₀ Cl ₆	45-446	0.00548	-0.00810	0.9998	7.8	4.2
C ₁₂ H ₁₉ Cl ₇	9.3-373	0.0187	-0.0260	0.9984	2.6	7.5
C ₁₂ H ₁₈ Cl ₈	5.9-237	0.0302	-0.0423	0.9994	1.4	6.9
C ₁₂ H ₁₇ Cl ₉	3.9-155	0.0695	-0.0577	0.9990	0.8	5.6
C ₁₃ H ₂₃ Cl ₅	40-404	0.000425	-0.000714	0.9991	17.6	9.5
C ₁₃ H ₂₂ Cl ₆	42-425	0.00357	0.00332	0.9998	13.9	7.9
C ₁₃ H ₂₁ Cl ₇	9.5-378	0.00767	0.000342	0.9990	3.8	8.7
C ₁₃ H ₂₀ Cl ₈	7.5-298	0.014	-0.0171	0.9995	2.8	9.7
C ₁₃ H ₁₉ Cl ₉	4.7-188	0.0267	-0.0243	0.9997	0.9	5.0

^a 20, 100, 200, 500, 1000 and 2000 ng/mL of polychlorinated decanes and undecanes; 50, 200, 500, 1000 and 2000 ng/mL of polychlorinated dodecanes and tridecanes

^b 2 μl injection in ECNI/MS

^c RSD: relative standard deviation

References:

- (1) *alkanes, C10-13, chloro*; European Union Risk Assessment Report 2008, Volume: 81; European Chemicals Bureau; 2008
- (2) *Study contract on "support related to the international work on Persistent Organic Pollutants (POPs)" management option dossier for Short Chain Chlorinated Paraffins (SCCPs), 12 June 2007"*; NV.D.1/SER/2006/0123r; DG Environment; European Commission; 2007.
- (3) Nakanishi, J.; Tsunemi, K. *Short-chain Chlorinated paraffins*; Maruzen Co., Ltd.; Tokyo, Japan, 2005.
- (4) Yuan, B.; Wang, Y.; Fu, J.; Jiang, G. Evaluation of the pollution levels of short chain chlorinated paraffins in soil collected from an e-waste dismantling area in China. *Organohalogen Compd.* 2009, 71, 3106-3109.
- (5) Yuan, B.; et al. An analytical method for chlorinated paraffins and their determination in soil samples. In *Chinese Science Bulletin*; Science China Press, co-published with Springer, 2010; Vol. 55, pp 2396-2402.
- (6) Koizumi, A.; et al. Paradoxical increases in serum levels of highly chlorinated PCBs in aged women in clear contrast to robust decreases in dietary intakes from 1980 to 2003 in Japan. *Environ. Health Prev. Med.* 2009, 14, 235-246.

- (7) Wada, Y.; et al. Secular trends and geographical variations in the dietary intake of polybrominated diphenyl ethers (PBDEs) using archived samples from the early 1980s and mid 1990s in Japan. *J. Occup. Health* 2005, 47, 236-241.
- (8) Iino, F.; Takasuga, T.; Senthilkumar, K.; Nakamura, N.; Nakanishi, J. Risk assessment of short-chain chlorinated paraffins in Japan based on the first market basket study and species sensitivity distributions. *Environ. Sci. Technol.* 2005, 39, 859-866.
- (9) Takasuga, T.; Iino, F.; Abe, M.; Yoshida, K.; Nakanishi, J.; Senthilkumar, K. Preliminary Study of Polychlorinated n-Alkanes in Standard Mixtures, River Water Samples from Japan by HRGC-HRMS with Negative Ion Chemical Ionization. *Organohalogen Compd.* 2003, 60, 424-427.
- (10) Tomy, G. T.; Stern, G. A.; Muir, D. C. G.; Fisk, A. T.; Cymbalisty, C. D.; Westmore, J. B. Quantifying C10-C13 polychloroalkanes in environmental samples by high-resolution gas chromatography electron capture negative ion high resolution mass spectrometry. *Anal. Chem.* 1997, 69, 2762-2771.
- (11) Zencak, Z.; Borgen, A.; Reth, M.; Oehme, M. Evaluation of four mass spectrometric methods for the gas chromatographic analysis of polychlorinated n-alkanes. *J Chromatogr A* 2005, 1067, 295-301.

Table 2. Recovery and detection limits for SCCPs analysis in food composite samples

SCCPs congeners	Method	non-fortified sample		Concentration of fortified sample (pg g ⁻¹) (n=7)	Recovery and (reproducibility) % (RSD%) ^b (n=7)
	detection limit ^a (ng mL ⁻¹)	^a (pg g ⁻¹)	Fortified amount (pg)		
C ₁₀ H ₁₇ Cl ₅	400	ND	46000	2153±42	94 (1.9)
C ₁₀ H ₁₆ Cl ₆	200	ND	20000	895±33	90 (3.6)
C ₁₀ H ₁₅ Cl ₇	50	ND	3900	182±7.2	93 (4.0)
C ₁₀ H ₁₄ Cl ₈	20	ND	620	33±1.7	106 (5.2)
C ₁₀ H ₁₃ Cl ₉	10	ND	230	13±1.3	117 (9.8)
total C ₁₀ Cl _x	400	ND	71000	3276±68	92 (2.1)
C ₁₁ H ₁₉ Cl ₅	500	ND	40000	2227±97	111 (4.4)
C ₁₁ H ₁₈ Cl ₆	300	ND	42000	2257±70	107 (3.1)
C ₁₁ H ₁₇ Cl ₇	100	ND	15000	752±33	100 (4.4)
C ₁₁ H ₁₆ Cl ₈	50	ND	2700	145±4.2	107 (2.9)
C ₁₁ H ₁₅ Cl ₉	20	ND	120	<20	-
total C ₁₁ Cl _x	500	ND	100000	5390±159	108 (2.9)
C ₁₂ H ₂₁ Cl ₅	600	ND	69000	3905±169	113 (4.3)
C ₁₂ H ₂₀ Cl ₆	400	ND	78000	4326±139	111 (3.2)
C ₁₂ H ₁₉ Cl ₇	200	ND	44000	2619±56	119 (2.1)
C ₁₂ H ₁₈ Cl ₈	100	80	8900	522±14	99 (3.2)
C ₁₂ H ₁₇ Cl ₉	50	71	740	116±5.2	124 (11.3)
total C ₁₂ Cl _x	600	151	200000	11488±212	115 (1.8)
C ₁₃ H ₂₃ Cl ₅	900	ND	63000	3985±222	127 (5.6)
C ₁₃ H ₂₂ Cl ₆	700	ND	69000	3915±181	113 (4.6)
C ₁₃ H ₂₁ Cl ₇	300	ND	46000	2905±86	126 (3.0)
C ₁₃ H ₂₀ Cl ₈	200	ND	17000	1139±38	134 (3.3)
C ₁₃ H ₁₉ Cl ₉	50	84	4100	250±9.3	81 (5.6)
total C ₁₃ Cl _x	900	84	200000	12194±240	122 (2.0)

^a 2 µl injection in ECNI/MS

^b 20 g food composite sample was extracted and 2 mL aliquote was analyzed.

^c RSD: relative standard deviation