Development of an analytical method for the detection of PBDD/DF in environmental samples.

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Introduction

The rapid increase in the usage of brominated flame retardants (BFR) the last two decades will undoubtedly result in more bromine containing waste. Both in laboratory and pilot scale experiments the formation of PBDD/DFs during thermal treatment of the BFRs or BFR containing waste has been shown[i,ii]. Potentially this might result in rising levels of these compounds in the environment. PBDD/DFs exhibit similar properties as chlorinated PCDD/DFs although they have a larger molecular weight, somewhat lower water solubility, lower vapour pressure, and are more lipophilic. PBDD/DFs are expected to bio accumulate in a similar way as their chlorinated homologues. The persistence of PBDD/DFs might however be somewhat less due to increased photolytic degradation. Toxicologically they behave again similar to the more extensively studied chlorinated dioxins and furans and show nearly the same dioxin like toxicity as their chlorinated homologues as recently reviewed by Birnbaum[iii] et al. Despite their dioxin like toxicity and potential large emission sources, only limited information is available on levels of these compounds in the environment. This is probably due to historical low levels and potential analytical difficulties. Several methods adapted from the analysis of chlorinated dioxins have however been published in the past[iv,v,vi], and recently several new methods have been published[vii,viii]. Potential problems are interferences of BDEs during clean up and extraction or high resolution GC/MS analysis. Another problem might be thermal breakdown of the target compounds or Deca BDE to PBDFs. General human population background data is until recently not known and only occupational exposure data showed measurable amounts of PBDD/DFs[ix,x]. No PBDD/DFs were found in salmon (< 0.3 ppt), osprey (<0.5 ppt) or human milk (<1 ppt) from Sweden by Wiberg et al in 1992[xi] or in carp[xii] and sediment samples[xiii] from the US. Recently both 2,3,7,8-TeBDD and 2,3,7,8-TeBDF were found in Japanese sediment at levels of 0.3-3.2 pg/g by Choi et al[viii]. The same group also found measurable levels of PBDD/DF in human samples from Japan[xiv]. Levels of 0.1-4.2 pg/g were found in 10 human adipose samples from 2000, surprisingly levels in the same range 1.6-4.3 pg/g lipids were found for 10 samples from 1970. Here we present the validation of a traditional open column extraction method for the analysis of PBDD/DF in addition to SFE-LC extraction of PBDD/DFs in biological samples.

Material and methods

Several different configurations of different open columns (multilayer silica, AlOx, Florisil and carbon) were tested for both the extraction and clean up of human adipose samples. The sample consisted of a homogenized human adipose tissue sample which normally serves as a QA/QC sample for chlorinated dioxin and furan analysis and was obtained from the University Hospital Örebro in 2002. The QA/QC sample was stored at – 18 ˚ C until usage. The configuration of the extraction column, the multilayer silica column and the carbon column used in the final experiments is described below. Glass pipettes (50 ml) were plugged with glass wool and filled with 6 or 20 g of homogenized sample (1:4 sample/Na_2SO_4) and spiked with a 50 ul of a mix containing Mono-through Penta PBDD/DF at a concentration of 10-50 pg/ul for the recovery experiments or 50 ul of a internal standard mix containing ^{13}C labeled 2,3,7,8-TeBDD at a concentration of 2.5 pg/ul. The samples were eluted with n-hexane/dichloromethane (1:1, v/v) and after evaporation of the solvent the amount of fat was determined gravimetrically. The fat samples were dissolved in ~5 ml n-hexane and added to a multilayer silica column consisting of KOH silica, neutral activated silica gel, 40 % H_2SO_4 silica gel, 20 % H_2SO_4 silica gel, neutral activated silica gel and activated Na_2SO_4. This column was eluted with hexane which after collection was evaporated to around 1 ml using an evaporator. This volume was added to a 25 ml glass column containing Carbopack C dispersed on Celite 545. The non planar fraction was eluted with 10 ml hexane, while the planar fraction containing the PBDD/DFs was eluted with 80 ml toluene. After the addition of a recovery standard (^{13}C labeled 1,2,3,4-TeCDD) the samples were evaporated and transferred to an amber glass auto sampler vial in 25ul of tetradecane. The extracts were stored in – 18˚C until HRGC-HRMS analysis.

Supercritical fluid extraction (SFE) was tested by adding the PBDD/DF mix to a standard SFE cell (10 ml) filled with 5ml Na_2SO_4 and 5 ml AlOx. During dynamic extraction at 40 ºC and 280 atm with CO_2 for 45 minutes the target compounds were collected on a solid phase trap containing AX-21 carbon on ODS silica. After sample extraction this trap was eluted with hexane and finally with toluene to elute the PBDD/DFs.

HRGC/HRMS analysis was performed on a Micromass Autospec Ultima operating at 10 000 resolution using EI ionization at 35 eV. All measurements were performed in the selective ion recording mode (SIR), monitoring the two most abundant ions of the molecular bromine cluster. Several different GC columns were used including a 30 m Equity-5 (0.25 mm id, 25 um), a 30 m DB-5MS (0.25 mm id, 25 um) or a 60m BPX-5 (0.32 mm id, 25 um). Splitless injection was used to inject 1ul of the final extract on the GC column, several different GC temperature programming was used to optimize the response (and minimize degradation in both the injector and on the column) depending on column length and GC performance. All recoveries and levels were calculated against ^{13}C labeled 2,3,7,8-TBDD, which might in some cases result in variation of the relative response factors when congeners of different brominating level were analyzed. Detection levels were calculated at a S/N ration of 3, corrected for recovery of the internal standard.

Results and discussion

The test of the elution of the PBDD/DF mix on the different columns showed generally good results after adjusting the elution volumes. As an example the results for the multilayer silica and the carbon column are shown in Table 1. Similar results were also obtained for the AlOx and Florisil columns. The results for the lower brominated PBDD/DFs, both mono and di brominated congeners, is somewhat low probably due to evaporation losses. Although evaporation losses were minimized as much as possible, and improvement was shown in later experiment this could not completely be avoided with the instrumentation used. Another observation is that because only one internal standard (^{13}C labelled 2,3,7,8 TeBDD) was available at the time of the method development and was used for all experiments the results for the PBDD/DFs at other bromination levels might be somewhat less accurate. The GC/MS reponse of brominated dioxins and furans seem to drop much more from one bromination level to another than their chlorinated homologues.

T*able 1. Recovery after elution on multilayered silica and carbon column of a mix of PBDD/DF (10-50 pg). Recovery calculated against ^{13}C 2,3,7,8-TeBDD.*

Sample	**DL-03-003:1**	**DL-03-003:2**	**DL-03-003:3**	**DL-03-003:4**	**DL-03-003:5**	**DL-03-003:6**
Recovery	Silica	Silica	Silica	Carbon	Carbon	Carbon
1-MoBDD	16%	14%	14%	25%	33%	30%
4-MoBDF	41%	52%	32%	18%	23%	22%
2,7-&2,8-DiBDD	55%	60%	46%	52%	102%	62%
2,7-DiBDF	56%	61%	47%	48%	69%	60%
2,8-DiBDF	54%	60%	43%	51%	75%	59%
2,3,7-TriBDD	75%	76%	67%	82%	100%	83%
2,3,8-TriBDF	74%	76%	64%	77%	96%	79%
2,3,7,8-TeBDD	108%	104%	108%	106%	135%	115%
1,3,7,8-TeBDD	96%	99%	97%	103%	124%	106%
1,2,3,4-TeBDD	103%	105%	107%	92%	107%	98%
1,3,6,8-TeBDD	82%	86%	83%	91%	107%	91%
1,3,7,9-TeBDD	104%	107%	102%	107%	131%	116%
2,3,7,8-TeBDF	102%	103%	101%	102%	128%	105%
1,2,3,7,8-PeBDD	200%	139%	210%	91%	106%	104%
2,3,4,7,8-PeBDF	150%	123%	158%	93%	112%	103%
1,2,3,7,8-PeBDF	209%	144%	227%	103%	119%	118%

In Table 2 results from the spiking experiments of the human adipose tissue and the SFE-LC extraction are shown. The open column extraction of 1 g of the fat sample showed good results after clean up through the multilayer silica column and the carbon column, also the recovery of the lower brominated PBDD/DFs was somewhat improved. In addition as can be seen from Table 2 it was possible to extract the PBDD/DFs from Na_2SO_4 during SFE extraction, however the SFE of these high molecular compounds still needs some further method development. Screening of 1g of adipose tissue (open column extraction) did not result in any detectable amounts of the target

compounds. A larger sample size is therefore necessary which was easier to achieve by up scaling of the open column method.

A total of 4 g of the QA/QC human adipose tissue was analysed in order to lower the detection limit of the method. Preliminary result of the GC/MS analysis of this sample did not find any 2,3,7,8-PBDD or 2,3,7,8-TeBDF at a detection level of 0.1 pg/g.

Table 2. Results recovery experiment after addition of a PBDD/DF mix (10-50 pg) to 1 g of human adipose tissue. Open column extraction followed by acid/base silica and carbon column clean up and SFE extraction (40 ºC, 280 atm, 45 min dynamic CO_2) after addition to Na_2SO_4. Recovery calculated against ^{13}C 2,3,7,8-TeBDD.

Sample	**DL-03-003:27**	**DL-03-003:28**	**DL-03-003:29**	**DL-03-003:36**	**DL-03-003:37**	**DL-03-003:38**
Recovery				Adipose*	Adipose*	Adipose*
1-MoBDD	7%	69%	7%	46%	39%	42%
4-MoBDF	5%	69%	6%	63%	48%	51%
2,7-&2,8-DiBDD	9%	72%	11%	100%	94%	97%
2,7-DiBDF	10%	65%	9%	90%	87%	87%
2,8-DiBDF	8%	74%	9%	97%	92%	97%
2,3,7-TriBDD	32%	72%	28%	106%	111%	112%
2,3,8-TriBDF	22%	70%	21%	107%	105%	111%
2,3,7,8-TeBDD	65%	65%	58%	122%	131%	132%
1,3,7,8-TeBDD	39%	66%	33%	108%	128%	125%
1,2,3,4-TeBDD	11%	25%	12%	105%	117%	120%
1,3,6,8-TeBDD	16%	64%	18%	119%	103%	127%
1,3,7,9-TeBDD	32%	65%	33%	125%	137%	124%
IS ^{13}C 2,3,7,8-TeBDD	NA	NA	NA	125%	139%	133%
2,3,7,8-TeBDF	67%	68%	61%	122%	136%	139%
1,2,3,7,8-PeBDD	64%	59%	56%	85%	105%	123%
2,3,4,7,8-PeBDF	53%	32%	50%	95%	111%	125%
1,2,3,7,8-PeBDF	31%	43%	33%	95%	115%	133%

* Calculated against RS ^{13}C 1,2,3,4-TeCDD.

Conclusion

A traditional chlorinated dioxin and furan open column extraction and clean up method was successfully adapted for the analysis of PBDD/DFs. Preliminary results when using this method on 4 g of human adipose tissue did not show any 2,3,7,8-TeBDD/DF at a detection level of 0.1 pg/g.

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