COMPARISON OF LEVELS OF PCDD/Fs AND NON-*ORTHO* PCBs IN PCB 153 FROM SEVEN DIFFERENT SUPPLIERS

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Introduction

Twelve PCBs with dioxin-like (DL) properties have been carefully studied through the years to facilitate risk assessment and they have been assigned WHO-TEF values [1] based on their relative toxicity and endocrine effects compared to 2,3,7,8-TCDD. From a toxicological point of view, the non-dioxin-like PCBs (NDL-PCBs) are less characterized but usually account for more than 90% of the total mass of PCBs in food samples [2]. Furthermore, over 90% of the NDL-PCB exposure in the general population is via food and the average daily intake can be estimated to be 10-45 ng/kg (bw)/day according to the European Food Safety Authority EFSA [3]. The EFSA committee concluded that a proper risk assessment of this abundant and environmentally significant class of compounds could not be accomplished. In 2006, the European Commission initiated a project which has as its aim to better examine the toxicity of NDL-PCBs: ATHON- "Assessing the toxicity and hazard of non-dioxin-like PCBs present in food". The ATHON project will perform all *in vivo* and *in vitro* studies with ultra pure PCBs with known levels of DL-PCBs, PCDD/Fs and total TEQ-levels. As a first step in this study the major suppliers of PCB 153 were identified and the aim of the research was to investigate if there were any clear differences in the quality of their products based on possible impurities of PCDD/Fs and non-*ortho* PCBs. PCB 153 was selected because of its relatively high presence in environmental compartments and biota and since it has been the most frequently studied NDL-PCB in a few major in vivo studies [2]. Impurities of PCDD/Fs and DL-PCBs, even at trace levels, in PCB 153 may make a significant contribution to the effects seen in *in vivo* studies as the highest concentrations being studied are at mg/g (bw)/day during a period of time. These high daily exposure levels in combination with possible accumulation of toxic impurities may by time pose a threat to the significance of observed effects. Within the ATHON project all NDL-PCBs used for both *in vitro* and *in vivo* tests are analyzed and in many cases purified to remove possible traces of PCDD/Fs and non-*ortho* PCBs.

Materials and Methods

Various amounts of PCB 153 were purchased from seven different suppliers viz. Accustandard (New Haven, USA), Dr Ehrenstorfer (Augsburg, Germany), Ultra Chemicals (New Jersey, USA), Chemical Service (Muskegon, USA), Cambridge Isotope Laboratories (Andover, USA), Chiron AS (Trondheim, Norway) and Sigma-Aldrich (Seelze, Germany). An aliquot of 10-20 mg of crystalline PCB 153 from each supplier was dissolved in 2ml *n*-hexane using an ultra sonic bath and 40 µl of an internal standard mixture containing the 17 2,3,7,8-substituted ^{13}C-labelled PCDD/Fs was added. The dissolved PCBs were then transferred to glass columns (Ø 16mm) with ends cut at both sides. The glass columns were filled with glass wool at either side with 3g of activated carbon (NORIT, Amersfoort, The Netherlands) mixed with Celite® (Fluka, Buchs, Switzerland) in the proportions 7.9/92.1 in between. Before use, the columns were washed with 30 ml dichloromethane (DCM)/methanol/toluene 15/4/1 (v/v/v), 10 ml DCM and 30 ml *n*-hexane. PCB 153 and impurities from other NDL-PCBs were eluted with the first fraction using 280 ml *n*-hexane as eluent. The columns were then turned upside down and the PCDD/Fs and non-*ortho* PCBs were eluted with 280 ml of toluene. 100µl of tetradecane as keeper was added and the *n*-hexane was removed by rotary evaporation. The samples were then transferred with *n*-hexane to washed, miniature, multi-layer silica columns (∅ 5 mm) filled with, from the bottom: glass wool, KOH-silica, deactivated silica, 40% H_2SO_4-silica and dry Na_2SO_4 (s), and then eluted with 8 ml *n*-hexane. Prior to injection 40µl of a recovery standard mixture with ^{13}C-labeled 1,2,3,4-TCDD, 1,2,3,4,6-PeCDD, 1,2,3,4,6,9-HxCDF and 1,2,3,4,6,8,9- HpCDD was added. Finally, the samples were evaporated to *ca* 100 µl under a gentle stream of nitrogen. The quantification standard was prepared using 1-ml aliquots from a dilute native standard

(Wellington laboratories) containing 100pg of TCDD/Fs, 500pg of Pe-HpCDD/Fs and 1000 pg OCDD/F. The samples were analyzed using a 60m × 0.25mm × 0.25µm DB-5MS column (J&W Scientific) with helium as carrier gas on a GC-HRMS system consisting of an HP 6890 interfaced with a Waters AutoSpec ULTIMA NT 2000D high resolution mass spectrometer. Quantification was performed according to the isotope dilution technique.

Results and Discussion

The limit of impurities for individual PCDD/Fs and non-*ortho*-PCBs congeners was in the ATHON project set to 0.0001%, i.e. 1µg/g PCB for congeners with a TEF ≥ 0.1. In the present study, extremely low levels of PCDDs could generally be found in the samples (Table 1). 2378-TCDD was below the detection limit in all samples. Among the PCDFs, 2378-TCDF and the PeCDFs could be found in all samples, whereas the hepta- and octa-CDFs were found at very low levels, close to the detection limit. PCB 77 was found in ng/g levels in most samples in comparison with PCB169 that could not be detected in most samples.

Table 1. Concentrations and WHO-TEQs[1] in PCB 153 from seven suppliers.

Supplier	Accustandard		Dr Ehrenstorfer		Ultra Chemicals		Chemical Service		CiI		Chiron AS		Sigma-Aldrich	
Congener	pg/g	TEQ	pg/g	TEQ	pg/g	TEQ	pg/g	TEQ	pg/g	TEQ	pg/g	TEQ	pg/g	TEQ
2378 TCDD	nd	0	nd	0	nd	0	nd	0	nd	0	nd	0	nd	0
12378 PeCDD	nd	0	nd	0	nd	0	nd	0	nd	0	nd	0	nd	0
123478 HxCDD	nd	0	nd	0	nd	0	nd	0	nd	0	nd	0	350	35
123678 HxCDD	nd	0	nd	0	nd	0	420	42	430	43	nd	0	340	34
123789 HxCDD	nd	0	nd	0	nd	0	170	17	160	16	400	40	nd	0
1234789 HpCDD	nd	0	nd	0	520	5	570	6	530	5	160	2	350	4
OCDD	nd	0	nd	0	nd	0	nd	0	nd	0	120	0	390	0
2378 TCDF	7800	780	5700	570	1250	125	1270	127	1550	155	650	65	770	77
12378 PeCDF	nd	0	700	21	120	4	124	4	160	5	1370	41	420	13
23478 PeCDF	4000	1200	4200	1260	1500	450	1700	510	1920	576	1280	384	260	78
123478 HxCDF	nd	0	6300	630	870	87	nd	0	nd	0	730	73	240	24
123678 HxCDF	nd	0	nd	0	nd	0	nd	0	nd	0	280	28	290	29
234678 HxCDF	420	42	nd	0	790	79	741	74	790	79	270	27	440	44
123789 HxCDF	190	19	nd	0	340	34	263	26	270	27	450	45	720	72
1234678 HpCDF	nd	0	550	6	nd	0	117	1	nd	0	320	3	480	5
1234789 HpCDF	nd	0	nd	0	nd	0	nd	0	nd	0	130	1	200	2
OCDF	650	0	nd	0	nd	0	nd	0	nd	0	250	0	390	0
PCB 77	55000	6	6900	1	960	0	576	0	690	0	1060	0	3300	0
PCB 81	nd	0	320	0	nd	0	nd	0	nd	0	nd	0	nd	0
PCB 126	nd	0	6200	620	580	58	977	98	1940	194	nd	0	2300	230
PCB 169	nd	0	nd	0	nd	0	nd	0	nd	0	nd	0	230	2
WHO-TEQ pg/g		**2047**		**3107**		**842**		**905**		**1100**		**709**		**649**

nd = not detected.

[1]According to the TEF-values by van den Berg et al 2006. Values are calculated per g of PCB153.

The total WHO-TEQ ranged from 649 pg/g (Sigma –Aldrich) to 3107 pg/g (Dr. Ehrenstorfer). This means that all the tested PCB 153 materials have concentrations of PCDD/Fs and non-*ortho*-PCBs significantly below the set ATHON limit. It should, however, be stressed that this data derives from only a single analysis of one batch from each of the different suppliers. Results (not presented here) of analysis of different batches of other NDL-PCBs from the same supplier showed that there can be larger differences in impurity levels of PCDD/Fs and non-*ortho*-PCBs between batches from the same supplier than between samples from different suppliers. The low amounts analysed also contribute to the total uncertainty of the analysis. However, these materials are very expensive therefore larger sample sizes were not considered. Hence, we can conclude that there are no larger differences in PCDD/F impurities of the tested materials considering the total WHO-TEQ, as can be seen in Table 1 where only very small amounts of PCDDs are present and those that are detected are close to the limits of detection for the method. The main contributor to the total TEQ in six out of the seven evaluated PCB 153 materials was 2,3,4,7,8-PeCDF (41-59%; Figure 1). The exception was Sigma-Aldrich where 2,3,4,7,8-PeCDF contributed to 12% of the total TEQ and instead PCB 126 was the main contributor with 35 % of the total TEQ. Also TCDF was a high contributor to the total WHO-TEQ and could be detected in relatively high

concentrations in all evaluated materials. When comparing the limit of impurities set in ATHON at 0.0001% for a individual PCDD/F congener with higher TEF-value than 0.1, it can be concluded that all evaluated materials in this study is of a quality well below the limit.

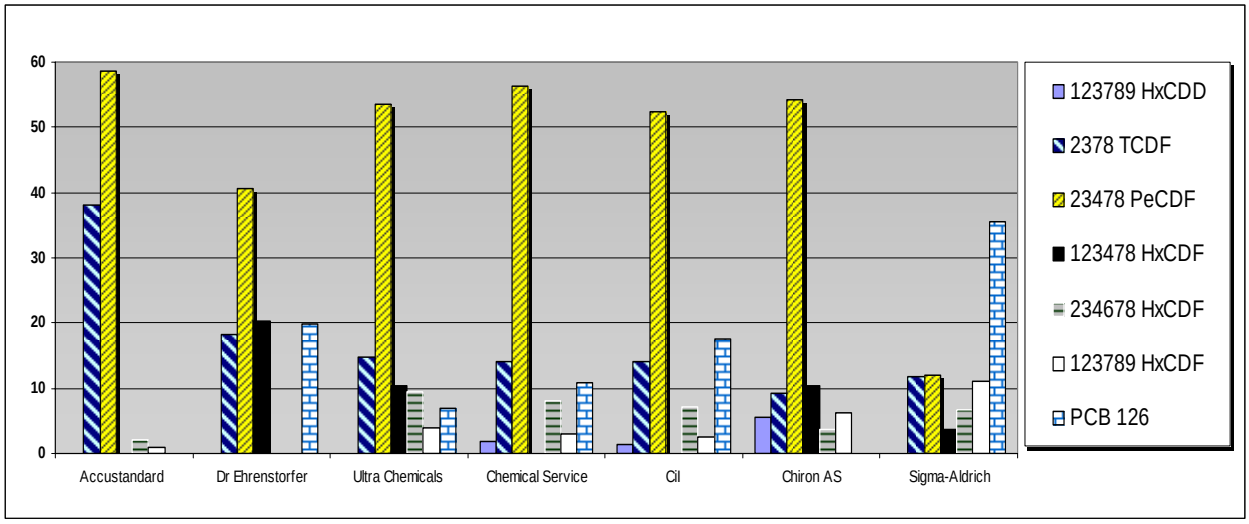

Figure 1. Congeners with highest contribution to total WHO-TEQ in %, shown for PCB 153 from 7 different suppliers.

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