

ANNEX 1 of Horizontal desk study 27 Brominated Flame Retardents

PBDEs in sludge, waste, soil and sediment.

This paper has been written for the Horizontal Standards on Organic Micro-pollutants for Implementation of EU Directives on Sludge, Soil, and Treated Bio-waste (HORIZONTAL-ORG)

Ethel Eljarrat, Göran Marsh and Damià Barceló

This paper is based on the following documents:

Standard draft

ISO/TC 147 Water quality – Determination of selected polybrominated diphenyl ethers in sediment and sewage sludge by extraction and GC-MS

Review articles

Eljarrat, E., Barceló, D., Sample handling and analysis of brominated flame retardants in soil and sludge samples. TrAC, In press.

de Boer, J., Law, R.J., Developments in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons. J. Chromatogr. A 1000 (2003), 223-251.

Articles

Vetter, W., Stoll, E., Garson, M. J., Fahey, S. J., Gaus, C., Müller, J. F. Sponge halogenated natural products found at parts per million levels in marine mammals. Environ. Toxicol. Chem. 21, (2002), 2014-2019.

Marsh, G., Athanasiadou, M., Bergman, Å., Asplund, L., Identification of hydroxylated and methoxylated polybrominated diphenyl ethers in Baltic Sea salmon (*Salmo Salar*) blood. Environ. Sci. Technol., 2004, 38, 10-18.

Eriksson, J., Green, N., Marsh, G., Bergman, Å. Photochemical decomposition of fifteen polybrominated diphenyl ether congeners in methanol/water. Environ. Sci. Technol., 2004, 38, 3119-3125.

1. Introduction

PBDEs are measured in several matrices. For environmental purposes it is necessary that the methods applied in these matrices are comparable and making use of the same principles and instrumentation. The project HORIZONTAL has been started to develop horizontal and harmonised European standards in the field of sludge, soil, contaminated soil and treated biowaste. As shown above, several articles and one standard draft are available for the measurements of PBDEs in different environmental matrices. Depending on the properties of the matrix, different or slightly different steps in the method can be necessary. In a standard all steps must be clear and hence, a decision structure is described in this discussion paper. Later on, all the steps that are necessary to analyse the different matrices can be described in more detail. This should be done by using the technical content in the literature mentioned above.

The analysis of PBDEs can be described with the following steps

- Pre-treatment of the sample and preparing of the test portion
- Extraction
- Clean-up
- Measurement by GC in combination with ECD or MS
- Requirements for identification and calculations

In the following, the steps and decisions to be made are described

2. Pre-treatment of the sample and preparing of the test portion

For the analysis of PBDEs, the following relevant sample types are distinguished

- Sediment
- Sludge
 - Sewage sludge
 - Industrial sludge
- Bio-waste
- Soil

Sampling of sewage sludge must be according to ISO-5667. Storage and transport of sludge, bio-waste and soil must be carried out in the dark at approximately 4°C. Pre-treatment must be done immediately in the laboratory by homogenising, freezing and freeze-drying.

PBDEs are sensitive for UV light and sunlight and undergo photochemical degradation if exposed. In general, the photochemical reaction rate increase with increasing number of bromine substituents, and thus are the perbrominated BDE-209 the most sensitive. Great care must be taken to avoid photochemical degradation of PBDEs throughout the analysis and the samples should be as kept in dark as far as possible. UV filters may be placed at windows and below fluorescent lights.

For the extraction of the sample, it is necessary that the starting material is comparable. This is true for waste and soil. Pre-treatment methods exist to deliver a dry or relatively dry test portion. Work has to be done for sludge. A freeze dry method is available, but there may be simpler approaches (centrifugation, filtration), which also can be used to remove the main part of the water. For the extraction of PBDEs from solid materials it is necessary that the test portion contains less than 25% of water.

3. Extraction

A traditional extraction method used for PBDE applications is based on a centrifugation methodology. The extraction has been carried out using acetone and acetone/hexane mixtures as solvent extractors. A robust liquid-solid extraction technique, which has been commonly used for different matrices and has been used for sewage sludge and biosolids treated sludge for PBDE analysis is the Soxhlet extraction. Typical solvents in Soxhlet extraction of PBDEs from sewage sludge are toluene and hexane/acetone. The extraction time can be from 12 to 24 h. Pressurized liquid extraction (PLE), commonly named accelerated solvent extraction (ASE) can also be used for the extraction of PBDEs. CH_2Cl_2 or hexane/ CH_2Cl_2 is delivered into an extraction cell containing the sample, which then is brought to an elevated temperature (100 to 150°C) and pressure (1000 to 1500 psi). The entire extraction process is fully automated. PBDE recoveries obtained using PLE method are similar to those obtained using the conventional Soxhlet extraction. The use of solid phase microextraction (SPME) may also be used. Similar to PLE this technique allows efficient extraction, reduced solvent consumption

and analysis time, and it is easily automated. SPME uses headspace extraction and hard contamination of the chromatographic system is prevented.

Of these four extraction methods (centrifugation, Soxhlet, PLE and SPME), Soxhlet and PLE should preferably be used in advance of centrifugation. SPME may be a good alternative in the future since no clean-up might be necessary. However, SPME needs to be further examined. In Table 1 shows the extraction techniques that have been applied for the analysis of BFRs in sewage sludge, soil and biosolids treated sludge samples.

This will lead to the following generalised procedures. In a following document procedures from relevant papers will be used.

Soxhlet/ASE Extraction of a dry test sample with hexane, acetone or toluene. Addition of internal standards to the extract.

Table 1. Sample extraction techniques applied to the analysis of BFRs in sewage sludge, soil and biosolids treated sludge samples.

Matrix	Extraction method	Solvent	Time	References ^a
Sewage sludge	Centrifugation	Acetone Acetone/hexane (1:3)	60 min 30 min	Nylund et al 1992, Sellström et al. 1999, Öberg et al. 2002
Sewage sludge	Soxhlet	Toluene	18 h	Hagenmaier et al. 1992
Sewage sludge	Soxhlet	Hexane:acetone (3:1)	12 h	De Boer et al. 2003
Sewage sludge	Soxhlet	Toluene	24 h	Kohler et al. 2003
Sewage sludge	Soxhlet	Toluene	Not described	Moche et al. 2003
Biosolids treated sludge	Soxhlet	Toluene	12-16 h	Kolic et al. 2003
Soil, sewage sludge	PLE ^b	CH ₂ Cl ₂	100°C, (1000 psi, 2 cycles)	Hale et al. 2002
Sewage sludge	PLE	Hexane:CH ₂ Cl ₂ (1:1)	100°C, (1500 psi, 3 cycles)	Larrazabal et al. 2004
Sludge	SPME ^c	–	–	Salgado et al. 2003

^aReferences: Nylund, K., Asplund, L., Jansson, B., Jonsson, P., Litzén, K., Sellström, U. Chemosphere 24 (1992) 1721. Sellström, U., Kierkegaard, A., Alsberg, T., Jonsson, P., de Wit, C. Organohalogen Compd. 40 (1999) 383. Öberg, K., Warman, K., Öberg, T. Chemosphere 46 (2002) 805. Hagenmaier, H., She, J., Benz, T.,

Dawidowsky, N., Düsterhöft, N., Lindig, C., *Chemosphere* 25 (1992) 1457. de Boer, J., Wester, P.G., van der Horst, A., Leonards, P.E.G., *Environ. Pollution* 122 (2003) 63. Kohler, M., Zennegg, M., Gerecke, A.C., Schmid, P., Heeb, N. *Organohalogen Compd.* 61 (2003) 123. Moche, W., Stephan, K. *Organohalogen Compd.* 61 (2003) 147. Kolic, T.M., MacPherson, K.A., Reiner, E.J., Ho, T. *Organohalogen Compd.* 61 (2003) 175. Hale, R.C., La Guardia, M.J., Harvey, E., Mainor, T.M., *Chemosphere* 46 (2002) 729. Fabrellas, B., Larrazabal, D., Martínez, M.A., Eljarrat, E., Barceló, D. *Organohalogen Compd.* 66 (2004) 3755. Salgado, C., Chao, M.G., Jares, C.G., Cela, R., Llompart, M., Proceedings of the Third Scientific Meeting of the Spanish Society of Chromatography and Related Techniques, Almería, Spain, November 19-21, 2003, pp 131. ^bPLE = pressurized liquid extraction; ^cSPME = solid phase microextraction

4. Clean-up

Clean-up has to be used if compounds are present that can interfere (co-elute in any extent) with the PBDE congeners of interest. Depending on the different matrices, a variety of sample clean-up procedures may be suitable. The complexity of sewage sludge matrices implies the use of multi-step purification methods. Examples of purification techniques applied to sewage sludge samples are given in Table 1. Sometimes, soil and sewage sludge extracts contain relatively large amounts of elemental sulphur, which would disturb the GC analysis, and must be removed. Typical methods for sulphur removal are treatment with copper powder, silica modified with AgNO₃ in a multi-layer silica column, or desulfuration with mercury. Optionally, elimination of elemental sulphur could be done simultaneously with the extraction procedure, by addition of copper powder to Soxhlet beaker or to PLE extraction cell. Sewage sludge samples contain high level of organic matter that disturbs the well operation of purification via column chromatographic procedure. For this reason, in most cases, the purification step starts by a treatment with concentrated sulfuric acid to remove organic matter. Clean-up stage may then be performed with the aim of purification as well as fractionation of the extract, using a great variety of adsorbents (silica, Florisil, alumina ...). However, these conventional multi-step purification methods are time consuming. For this reason an automated cleanup system based on the sequential use of multilayer silica and basic alumina adsorbents respectively, pre-packed in Teflon columns may be used. The automated system configuration consists of a valve module, a valve drive module and a pump. The whole system is computer controlled and can be programmed as required (i.e. volume, flow rates, direction of solvent flow ...). This system is regarded as an alternative that offsets most of the disadvantages of the conventional cleanup methods given its capacity for processing automatically different samples simultaneously in approx. one hour. In Table 2

examples of clean-up procedures that have been applied for the analysis of PBDEs in sewage sludge and soil samples are shown. As mentioned above, no clean-up may be necessary then SPME is used as the extraction method, but additional studies have to be done to establish this fact.

Table 2. Sample clean up techniques applied to the analysis of PBDEs in sewage sludge and soil samples.

Matrix	Clean-up	References ^a
Sewage sludge	- Conc. H ₂ SO ₄ treatment - Multilayer silica + alumina - GPC - Basic alumina	Hagenmaier et al. 1992
Sewage sludge	- GPC ^b : two columns - H ₂ SO ₄ treatment - Extraction with pentane - Silica	De Boer et al. 2003
Sewage sludge	- Conc. H ₂ SO ₄ treatment - Desulfuration with mercury - Multilayer silica + basic alumina	Kohler et al. 2003
Sewage sludge	- 4 columns	Moche et al. 2003
Sewage sludge	- Conc. H ₂ SO ₄ treatment	Nylund et al 1992, Sellström et al. 1999, Öberg et al. 2002
Soil, sewage sludge	- Envirosep® size-exclusion - Silica	Hale et al. 2002
Sewage sludge	- Conc. H ₂ SO ₄ treatment - Automated purification: acidic silica, basic alumina	Larrazabal et al. 2004

^aReferences: Hagenmaier, H., She, J., Benz, T., Dawidowsky, N., Düsterhöft, N., Lindig, C., Chemosphere 25 (1992) 1457. de Boer, J., Wester, P.G., van der Horst, A., Leonards, P.E.G., Environ. Pollution 122 (2003) 63. Kohler, M., Zennegg, M., Gerecke, A.C., Schmid, P., Heeb, N. Organohalogen Compd. 61 (2003) 123. Moche, W., Stephan, K. Organohalogen Compd. 61 (2003) 147. Nylund, K., Asplund, L., Jansson, B., Jonsson, P., Litzén, K., Sellström, U. Chemosphere 24 (1992) 1721. Sellström, U., Kierkegaard, A., Alsberg, T., Jonsson, P., de Wit, C. Organohalogen Compd. 40 (1999) 383. Öberg, K., Warman, K., Öberg, T. Chemosphere 46 (2002) 805. Hale, R.C., La Guardia, M.J., Harvey, E., Mainor, T.M., Chemosphere 46 (2002) 729. Fabrellas, B., Larrazabal, D., Martínez, M.A., Eljarrat, E., Barceló, D. Organohalogen Compd. 66 (2004) 3755. ^bGPC = gel permeation chromatography

5. Chromatographic separation

5.1 Choice of detector

MS detectors are allowed. GC coupled to either negative chemical ionisation (NCI) or electron ionisation (EI) low resolution mass spectrometry (LRMS) are the alternatives more frequently used for PBDE analyses. NCI-LRMS is by far the most popular technique due to high levels of sensitivity provided. However, if high specificity and accuracy in quantification is needed (for instance, by using isotopic dilution method), EI-LRMS shows better performance, at the expense of lower sensitivity. Another MS detection technique that may be used is the quadrupole ion trap (QIT)-MS in a tandem mode, which has been shown to have good selectivity and to be close to NCI in sensitivity. GC-high resolution mass spectrometry (HRMS) based analytical methods are also available for determination of congener specific PBDE compounds, providing the most selective and sensitive method. However sophisticated and expensive instruments which require a trained personnel and frequent maintenance are needed for HRMS. Time of flight (ToF)-MS is a detector that combines good sensitivity, accuracy and identity confirmation, but the instruments are expensive. However, more studies related to PBDE analysis are needed. A comparison of the performance with the different MS approaches NCI-MS, EI-LRMS, EI-HRMS, QIT-MS and ToF-MS that can be used in PBDE analysis are shown in Table 3. In Table 3 examples of detection techniques that have been applied for the analysis of PBDEs in sewage sludge samples are shown.

Table 3. Comparison of the performance of different MS approaches used in PBDE analysis.

Technique	Sensitivity	Identity Confirmation	Accuracy	Cost
NCI-MS	++	+ (bromine ion)	++ (internal standard)	+
EI-LRMS	+	++ (nominal molecular mass)	+++ (isotopic dilution)	+
EI-HRMS	+++	+++ (exact molecular mass)	+++ (isotopic dilution)	+++
QIT-MS	++	++ (nominal molecular mass)	+++ (isotopic dilution)	+
ToF-MS	++	+++ (full scan)	+++ (isotopic dilution)	++

5.2 Choice of column

Good separation can be obtained for most PBDE congeners using 50 m columns. However, more studies are required in order to determine potential co-elutions between PBDE congeners, since all 209 PBDE congeners are not available at present and have thus not been evaluated for co-elutions. Even though no co-elutions between the major important PBDE congeners i.e. BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183 and BDE-209 and other PBDE congeners have been reported, attention must be paid order to determine potential co-elutions of PBDE congeners that may appear e.g. PBDE congeners that may have been formed from any kind of transformation reactions such as photochemical degradation. BDE-209 should receive special attention because of its sensitivity for higher temperatures and the higher susceptibility for degradation in the GC system. Analyses can be carried out using short GC columns (10-15 m) avoiding long exposures to elevated temperatures. The film thickness of the short column should preferably be 0.1-0.2 μm , with the aim not to extend the exposure to high temperatures unnecessarily. This means that the analysis of BDE-209 should occur separately from the analysis of the rest of PBDE congeners.

5.3 Use of internal standard

Internal standards (IS) have to be used. IS for GC-MS in the EI mode using the isotopic dilution technique are,

- ^{13}C -BDE-47 for tetraBDEs
- ^{13}C -BDE-99 for pentaBDEs
- ^{13}C -BDE-153 for hexaBDEs
- ^{13}C -BDE-183 for heptaBDEs
- ^{13}C -BDE-209 for BDE-209

IS for GC-MS in the NCI mode are,

- BDE-77 and BDE-181 for tetraBDEs, pentaBDEs, hexaBDEs and heptaBDEs measuring m/z 79, 81.
- ^{13}C -BDE-209 for BDE-209 using the isotopic dilution technique, measuring m/z 494.7, 496.7 for IS and m/z 484.7, 486.7 for native BDE-209.

6. Identification and quantification

6.1 Identification

Identification is based on relative retention times (RRTs) and masses. The principle of identification points will be followed (EU 2002⁵ and ISO/DIS 22892). Three identification points are necessary. For MS this means the RRT and two masses. Additional evidence is allowed. The absence of the peak in the chromatogram means a value below detection limit. Additional identification could be to calculate the isotopic ratio between two masses from the same cluster i.e. m/z 79, 81. The relative isotopic peak ratios must be within $\pm 15\%$ of the theoretical values. If identification is difficult an alternative clean-up may be necessary.

6.2 Calibration

Linear calibration curve with at least 5 points is necessary. The calibration curves will determine the range of linearity and concentrations in the analysis must be in the established range. If not, a dilution or concentration of the extract must be carried out before injection in the GC-MS system.

6.3 Interferences

Following substance groups should be taken into consideration as possible interferences.

- Polychlorinated biphenyls (PCBs)
- Polychlorinated naphthalenes (PCNs)
- Polychlorinated diphenyl ethers (PCDEs)
- Methoxylated polychlorinated diphenyl ethers (MeO-PCDEs)
- Polybrominated biphenyls (PBBs)
- Methoxylated polybrominated diphenyl ethers (MeO-PBDEs)
- Halogenated bipyrrols

Following relevant examples of co-elutions on non-polar GC columns are known to occur;

PCBs: CB-180 may interfere with BDE-47 when EI-MS is applied (see Figure 1).

PBBs: BB-153 may interfere with BDE-154 when ECI-MS is applied.

TBBPA may interfere with BDE-153 when ECI-MS is applied.

Other examples of co-elutions on non-polar GC columns of polybrominated compounds have been reported from environmental samples from biota, but their presence in sediment, sludge and soil is unknown.

MeO-PBDEs: 5-Cl-6-MeO-BDE47 and 6'-Cl-2'-MeO-BDE68 may interfere with BDE-99 when ECI-MS is applied.

DiMeO-PBDE: 2',6-DiMeO-BDE68 may interfere with BDE-99 when ECI-MS is applied.

Figure 1. Total ion current obtained following the co-injection of PBDE (---) and PCB (—) standard mixtures. Hepta- and octa-CBs eluted within the chromatographic window defined for tetra- and penta-BDEs. BDE-47 and CB-180 eluted at the same retention time.

