IDENTIFICATION OF HEXABROMOCYCLODODECANE IN BUILDING MATERIAL

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Introduction

The impact of Brominated Flame Retardants (BFRs) on the environment and their potential risk for animal and Human health is a present time concern for the scientific community. Diet is considered as a major route of exposure for Human, particularly through animal product consumption, such as seafood products[1]. Past monitoring plans also highlighted terrestrial livestock animals as contributing to food exposure to BFRs. Aside feed, other sources might be considered, such as fire-proofed materials. Therefore, in order to implement efficient measures to decrease contamination levels of BFRs in terrestrial animal products, a better knowledge is required regarding exposure of livestock in the farming environment.

The aim of the present project was to investigate the potential sources of BFRs in a farm building environment, which can possibly contaminate the livestock. To this purpose, a screening was realized at two sites, representative to poultry and pork productions, using a field X Ray Fluorescence (XRF) instrument. The first site was a demonstration hall collecting all kind of materials used in broiler and laying hen production buildings (Ploufragan, France). The second one was a farm dedicated to pork experimentations (Romillé, France).

Based on screening results, samples of insulation materials were collected and analyzed by LC-MS/MS and GC-HRMS for the presence of hexabromocyclododecane (HBCD) and decabromodiphenylether (decaBDE), respectively. Additionally, direct analysis by atmospheric ion source DART was used to confirm previous results and investigate unknown brominated substance(s).

Materials and methods

X Ray Fluorescence (XRF)
Handheld XRF instrument (Bruker) was manipulated by an authorized expert, in order to access elemental composition for atomic number above 12 (magnesium). Particular attention was paid to bromine element and calibration test was performed using HBCD technical powder (>95%, Fluka). Identification of bromine was considered as a serious indication for the presence of BFR. Particular attention was paid to antimony as well since it is well described that antimony oxide (Sb_2O_3) is frequently associated to BFR as synergist. More than 150 screening tests were performed on many kinds of materials, including walls, insulation, ventilation, feeders, drinkers, pipes, wires, nests, *etc*.

Building isolation materials
Collected insulation materials (n=33), mainly organic polymers, were obtained from various sites dedicated to broiler and laying hen productions, on the French territory. Aliquots were sampled "at heart" (elimination of surfaces) and sliced using a surgical blade. Five to 35 mg of material were extracted by 10 mL of dichloromethane (DCM, Picograde®, LGC Promochem) in a 15 mL flask during a few days. Appropriated dilution of liquid phase was performed prior to HBCD and decaBDE analyses.

Standards
Native α-, β-, γ-1,2,5,6,9,10-HBCD, decaBDE and their ^{13}C-labeled surrogates were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Working solutions were prepared in toluene (Picograde®, LGC Promochem). Glassware was baked at 400 °C during 4 h prior to use, for analytical contamination purpose.

Analysis of HBCD
HBCD isomers were analyzed by LC-ESI(-)-MS/MS, according to a method routinely applied in the frame of research projects and French monitoring plans dedicated to food safety. Briefly, extracts were spiked with ^{13}C-labeled internal standards and reconstituted in 50 µL of a methanol/water mixture (80:20, v/v). Ten microliters were injected on a Hypersil Gold column (100 mm x 2.1 mm, 1,9 µm, Thermo Scientific) using a 1260 pumps series (Agilent Technologies). Mobile phase was constituted of water containing ammonium acetate at 20 mM (A) and methanol/acetonitrile 1:1 (v/v) (B). The gradient started in isocratic conditions at 70% B during 9.5 min and increased to 100% B at 14 min. Electrospray ion source was operating in the negative mode and coupled to a triple quadrupole (6410, Agilent). Acquisition was set in the MRM mode, based on $[M\text{-}H]^- > [Br]^-$ transitions. Identification was based on two signals (retention time, ratio) and quantification was based on isotopic dilution principle.

Analysis of decaBDE
decaBDE was analyzed by GC-EI(+)-HRMS, according to a method routinely applied in the frame of research projects and French monitoring plans dedicated to food safety. Briefly, extracts were spiked with ^{13}C-labeled internal standards and reconstituted in 20 µL of toluene. Two microliters were injected on a RTX-1614 column (15m x 0.25 mm, 0.10 µm). Electron impact source was set at 70 eV and coupled to electromagnetic sectors (JMS800D, Jeol, Japan, R=10,000). Identification was based on two signals (retention time, ratio) and quantification was based on isotopic dilution principle.

Ambient mass spectrometry
A Direct Analysis in Real Time (DART, IonSense) atmospheric ion source was connected to an LTQ-Orbitrap instrument (Thermo Scientific) operating in the negative mode at R=30,000 FWHM. Source temperature was set at 250 °C. Sample extracts were loaded on mesh containing 10 spots, allowed to dry and placed on a sample tray. Mesh were carefully washed prior to use and checked for any cross contamination.

Results and discussion

On site XRF screening for bromine
Among material surfaces screened, only some insulation materials revealed the presence of bromine. Observed levels were in the range 2-4.3%. Antimony was not observed. Therefore, insulation materials were considered as unique potential sources of BFRs (excluding feed which was out of scope). Unfortunately, about half the insulation materials tested were not dense enough for a measurement, due to security reasons (X rays should not be emitted in air).

decaBDE
Results of analysis of insulation materials (n=23) did not show the presence of decaBDE above 0.05% (w/w), assuming that decaBDE would at least partially migrate from the polymer matrix to the DCM since it used as a filler. Therefore, it was concluded that it is unlikely that decaBDE is present in investigated farm buildings.

HBCD
Interestingly, 11 out the 33 samples were completely dissolved by DCM, all mentioned as polystyrene based polymers. Results of analysis of insulation materials (n=33) revealed the presence of extracted HBCD above 1 ppm (w/w, each isomer) in 6 samples (Table 1), all among the 11 polystyrene foam polymers. This observation is consistent with the fact that HBCD market is 80% dedicated to manufacture of expanded (EPS) and extruded (XPS) polystyrene foams for insulation of buildings[2]. The complete dissolution allowed not only for a precise quantification of HBCD in extracts but in insulation material itself as well. Matching between bromine XRF

value (when available) and HBCD content found by LC-MS/MS was fairly good, assuming that HBCD content in bromine is 75%.

In terms of HBCD isomer patterns, the β-isomer was quite stable in the 6 samples (11-15%), but α- and γ-isomers showed an important variability. Indeed, one sample was dominated by γ-isomer (70%, 13.1130.3). This sample was also the one showing the lowest total content in HBCD (0.61%). The 5 other samples were dominated by α-isomer (60-80%). Assuming that technical HBCD is dominated by γ-HBCD, this observation is of importance to understand isomer profile of contamination sources. Indeed, a PS insulation material is not necessarily reflecting the technical isomer profile. One hypothesis would be that insulation material might be affected (isomerization, selective degradation) by time and temperature effects along its use but the main hypothesis to be explored is the isomerization during the manufacture process, under high pressure and temperature conditions. These conditions are presumably above conditions required for isomerisation from γ- to α-HBCD.

Table 1: Quantification of HBCD isomers and/or bromine element in insulation materials (n=7 positive out of 33 screened) by LC-ESI(-)-MS/MS according to isotopic dilution method and by handheld XRF, respectively. N/A: no value displayed by instrument due to low density of material involving security shutdown; LOD = 1 ppm; *: not dissolved in DCM (polyurethane foam).

Sample code	Br content	Extracted HBCD (%, LC-MS/MS)				HBCD pattern (%)		
	(%, XRF)	Total	α	β	γ	α	β	γ
M8F0	2.1	2.20	1.69	0.32	0.20	77	14	9
13.1130.3	N/A	0.61	0.11	0.07	0.43	19	11	70
13.1130.5	3	3.77	2.26	0.56	0.95	60	15	25
13.1130.8	N/A	3.64	2.91	0.45	0.28	80	12	8
13.1130.11	2	2.59	1.94	0.38	0.27	75	15	10
13.1994.4	N/A	3.85	2.99	0.54	0.33	78	14	8
13.1130.12*	4.3	<LOD	<LOD	<LOD	<LOD	-	-	-

Surprisingly, a polyurethane foam sample didn't reveal the presence of HBCD and decaBDE (13.1130.12) whereas bromine was identified at 4.3% by XRF, the highest observed content. Thus, the chemical nature of bromine in this sample raised a question.

Ambient mass spectrometry (DART)
Full scan acquisition in the range m/z 70-1000 unambiguously confirmed the presence/absence of HBCD in each sample extract (n=33).

The extract from the polyurethane foam containing bromine (13.1130.12) showed unexpected brominated isotopic clusters, confirming the presence of bromine in the corresponding insulated material. However, intensities of these clusters are relatively low in comparison to bromine content determined by XRF. It can be explained by a poor extraction yield by dichloromethane: polyurethane is a thermosetting plastic polymer and was not dissolved by DCM. The unknown brominated compound might also be covalently bound to the matrix, limiting its extraction. Another explanation relies to the ionization efficiency which might be relatively low. The analysis of the same extract by Electrospray (direct infusion) or by LC-ESI(+/-)-HRMS didn't revealed any brominated cluster (Accucore aQ 100 x 2.1 mm, 2.6 µm, acetonitrile/water gradient with acetic/acid 0.1%, coupled to Exactive instrument, Thermo Scientific), indicating that ESI might not be adapted for this unknown substance.

In details concerning this particular sample, four tribrominated clusters appeared to be well defined: 4 peaks at 2 uma distance with matching peak ratios. Most ^{13}C contributions were also observed. Table 2 shows tentative chemical formulas for these 4 clusters. The presence of numerous oxygen atoms excludes polybrominated diphenylethers or tetrabromobisphenol. No chemical structure was suggested. Two clusters were observed as well at higher m/z values but with lower intensities, tentatively suggested as tetrabrominated due to cluster

shape. However, mass difference between observed ions was not as good as expected and no chemical formula has been tentatively suggested. Further investigation is required to identify chemical structure of bromine in this sample.

Table 2: Tentative chemical formulas of observed isotopic clusters in sample 13.1130.12 using DART ionization and LTQ-Orbitrap instrument. a: m/z based on ^{79}Br signals; b: intensity based on base peak of cluster.

m/z[a]	Intensity[b]	Tentative Formula	Degree of Unsaturation	Deviation (ppm)
396.73597	13217	$C_8O_6Br_3$	7.5	1.678
454.77779	19827	$C_{11}H_6O_5Br_3$	7.5	1.802
484.78823	28685	$C_{12}H_8O_6Br_3$	7.5	1.847
542.83079	6928	$C_{15}H_{14}O_7Br_3$	7.5	2.373
558.82092	5950	-	-	-
616.86251	5510	-	-	-

Conclusion and perspectives

Potential sources (other than diet) of BFRs contamination of livestock in poultry and pork farms were investigated using handheld XRF to detect the presence of bromine at the surface of scanned materials in farm buildings. Only insulation materials revealed the presence of bromine. A set of 33 insulation materials was then sampled and analyzed. HBCD was identified and quantified by LC-MS/MS in 6 polystyrene samples at levels ranging from 0.61 to 3.85% (w/w). Isomer pattern showed two groups, dominated by α and γ isomers, respectively. Further investigation on process parameters influence on isomer pattern is scheduled. Additionally, unknown substance(s) was observed in polyurethane foam containing ~4.3% bromine, using DART-HRMS. Chemical formulas were suggested for some brominated clusters, involving many oxygen atoms, but further investigation is required for chemical structure identification.

References

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