Chlorinated paraffins in dietary supplement oil capsules from the German market

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Introduction

Chlorinated paraffins (CPs) are a highly complex mixture of polychlorinated *n*-alkanes which are usually subdivided into short-chain chlorinated paraffins (SCCPs, C_{10}-C_{13}), medium-chain chlorinated paraffins (MCCPs, C_{14}-C_{17}) and long-chain chlorinated paraffins (LCCPs, >C_{20}).[1] Like other anthropogenic halogenated substances, CPs were detected in the environment all over the world.[2,3] In addition to the exposure via inhalation of dust,[1] one major pathway for human exposure is food.[4]

Dietary supplements (DS) are concentrates of nutrients that are consumed alongside the regular diet.[5] Total DS sales accounted for 1.2 billion euros in Germany in 2015.[6] Several times, DS were analyzed for polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexabromododecane (HBCD), other brominated flame retardants (BFRs) and halogenated pesticides.[7–10] Our goal was to investigate the occurrence of CPs in various fatty DS available on the German online market and to evaluate human exposure for the consumer.

Materials and methods

Standards. Commercial analytical standard mixtures of SCCPs (51.5%, 55.5% and 63% Cl content) and MCCPs (42%, 52% and 57% Cl content), both at 100 ng/µL, were from Dr. Ehrenstorfer (Augsburg, Germany). Perdeuterated α-hexachlororcyclohexane (α-PDHCH), synthesized in our work group,[11] was used as recovery standard in the samples. MeOH-BDE 66 (BCIS), synthesized in our work group,[12] was used as surrogate standard in the samples and added prior to injection.

Chemicals. 2,2,4-trimethylpentane (*i*-octane, for pesticide residue analysis) was from Fluka Analytics (Seelze, Germany). Sulfuric acid (96-98%, for analysis *p.a.*) was from Carl Roth (Karlsruhe, Germany). Silica gel 60, sodium sulfite (anhydrous) (puriss., *p.a.*) cyclohexane and ethyl acetate were from Sigma-Aldrich (Seelze, Germany). Acetone (p.a., ≥99%) and *n*-hexane (for pesticide residue analysis, ≥99%) were obtained from Th. Geyer (Renningen, Germany).

Samples. DS capsules (n=17) with high fat content were ordered from online retailers. They were distributed into three distinct groups according to their health claims: (i) vitamin E supplements (n=8 samples, E1-8), (ii) n-3 fatty acid supplements (n=7, N1-7) and (iii) other supplements (n=2, O1&2).

Sample preparation. Oil of several capsules was pooled into one sample per brand. Approx. 0.5 g of the pooled oil was weighed into a glass centrifuge tube and dissolved in 10 mL *n*-hexane. The internal standard α-PDHCH (107 ng) and 5 mL of concentrated sulfuric acid were added to the sample. After shaking vigorously, the tube was centrifuged at 4000 rpm for 5 min to achieve complete phase separation. The acid phase was re-extracted twice with 5 mL *n*-hexane. The organic layers were combined and again treated with 2.5 mL of concentrated sulfuric acid. After re-extracting the acid phase twice with 5 mL of *n*-hexane, the combined organic layers were evaporated to ~1 mL and subjected to column chromatography as described elsewhere.[13] The sample extracts were brought to 1 mL or, for lesser contaminated samples, to 100 µL under a gentle nitrogen stream.

Quality control. All glassware was cleaned with detergent, distilled water, acetone and distilled cyclohexane/ethyl acetate prior to use. Each sample was prepared in duplicates, and deviations were <20%. One procedural sample

was prepared for each six samples. Mean recovery of the internal standard was at 88±27%, with all but three samples between 72% and 127%. Those three samples were corrected to a recovery rate of 100%.

Gas chromatography with electron capture negative ion mass spectrometry (GC/ECNI-MS). The measurements were performed on a 7890/5975C MSD system (Agilent, Waldbronn, Germany), with settings as described elsewhere.[13] CPs were quantified using a modified version of the method described by Reth *et al*. by monitoring the CPs of chain lengths C_{10}-C_{17} and 4-10 Cl in selected ion monitoring (SIM) mode.[14,15]

Results and discussion

CP concentrations in dietary supplements. Seven of the 17 samples (41%) showed CP levels between 266 and 48,200 ng/g fat (**Tab. 1**). These seven samples were contaminated with MCCPs, whereas four sample (24%) additionally contained SCCPs (**Tab. 1**). Especially vitamin E supplements (75%) were contaminated with CPs. Interestingly, the four vitamin E samples with the highest CP content were derived from palm oil. MCCPs were predominant (all >32,000 ng/g fat), and three of them additionally contained SCCPs up to 2,670 ng/g fat (**Tab. 1**). No other vitamin E sample was derived from palm oil. The two lowest contaminated samples E6 and E7, which were derived from soy bean oil and rice bran oil respectively, only contained MCCPs. This indicated that the presence of CPs was linked to the type of oil used in the dietary supplements. Only two DS based on vitamin E were free of CPs. By contrast, only one of seven (14%) *n*-3 fatty acid samples (N5) was contaminated with CPs. Sample N5 was the only DS sample with a higher level of SCCPs (1,900 ng/g fat) compared to MCCPs (514 ng/g fat). Lahaniatis detected CPs in five (35%) of 14 fish oil samples in 2002.[16] In these samples, SCCPs were dominant in three (60%) out of the samples.[16] Finally, O1 and O2 were free of CPs.

Tab. 1: sample content (c) and daily intake (DI) of SCCPs/MCCPs by dietary supplements

	SCCPs			MCCPs		
sample	**c [ng/g fat]**	**DI [µg]**	**[%] of ADI**	**c [ng/g fat]**	**DI [µg]**	**[%] of ADI**
E1	-	-	-	40,900	13.9	3.3%
E2	2,670	0.69	0.01%	45,500	12.8	2.8%
E3	543	1.74	0.02%	32,500	104	24.5%
E5	2,420	0.97	0.01%	36,500	14.6	3.4%
E6	-	-	-	1,020	0.265	0.06%
E7	-	-	-	266	0.106	0.03%
N5	1,900	1.90	0.03%	514	0.514	0.12%
mean*	465	0.33	0.00%	9,270	8.57	0.02%

*= calculated for all 17 samples (with n.d. = 30 ng/g fat)

Samples E6, E7 and N5 showed CP concentrations similar to those reported in fish, dairy products and vegetable oils (**Tab. 2**). However, the contamination level of samples E1-3 and E5 were more than one order of magnitude higher contaminated with MCCPs, which indicated an elevated threat for the consumer.

CP congener patterns. Sample N5 (made from fish oil as opposed to vegetable oil) was dominated by SCCPs and namely C_{11}-CPs which contributed ~40% to the SCCP content (**Fig. 2)**. C_{11} was also reported as the predominant CP chain length in other fish oil samples.[16] Hence, the CPs most likely originated from food chain enrichment by the fish. The low detection frequency in that group indicated that the other fish/seafood oils were probably refined before use in the capsules.[17] By contrast, the MCCP pattern of sample N5 resembled the composition of technical standards with its high abundance of C_{14}-CPs. Hence, MCCPs in N5 were possibly introduced during the production process. The congener patterns of samples E1-3 and E5-7 differed from the typical CP pattern of

foodstuffs which is usually characterized by higher proportions of shorter chain lengths (C_{10}/C_{11} in the case of SCCPs and C_{14} in the case of the MCCPs) and lower chlorinated homologs.[3,16,18] By contrast, the three samples containing SCCPs (E2, E3, E5) were dominated by C_{13} (>40%). Similarly, C_{14}-CPs were still the most abundant MCCP species in four of the samples, but they contributed <50% to the total CP content in all but one CP positive E sample. Moreover, the high proportion of longer chain lengths (>10% of both C_{16} and C_{17}) was unusual for environmental samples, where they were either low abundant or even absent.[1,2] Therefore, these products were most likely contaminated with CPs during the manufacturing process. Hence, a better control of processes in the food industry on use and possible transfer of CPs into the products is required.

Tab. 2: CP concentrations in foodstuffs

	matrix	mean	range [ng/g fat]	reference
C_{10-17}	DS	9,270 ng/g fat	<LOD**-48,200	this study
C_{10-20}	dairy products	300 ng/g ww*	-	19
C_{10-20}	vegetable oils	150 ng/g ww*	-	19
C_{10-13}	fats	140 ng/g fat	-	18
C_{10-13}	fish	170 ng/g fat	-	18
C_{10-17}	dairy products	-	<LOD**-63	20
C_{10-17}	fish	-	116-613	16
C_{10-17}	fish oil		131-431	16

* = wet weight; **= limit of detection

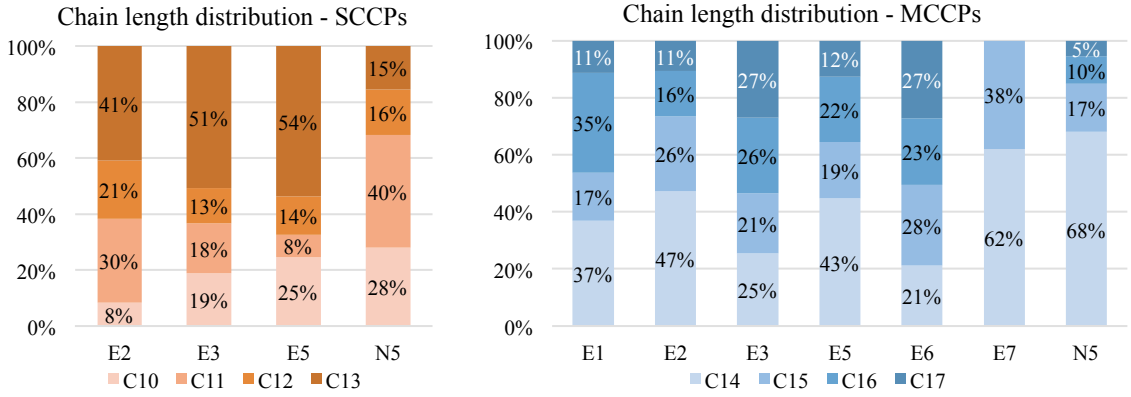

Fig. 2: chain length distribution of SCCP/MCCPs in CP-positive dietary supplement samples

Human exposure via consumption of dietary capsules contaminated with CPs. To estimate the daily CP intake via the examined DS, the fat content per capsule was either taken from the product label, or approximated by dividing the sample weight by the number of capsules used. By multiplying the CP concentration with the amount of fat per capsule and the recommended daily amount of capsules given by the manufacturers, the expected CP ingestion was calculated for each product (**Tab. 1**). These values were used to calculate the contribution of the CP containing samples to the acceptable daily intake (ADI) for an average European adult (body weight, 70.8 kg[21]).

As ADI for the SCCPs, the value of 100 µg/kg body weight[22] was used, whereas the ADI for MCCPs was set to 6 µg/kg body weight[23]. Daily intake of SCCPs via the screened products was <<1% of the ADI when consumed as recommended. The median dietary intake via contaminated samples of 0.33 µg SCCPs was almost one order of magnitude lower than the reported median daily intake via food (1.4 µg). On the other hand, MCCP intake from the highly contaminated dietary supplementary samples corresponded with >2% of the ADI. For sample E3 (>100 µg) even 24.5% of the ADI was reached (**Tab. 1**).This value resulted from the high fat content per capsule (0.8 g) and a suggested intake of up to four capsules a day. In this case, the mean intake of 8.57 µg MCCPs via dietary supplements was even higher than the respective value reported for food (3.0 µg).[24] When consumed regularly, contaminated DS may therefore contribute more to the human MCCP exposure than food.

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