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Congener-specific determination of polybrominated diphenyl ethers in biosolids by GC/HRSIRMS

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ABSTRACT

Biosolids from eight municipal sewage treatment plants were studied from the standpoint of determining PBDE congener target analytes in each sample. Targeted analytes were subjected to high specificity through the use of high resolution selected ion recording mass spectrometry (HRSIRMS). Three ions from the molecular ion cluster of each congener group were monitored at 10000 resolution and PCB#204 was used as the internal standard. The seven congeners determined ranged from low tens of μg/kg to several mg/kg in each sample. The general presence of PBDEs indicates that biosolids serve as an environmental sink for these compounds, and the application of biosolids to land and other uses must take into consideration the presence and fate of these compounds.

KEYWORDS

Biosolids;
PBDEs;
HRSIR;
Accurate mass.

INTRODUCTION

Biosolids (sewage sludge) represent the end product of bacterial digestion and applied treatments of raw sewage in a municipal sewage treatment facility[1]. The precipitated material from the aqueous solution consists of a relatively intractable mass made up of both inorganic and organic substances that have reached an environmental sink. The complete characterization of this material remains unfinished but studies by various workers have increased our knowledge about this material[2].

In recent times the interest has shifted from disposal of the biosolid material in landfills to the preferred use of processed material as a soil amendment[3]. The question of course arises as to the potential exposure to biota that would result from such an application in the environment as well as effects on human health that might arise from eating crops exposed to the amended soil by way of animals eating such crops before going to market or inhaling dust from such applications. Additionally, concern arises from any appearance of substances from biosolids entering into groundwater or ultimately into drinking water due to leachate[4].

The biosolids have usually undergone additional treatment to destroy pathogens either by heat or irradiation after the considerable bacterial degradation and additional process treatment that has taken place. Thus, the biosolids are designated Class A or Class B depending on the extent of elimination of pathogens[5]. Some proponents advocate the additional step of composting to remove/degrade the remainder of the objectionable compounds that are currently known to reside in biosolids[6].

A number of papers have now filled in some of our questions about the types of contaminants in biosolids. The review by Rogers[1] included mention of a variety of compounds including organochlorine pesticides (e.g.,

aldrin), PCBs, and chlorophenols. A large presence for nonyl phenols and surfactants was also mentioned. The large and diverse class of pharmaceuticals and personal care products (PPCPs) was also mentioned and most of these compounds (e.g., antibiotics) would be found in the polar fraction of sludge components. The synthetic musks are one group of PPCPs that partition with the liphophilic fraction. Methods were given with extraction and cleanup procedures also included.

Oberg et al. described the occurrence of PBDEs in over 100 sludge samples from Sweden where the predominant tetra, penta, and hexa congeners were found as well as the decabromodiphenyl ether[7]. Ying and Kookana pointed out that high levels of triclosan in biosolids could be a concern in soil applications[8]. Synthetic musks were determined in biosolids[9]. Nonylphenols, pthalates, and PCBs were determined in biosolids and soil in an effort to follow the fate of such contaminants after soil amendment using biosolids[10].

A number of papers have focused on the polar analytes (contaminants) found in biosolids. Giger et al. reported methodology for extraction and determination of antibiotics including the fluoroquinolone antibiotics. Extraction difficulties and relatively low recoveries were noted[11] and this contrasts with quantitative recovery of nonpolar compounds. Mottaleb and Brumley reviewed the separations used in determining PPCPs in a variety of environmental matrices[12].

There are two EPA methods relevant to our approach. Method 1668a is for PCBs and uses three ions from the molecular ion clusters for monitoring. Method 1614 for PBDEs uses two ions per molecular ion cluster. In both cases, quantitation is by isotope dilution so that extensive use is made of stable isotope-labeled compounds[13,14].

In this work we report using a new method involving GC/HRSIRMS in a survey of the occurrence of PBDE congeners in biosolid samples from eight municipal treatment facilities in the U.S.

EXPERIMENTAL

Chemicals

PBDE congeners were obtained from Accu standard (8 congener mix BDE-CM: 28, 47, 99, 100, 153, 154, 183, 209). PCB#204 was obtained from Chem Service.

Samples

Samples were obtained from eight municipal treatment facilities and stored in glass bottles in a freezer. Several gram portions were taken and air dried in a hood. The dried material was pulverized in a ball mill grinder (Reutsch) at 20 Hz for 2 min using a glass ball and glass-lined sample vessel.

Mass spectrometry

GC/HRSIR: A Waters-MicroMass AutoSpec Premier (P) was operated at 10000 resolution in EI mode (500 μA trap current, 250^{0}C source, high boiling PFK calibrant, 8kV accelerating voltage, 350 V photo multipler detector). Software was MassLynx 4.1.

Method for PBDEs

Extraction/Cleanup

The method uses a SW-846 approved extraction (Method 3545A)[15] of BS in methylene chloride/acetone (50/50, v/v) by pressurized liquid extraction (Dionex ASE200 80^{0}C, 15 min static, 100% purge, two extractions per sample). The extract is concentrated and then fractionated on silica using SPE to isolate the fraction containing the PBDEs. A 3 ml Si SPE cartridge (Phenomenex) is washed with hexane; sample is applied in 1 ml of hexane and then the sample is eluted with 2 ml hexane, 2 ml hexane/methylene chloride (50/50 v/v), 2 ml methylene chloride, and 2 ml acetone. The PBDEs are found in the hexane fraction and hexane/methylene chloride (90/10 v/v) fractions which are combined. Typical sample size was 0.5 g and the final volume was 1 ml with 100 μl of internal standard added (100 pg/μl). The average recovery for this method was published previously[16].

GC/HRSIR

A 30 m 0.25μm film 0.25 mm ID column (DB5 Agilent-JandW) was used with temperature programming: 60^{0}C for 1 min followed by 60-300@20^{0}C/min. SIR (30 msec dwell) divided into three retention time groups: Gp 1: 403.80470, 405.80265, 407.80061, 416.97063 (PFK lock mass), 429.76057 (IS), 483.71385, 485.71112, 487.70907; 5 to 13.6 min;

TABLE 1: Retention times of PBDE congeners

Congener no	RT
28	12.03
47	13.11
99	14.36
100	14.02
153	16.07
154	15.36
183	18.66
IS	12.93

TABLE 2: Congener levels in biosolids from eight treatment facillities determined in triplicate

Sample#→ Congener#↓	1	2	3	4	5	6	7	8
28	43.1	55.2	8.9	6.3	9.8	17.5	6.9	21.8
47	1677	1627	570	284	376	920	299	503
99	2471	2123	652	285	466	121.8	324	809
100	547	465	149	60.0	102	308	70.8	189
153	179	174	54.1	18.4	33.6	84.5	18.3	48.8
154	142	146	46.4	15.7	25.8	73.1	17.4	42.2
183	20.9	33.2	16.4	17.8	10.0	26.9	8.0	16.1

TABLE 3: Precision for determination of a single extract (sample #3, third replicate)

Congener #	µg/kg /+/-% RSD
28	9.84 / 1.1
47	591 / 6.5
99	729 / 4.6
100	165 / 4.1
153	60.3 / 6.0
154	50.3 / 4.4
183	20.0 / 6.5

TABLE 4: Supplemental data showing all three determinations of congener levels in biosolids from eight treatment facillities determined in triplicate (three separate portions of dried, ground material taken through the extraction, cleanup, and final separation/determination independently) in µg/kg.

Sample#6 Congener# 9	1	2	3	4	5	6	7	8
28	33.1	59.1	8.3	6.3	10.4	19.7	6.4	21.9
28	28.3	64.3	8.8	7.2	9.4	17.9	7.9	19.0
28	68.0	42.1	9.7	5.5	9.7	14.8	6.5	24.4
47	1311	1747	576	273	391	1001	268	674
47	1161	1775	538	328	352	1038	362	568
47	2559	1359	595	252	386	722	268	826
99	1565	2333	578	288	532	1244	296	719
99	1390	2586	665	330	486	1620	403	630
99	4458	1451	713	237	381	791	272	1079
100	362	491	130	61.4	117	328	62.9	168
100	311	568	155	69.2	105	378	88.4	147
100	968	330	162	49.4	83.4	218	61.0	251
153	115	170	49.1	17.6	42.2	91.4	18.7	46.5
153	107	240	54.5	21.7	32.5	108	22.9	36.8
153	317	112	58.7	15.8	26.0	54.2	13.3	63.1
154	99.2	143	42.6	15.6	29.8	79.9	16.3	39.1
154	91.1	196	47.2	17.6	24.8	88.0	21.4	32.9
154	236	98.1	49.3	13.9	22.8	51.3	14.5	54.5
183	10.9	27.2	14.6	12.7	11.3	28.7	7.9	14.9
183	17.4	50.3	15.6	11.4	10.0	32.4	9.3	12.9
183	34.4	22.1	19.1	29.4	8.7	19.7	6.8	20.6

Gp 2: 561.62367, 563.62163, 565.61958, 566.96642 (PFK lock mass), 641.53214, 643.53009, 645.52805; 13.70 to 18.0 min; Gp3: 719.44265, 719.44265 (PFK lock mass), 721.44060, 723.43856; 18.1 to 25.0 min.

RESULTS AND DISCUSSION

Confirmation of identity of PBDEs

The seven congeners included in this study are the most common congeners determined in environmental analysis. The retention times are given in TABLE 1 for the congeners. The compounds range from tribromodiphenyl ethers to heptabromodiphenyl ether congeners, and they were addressed using three retention time groups (tri-BDE and tetra-BDE, pentaBDE and hexa-BDE, and hepta-BDE).

Confirmatory requirements are addressed using three ions from the molecular ion cluster and their relative abundances. The supporting information from the presence of additional congeners strengthens the conclusion that we are dealing with the polybrominated diphenyl ethers. The additional selectivity of 10000 resolution adds to our certainty as to the elemental composition, and the congener is established with the agreement of the retention time observed in sample extracts with those of standards. HRSIRMS is more selective than low resolution MS[16].

Biosolids analyses

The analyses of the eight municipal treatment facilities were carried out in triplicate and the results are given in TABLE 2. Levels of PBDEs ranged from low µg/kg to hundreds of µg/kg or to low mg/kg levels. Congeners 47 and 99 were always found as the highest concentration contaminants among the congeners. The individual analyses of each of the biosolids samples showed some significant variations. It is suspected that biosolids material itself is highly heterogeneous so that these results must be viewed as representative but could also vary greatly as samples differ on various time scales of sampling.

Considerable variation is sometimes observed in the results for the three sampling set and this is attributed primarily to heterogeneity in the biosolids themselves.

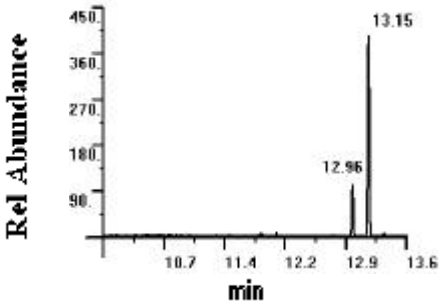

Figure 1: Group 1 total ion current responses (including m/z 405.80265 and m/z 485.71112 congener groups) showing congeners # at RT=12.05 (#28) and 13.15 (#47) and internal standard at RT=12.96

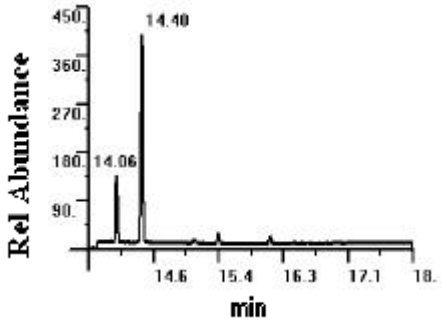

Figure 2: Group 2 total ion current responses (including m/z 563.62163 and m/z 643.53009) showing congeners # at RT=14.06 (#100) , 14.40 (#99) , 15.40 (#154) , and 16.11 (#153)

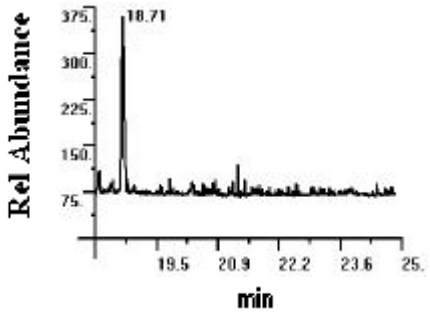

Figure 3: Group 3m/z 721.4406 ion current response showing congener #183 at RT=18.71

The reproducibility of a single determination is given in TABLE 3 for sample #3 (#3 replicate). This establishes a good estimate of precision for the single extract and can be compared to the variation found among replicate extractions of the same sample if so desired.

The precision of determination may not be as good as for isotope dilution studies where perhaps 2% precision or better may be obtained, but the RSDs are quite acceptable for this simpler and less costly approach. Supplemental data in TABLE 4 show the three independent determinations for each congener for all eight municipal sites and indicate sample heterogeneity.

The implications of these contaminant levels centers upon concern with the possible introduction of these contaminants, that have reached an environmental sink, back into the environment as a result of the application of biosolids to landfills or agricultural use. Leachate may also represent an additional path back into environmental transport processes.

Data examples

The following figures 1-3 illustrate the response for retention time groups 1, 2, and 3 respectively in sample 3 (third independent analysis). In general, the specificity is high for determining the congeners in biosolids despite the complexity of the matrix[16]. There are an enormous number of compounds present in this matrix, and this remains a fair characterization of the lipophilic fraction itself. The PBDEs exhibit some advantages because of their negative mass defect (high resolution monitoring thus eliminates many compounds with positive mass defect) and their relatively high molecular weights and longer retention times. Nevertheless, interferences were observed in low resolution monitoring with at least one congener group. Negative ion approaches also represent greater selectivity as does HRMS, but specificity may be lacking for lower brominated congeners since there may be little or no molecular anion produced[17].

Two figures below of mass spectra illustrate the agreement of spectra obtained from the monitored ions for a standard and for spectra obtained from sample extracts for congener #99. The theoretical relative abundance for the most abundant ions of the molecular ion cluster is 51.2:100:97.8, and observed experimental

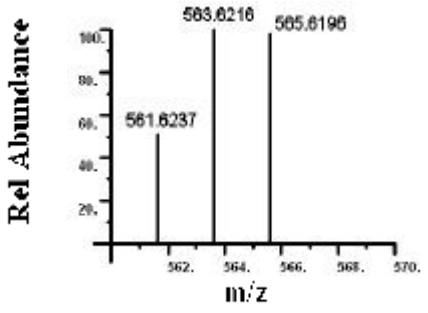

Figure 4: Mass spectrum of a standard of congener #99

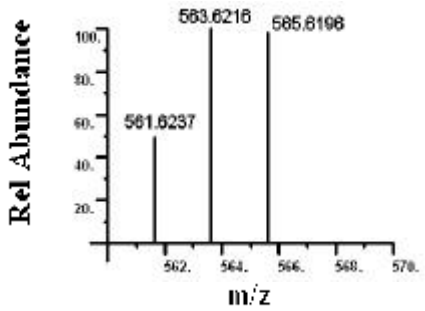

Figure 5: Mass spectrum of congener #99 obtained from a biosolid sample 3 (replicate 3)

values are within 2% of the calculated values.

Detection of other congeners and specificity

In addition to the monitored congeners, additional responses attributable to PBDEs were observed within the group windows. Specifically, in the first group window additional responses were observed for tribromo-BDEs at RT=11.88 and 12.07 min., and tetrabromo BDEs were observed at RT=12.71 and 13.33 min. In the second group window an additional response was observed for a pentaBDE at RT=15.12 min and responses were observed for hexaBDEs at RT=15.45 and 16.15 min.

The silica gel cleanup affords an extract that has removed the more polar coextractives that include the fecal sterols and sterones[16]. This enhances the specificity of the method but still allows detection of additional congeners beyond the commonly occurring compounds. The additional congeners were estimated at levels below 100 ppb based on responses of similar congeners.

CONCLUSION

These results confirm the important contribution that PBDEs make to the contaminant levels of biosolids in a ubiquitous manner. Biosolids thus constitute an important environmental sink for PBDEs. The levels reported should enable risk assessors to evaluate the application of biosolids to land use in conjunction with the potential exposure of biota to these compounds. This method differs from Method 1614 by only using a single internal standard and thus is much cheaper to carry out, while it maintains greater confirmative power using three ions from the molecular ion cluster.

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