Simultaneous and Reliable Determination of PCDD/F, PCB, PBDE and PBDD/F in Food and Feed with a Short Automated Sample Preparation

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Introduction

Currently there is an increasing public awareness of food and feed quality, especially concerning contaminants, such as polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/F), polychlorinated biphenyls (PCB) or polybrominated diphenylethers (PBDE) and their combustion metabolites polybrominated dibenzo-p-dioxins/dibenzofurans (PBDD/F). The EU Commission published several recommendations like the Recommendation 2013/711/EU amended by Recommendation 2014/663/EU on the reduction of the presence of PCDD/F and PCBs in feed and food. There, they recommend that the presence of PCDD/F, dioxin-like PCBs and non-dioxin-like PCBs in free-range eggs, organic eggs, lamb and sheep liver, Chinese mitten crab, dried herbs and clays as food supplements should be subject to an increased monitoring. Even the crisis in Germany in the end of 2010 once again put focus on PCDD/F as a result of contamination of feeding stuffs [1] and the impact on the food production. Thousands of farms were blocked until an analysis showed the compliance with the maximum levels. Of new interest are the brominated compounds, which are intensively studied. Main classes of flame retardants are PBDEs which are persistent and lipophilic like the PCDD/F, and therefore bio accumulative. Acute toxicity of PBDEs is lower than that of dioxins but they are suspected to cause neurological deficits and disorders of the hormonal system. PBDEs have an eco-toxicological potential and are detected in various environmental compartments. Toxicity of PBDD/F is similar to their chlorinated equivalents [2]. According to Recommendation 2014/118/EU Member States should monitor brominated flame retardants in food. Levels of brominated flame retardants in food of animal origin could be related to the presence of these substances in animal feed or in materials which are used in the surroundings of the animals and to which they have direct contact.

In order to handle the analysis of these compounds a rapid and reliable method must be available to give relevant information for evaluation. There is a need of automatization to get purified extracts within 60 minutes for all the stated compounds in a single run. We carried out a method with the *DEXTech Plus* device to analysis these compounds in a single clean up procedure and with two fractions. The results are presented in this abstract.

Methods and Materials

Samples: Two different laboratories, the Bavarian Health and Food Safety Authority (LGL) and the Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA-MEL) analysed different samples from the local market with the same automated DEXTech system (*alumina setting).*

Reagents:

Native and ^{13}C-labelled PCDD/F, PCB, PBDE and PBDD/F standards were purchased from Promochem or Campro, Germany

Solvents used were of quality grade "Nanograde" and purchased from Promochem, Germany

Apparatus:

GC-HRMS: Agilent HP 6890/Micromass AutoSpec Ultima HRMS or GC-HRMS (Thermo DFS) were used for analysis of extracts.

Extraction procedures:

Due to their lipophilicity the determination of PBDE, PBDD/F, PCDD/F, and PCB started with fat extraction. For different matrices different extraction techniques are used. It covers the wide spectrum from manual to automatic extraction. For pasty matrices, it could be done quite unproblematic with cold extraction by organic solvents with sodium sulfate and glass granulates. For milk, it is better to use liquid/liquid extraction or an automatic extraction under pressure and elevated temperature (PSE; Büchi Speed Extractor^{TM}) using a mixture of polar and nonpolar solvents, like toluene/ethanol or n-hexane/acetone. Extracts were concentrated using a rotary evaporator and in a final step under a gentle nitrogen flow.

Clean-up:

Automated sample preparation was done by a DEXTech Plus device (scheme see Figure 1)

The extracted samples are resolved in 10 ml n-hexane and loaded directly into the sample loop of the system. For up to 2 ml toluene there is no influence on the separation. If the extracted material contains no fat it is advisable to add a keeper like nonane. The ready-to-use LCTech columns (acid silica, alumina and activated carbon) are unpacked and placed into the column holder. The system starts with the injection of the sample and collects the fractions per sample. *The automated clean-up method was originally developed for PCDD/Fs as well as PCBs. Besides mono-ortho- and ndl-PCBs, PBDEs are eluted in pear shape flask 1, wherefore this fraction is analyzed for PBDEs. Chlorinated and brominated dioxins eluate together with the non ortho PCB in fraction 2.*

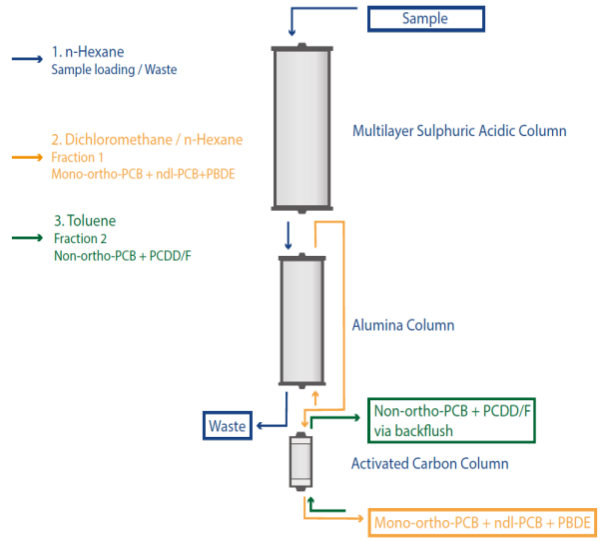

Figure 1: DEXTech (alumina column)

Table 1: Solvent consumption and time for the clean-up procedure; the conditioning steps are optional and used by reuse of the carbon column

			minute	*flow ml*
conditioning				
S1	n-hexane		12	7
S1/S2	n-hexane		5	7
S3	toluene		0	7
S3	DCM/n-hexane		3	3
fractionation				
S1	n-hexane	forward fraction S1	5	7
S1/S2	n-hexane	forward fraction 1	17	7
S2/S3	DCM/n-hexane	fraction 1	8	3
S3	toluene	fraction 2	10	1
N_2			5	

Fraction 1: 24 ml dichloromethane (DCM)/n-hexane (1:1) containing mono-ortho PCB, ndl-PCB and PBDE
Fraction 2: 10 ml toluene containing non-ortho PCB, PCDD/F and PBDD/F

GC/MS Analysis (CVUA MEL):
a) GC-HRMS: Agilent 6890 GC/Micromass Autospec Ultima HRMS
PCB: Injector: 100 °C, 2 µl splitless, up to 300 °C; column: Agilent ZORBAX HT-8 Column 50 m × 0.22 mm, 0.25 µm film thickness; Temperature program: 80 °C (3.0 min hold), 20 °C/min to 160 °C, (0 min), 4 °C/min to 300 °C (8 min), (Total run time = 50.0 minutes)
PCDD/F: Injector: 280°C, 1 µl splitless; column: DB-5MS (J&W) 60 m, 0.15 µm film thickness, 0.25 mm ID; Temperature program: 75°C (3 min) - 195°C (15°C/min) - 270°C (3°C/min)
PBDE: Injector: 275°C, 1 µl splitless ; column: Agilent DB-5MS (J&W) 15 m, 0.10 µm film thickness, 0.25 mm ID; Temperature program: 80°C (1,5 min) - 320°C (20°C/min) - 320°C (4 min)
Carrier gas: helium, pressure: 2 bar; MS-Resolution: 10000

Results and discussion
With the DEXTech Plus device with alumina setting it is possible to analyze the different compounds PCDD/F, PCB and PBDE in a single run and to separate all compounds into two fractions within 60 minutes. Through this method it is possible to analyze more samples in a shorter time with less solvent consumption.
The recovery for the PCDD/F and PCB are shown in previous publications [1, 3, 4]. In all experiments the average recoveries of PCDD/F and PCB ranged between 63-102% [1, 3, 4]. The percentages of the PCDD/F and PCB recoveries are in good agreement with the legislation requirements and are between the requested limits of 60 % to 120 %. The recoveries of the PBDE are shown in Table 2. They are also in a range (mean) from 59 to 101 % and only with a standard deviation around 20 %. Especially BDE-209 has sometimes lower recoveries. But this is not depending on the clean-up procedure. It is an effect of the behavior of the BDE-209 on GC-column. Chromatograms of the analysis are given in Figure 2. The measurements show that the extracts are well cleaned.

Table 2: recoveries of the PBDE in 95 different food and feed samples

Compound	mean recovery (%)	Standard deviation (%)	Max (%)	Min (%)
BDE-28	86	15	122	62
BDE-47	93	19	136	55
BDE-100	86	20	129	52
BDE-99	101	26	164	54
BDE-154	95	10	111	58
BDE-153	98	11	124	64
BDE-183	80	23	115	44
BDE-209	59	20	114	19

Comparison of internal standards of sample extracts and calibration solutions (Figure 2) show further that the degradation, especially of the thermo-labile BDE-209 (degradation to nona- and octa-BDE), was not as pronounced as suspected [5]. The detection of the low content of degradation products compared to the much more concentrated

BDE-209 illustrates the robustness of this method for the determination of PBDEs. The maximum abundance in the samples (cow's milk and baby food) was 5 to 10 while the internal standards had an abundance of 1000 (factor 100 to 200). The reference mass trace did not show a break in the baseline, which could be a sign of pollution. Based on these good results, the method was implemented in a second laboratory and the results were compared.

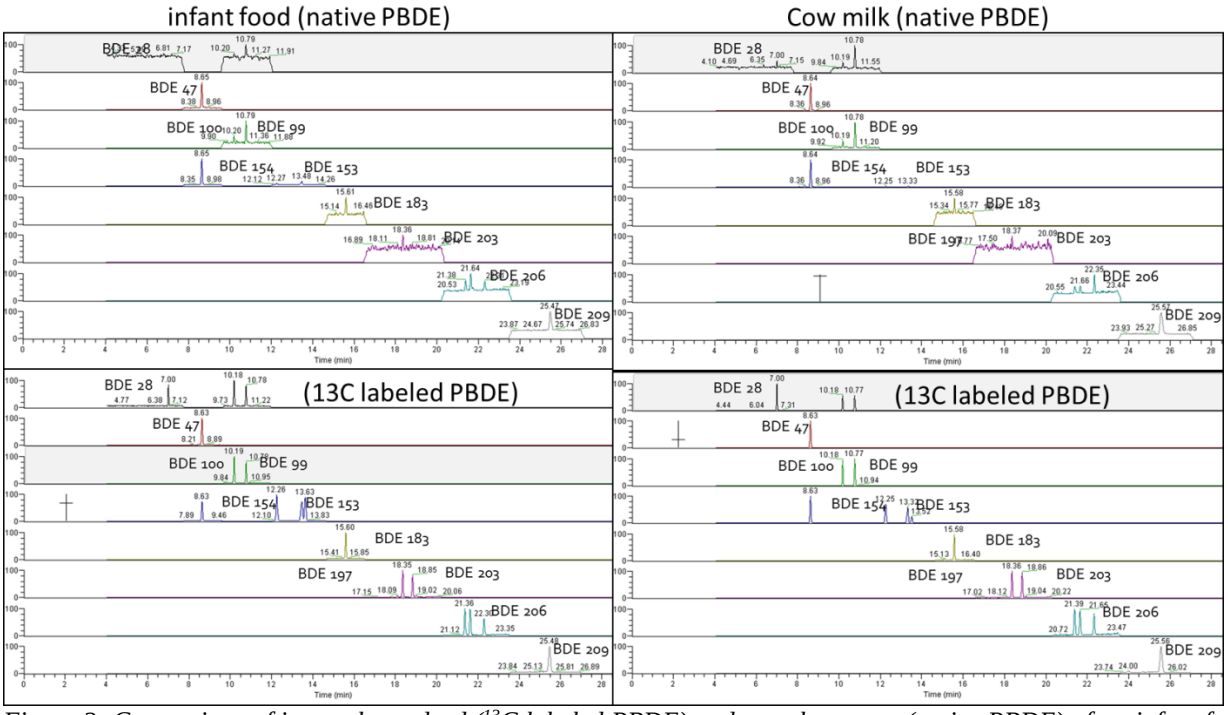

Figure 2: Comparison of internal standard (^{13}C labeled PBDE) and sample extract (native PBDE) of an infant food and a cow milk (LGL). Both samples have only background contamination (infant food ∑ PBDE (upperbound (ub)) 11 pg/g fresh weight, cow milk ∑ PBDE (ub) 20 pg/g fresh weight).

The comparisons between the two laboratories for a bovine liver sample and an infant food sample are shown in Table 3. The calves were obviously contaminated through an electro technical installation in the vicinity of the animals which were used as a playing toll on which the animals suck on. As can be seen there is a good agreement between the two laboratories and except for BDE 209 the results are within a range of +/- 10 %. In infant food samples, which are contaminated with BDE 209 in the laboratory through the output of a freeze drying pump the comparison of BDE 209 is also in a range between +/- 10 %. This shows that the comparability of the laboratories at high concentrations of BDE 209 is given. Attention should be paid to the blank values of the BDE congeners, in particular the highly concentrated BDE-209. To receive the real PBDE-concentrations the blank-values have to be considered. For a better presentation of the total PBDE-burden the individual concentrations, measured in pg/g fresh weight, are summarized to a sum upper bound concentration (Table 4).

Table 4 shows further samples of different food commodities with their dedicated amount. It shows that food of plant origin or feed is normally low contaminated. Food of animal origin can also be low contaminated like the liver from calves, pigs and hens. The calf's liver from Table 3 was not included in the evaluation of the other livers, because there was a real contamination and not back ground pollution. Meat samples can be low contaminated like the liver samples, but someone show higher contents. In this cases there are wide ranges between minimum and maximum values respectively mean values are substantially higher than medians for a sufficiently large sample group.

*Table 3: calculated amount of three different samples from the LGL and CVUA MEL: calf's liver and two different infant food (infant food: one sample * contaminated through the freeze drying pump, the second sample background contamination)*

matrix	Compound	Amount (LGL) [pg/g fresh weight]	Amount (CVUA) [pg/g fresh weight]	Mean [pg/g fresh weight]	Deviation from the mean value [%]
calf's liver (not included in table 4)	*BDE-28*	20.7	22.5	21.6	4.1
	BDE-47	426	420	423	-0.7
	BDE-99	1335	1550	1442	7.5
	BDE-100	74	90	82	9.7
	BDE-153	1006	1114	1060	5.1
	BDE-154	229	258	243	6.0
	BDE-183	17.4	17.0	17.2	-1.1
	BDE-209	99	41	70	-41
infant food*					
carrots with potatoes and salmon	*BDE-209**	1024	1006	1015	0.9
	BDE-209	8.1	5.1	6.6	22.7
Carrots with corn and calf	*BDE-209**	529	560	545	-2.8
	BDE-209	5.1	5.2	5.3	0

The levels in the fish samples are most noticeable. They vary between "undetected" to the highest levels ever found of 9000 pg / g fresh weight. This group is also the most diverse group. For example, fish from the Baltic Sea (Herring) or fish from the river Elbe, which are exposed to high environmental contamination, and fish from the pond industry, which do not have any water pollution, are represented in this group.

Table 4: different food/feed matrices with their sum PBDE upper bound values

matrices	liver	feed/corn	fish	meat	dairy milk	infant food	breast milk
number	*43*	*16*	*74*	*18*	*43*	*49*	*100*
	Sum PBDE upperbound pg/g fresh weight						
Min	2.6	4.7	0.33	3.5	5.7	9.2	9.7
Max	44	12.2	9496	232	21.5	116	3526
mean	9.2	7.5	463	66	13.8	40	157
median	6.9	7.3	176	14.4	13.6	31	81
90th percentile	19	10.7	1008	178	20.9	80	265

Conclusion

With the automatic purification system (*DEXTec Plus*; alumina setting) it is easy to analyze PCDD/F, PCB, PBDE and PBDD/F in one approach with adequate clean extracts. Reproducibility over all matrices is excellent with recoveries between 59 - 101%. The detection of the only low content of degradation products compared to the much more concentrated BDE-209 illustrated the robustness of this method for the determination of PBDE. The determination of BDE-209 is possible and reproducible together with the measurement of the other PBDE on a short column. The comparability of this method has been tested by two laboratories. The results, with a deviation of +/- 10%, have shown that the method is robust to collect data for the monitoring proposed by the EU.

References:

1. Bernsmann, T., Möhlenkamp, U., Fürst, P., Aulwurm, U., Baumann, M. (2013); Organohalogen Compounds Vol. 75, 728-732
2. Van den Berg, M., Denison, M., Birnbaum, L., DeVito, M., Fiedler, H., Falandysz, J., Rose, M., Schrenk, D., Safe, S., Tohyama, C., Tritscher, A., Tysklind, M., Peterson, R. (2013); Toxicol. Sci. 133 (2), 197-208
3. Bernsmann, T., Albrecht M., Fürst, P. (2014); Organohalogen Compounds Vol. 76, 1281-1284
4. Bernsmann, T., Albrecht M., Fürst, P. (2016); Organohalogen Compounds Vol. 78, 797-799
5. Albrecht M., Büchner K., Hilger B.,Stindl F. (2016); Organohalogen Compounds Vol. 78, 963-966