

GENERAL PAPERS

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(Pages 97-99 in *Preprints of Extended Abstracts*, Vol. 40 No. 2)

Symposia Papers Presented Before the Division of Environmental Chemistry
American Chemical Society
Washington, DC August 20-24, 2000

ENDOCRINE DISRUPTORS (OCTYLPHENOL, NONYLPHENOL, NONYLPHENOL ETHOXYLATES AND POLYBROMINATED DIPHENYL ETHERS) IN LAND APPLIED SEWAGE SLUDGE "BIOSOLIDS"

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Abstract

Sewage sludge "biosolids" from four publicly owned treatment works (POTWs) were found to contain potentially endocrine disrupting contaminants. Octylphenol (OP), nonylphenol (NP), nonylphenol ethoxylates (NPEOs) and polybrominated diphenyl ethers (PBDEs) were analyzed using enhanced solvent extraction, size exclusion chromatography, SPE, GC-ELCD and GC-MS. Levels of NP, NP mono- and diethoxylate detected in three of the four samples exceeded the land application limit of 50 mg/kg dry weight, set in Denmark. OP, which is significantly more estrogenic than NP, was detected at mg/kg levels. The samples also contained PBDE congeners with 4 to 5 bromines, similar to the commercial DE-71 formulation. The predominant PBDE congener observed was 2,2',4,4'-tetrabromodiphenylether (BDE-47), ranging from 499 -1050 µg/kg. Decabromodiphenylether was detected in only one of the samples, but at 588 mg/kg.

Introduction

In 1988, Congress amended the Marine Protection, Research, and Sanctuaries Act to prohibit open ocean dumping of industrial waste and sewage sludge. This ban prompted new legislation for the disposal of sludge ("The Standards for the Use or Disposal of Sewage Sludge," Title 40 CFR, Part 503, February 19, 1993). This rule sets regulations for the disposal of sewage sludge "biosolids" on lands designated for agricultural usage. (Currently the U.S. applies over 2 million dry metric tons of biosolids

annually.) However, Rule 503 does not require biosolids to be monitored for organic contaminants. (EPA is currently negotiating legislation to amend Rule 503 to include dioxins and co-planar PCBs.) These lipophilic compounds in wastewater may strongly sorb to solids. These solids are typically settled, de-watered and disposed of by landfilling, incineration, or applied as biosolids.

The worldwide production of alkylphenol ethoxylates (APEOs) exceeds 500,000 tons annually. These are used in detergents, paints, pesticides, textile, and personal care products. APEOs have been shown to degrade into more toxic and lipophilic compounds in wastewater treatment plants¹. Although APEOs are mainly associated with POTW, these compounds have also been detected in non-POTW effluents². APEOs bio-degrade by a step-wise shortening of the ethoxylate chains, creating a complex mix of compounds, including: short-chain ethoxylates, alkylphenoxy carboxylic acids (APECs), and alkylphenols (APs) such as nonylphenols (NPs). The APECs and longer chain APEOs are soluble in water. OP and NP, and the shorter chain APEOs have low water solubilities and tend to sorb to suspended solids or sediments¹. OP, NP and nonylphenol diethoxylate (NP2EO) have been reported to induce vitellogenin production in male trout at 10 µg/L for NP and NP2EO, and 3 µg/L for OP³. The threshold for expression of intersex (testis- ova) in Medaka was reported at 50 µg/L for NP⁴.

Polybrominated diphenyl ether (PBDE) production exceeds 40,000 tons annually. They are used as flame retardants in the plastic components of computers and televisions, circuit boards, and in polyurethane foam used in seats of cars, buses and furniture. The release rate of PBDE's into the environment is still under investigation. However they have been detected in fish tissue, sediments⁵ and in sewage sludge⁶. Hydroxylated-PBDEs resemble the thyroid hormones thyroxine and triiodothyronine and thus may interfere with normal physiological functions⁷.

Sampling and Method

Three of the biosolids used in this study originated from separate mid-Atlantic POTWs and were subjected to either lime stabilization or composting preceding distribution. Two were collected in the field, and the third sample was acquired from a local retail nursery. A fourth sample was obtained through mail-order from an international distributor which was heat-treated to destroy sludge pathogens, prior to distribution. All samples were analyzed for APs, NPEOs, and PBDEs (**Table 1 & 2**).

Percent solids were determined on each sample. All samples were freeze-dried, sieved (2000 µm) to remove large debris and stored in glass jars with Teflon lids at <4 °C, until analyzed. Enhanced solvent extraction (Dionix, ASE 200) was employed to extract the biosolids. Extraction conditions were: pressure @ 1000 psi, temperature @ 100 °C, heat 5 minutes, static 5 minutes, 60% flush, purge 180 seconds. Samples were subjected to two complete extraction cycles. The solvent volume used was approximately 30 mL of dichloromethane (DCM) for each extraction, sample size 5 g. The following surrogates were added to the sample prior to extraction:

perinaphthenone (for OP, NP, nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate) and PCB-204 (for 2,2',4,4'-tetrabromodiphenylether (BDE-47), 2,2',4,4',6-pentabromo- diphenylether (BDE-100), 2,2',4,4',5-pentabromodiphenylether (BDE-99) and decabromodiphenylether (BDE-209)). Extracts were reduced to 4 mL under nitrogen, and purified by size exclusion chromatography (Phenomenex^R, Envirosep-ABC, 350 x 21.1 mm. column, column flow rate 5 mL/min., DCM). The first 50 mL was discarded, removing high molecular weight lipids. The next 60 mL containing the compounds of interest (OP, NP, NPEOs, and PBDEs) were collected. This fraction was solvent exchanged to hexane. The partially purified extract was added to a silica column (2 g SPE columns) and separated, starting with a non-polar solvent (3 mL hexane) removing short chain alkanes, in the second fraction PAHs, PCBs, and PBDEs are separated by increasing the polarity of the extraction solvent (6 mL of 60:40 hexane/DCM). In the third fraction, the polar compounds OP, NP and NPEOs were removed with 10 mL of acetone. The second and third fractions were reduced in volume, solvent exchanged to toluene and transferred to GC auto-sample vials. Pentachlorobenzene (480 ng), was added to the second fraction (PBDEs) prior to GC-ELCD analysis and p-terphenyl (10 µg) was added to the third fraction (OP, NP, NPEOs) prior to being analyzed by GC/MS as internal standard.

Alkylphenol and ethoxylates were analyzed by high resolution capillary column with a mass spectrometer (Varian Saturn 2000, GC/MS) operated in the electron ionization (EI) mode with a mass range of 50-450 m/z⁺. Analytes were quantified with a five point linear regression calibration curve using the internal standard and selected ions for each compound of interest. Selected ions: OP 135 m/z⁺, NP 135 m/z⁺, NP1EO 179 m/z⁺, NP2EO 223 m/z⁺, perinaphthenone 152+180 m/z⁺, and p-terphenyl 230 m/z⁺. The analytical column was a 60 m, DB-5 (J&W Scientific) with a 0.25 µm film thickness and 0.32 mm inner diameter. Carrier gas was helium. GC program: initial column setting 75 °C, hold one minute, ramp at 4 °C/min., hold at 330 °C for 5 min., total run time 70 min., injector 315 °C, mass range collect from 50 to 450 m/z⁺.

PBDE extracts were subjected to high resolution capillary column with an electrolytic conductivity detector (ELCD). Analytes were quantified against the internal standard using a five point linear regression calibration curve. The same analytical column and carrier gas was used, as stated above. GC program: initial column setting 90 °C, hold one minute, ramp at 4 °C/min., hold at 320 °C for 39 min., total run time 97.5 min., injector 300 °C, detector cell 900 °C.

Results and Discussion

Of the APEO-related compounds, NP concentrations were the highest, ranging from 5.4 to 820 mg/kg (**Table 1.**). The total of the NP, NP1EO, and NP2EO concentrations detected in 3 of the 4 biosolids were 3- to 18- times higher than the Danish allowable land application limit of 50 mg/kg. In the biosolids tested, OP concentrations ranged from 0.206 to 7.49 mg/kg (**Table 1.**). OP had been previously shown to be 10- to 20-fold more estrogenic than NP and NP2EO⁸. Thus the OP concentrations, in the biosolids examined are sufficiently high at levels to be considered themselves a

concern from an estrogenicity standpoint.

PBDEs were also detected in the biosolids (**Table 2.**) BDE-47 was the predominant PBDE congener detected, ranging from 499 - 1050 µg/kg. The deca- and octa- formulation were also detected, but only in the heat-treated sample, (deca-bromodiphenylether at 588 mg/kg; the octa- formulation was not quantified in this report.) The other three biosolids showed no trace of the higher brominated formulation, even though the deca- and octa-BDEs make up 90% of the PBDE flame retardant market. All samples did contain the tetra and penta congeners ($\Sigma > 1$ mg/kg) (**Table 2.**) Their composition in the biosolids closely resemble the commercial formulation DE- 71, used primarily as a flame retardant in polyuretane foam.

According to the presented data, APs, NPEOs and PBDEs may enter the environment through the field application of biosolids. These contaminants are a concern for the terrestrial environment where biosolids are being applied, as well as potentially the aquatic environment. Possible for contamination from runoff requires further investigation. However, it has been previously shown that NP and NPEOs can leach from biosolids⁹. Further studies need to be conducted on the accumulative effects of endocrine disruptors on both terrestrial and aquatic organisms and on pathways for these compounds to enter the environment.

Table 1. APs and NPEOs (mg/kg dry weight) in four biosolids.

Alkylphenols and Ethoxylates	Compost (40 lb bag) n=3 mg/kg (%SD)	Compost (bulk) n=3 mg/kg (%SD)	Lime Stabilization n=3 mg/kg (%SD)	Heat Treatment n=3 mg/kg (%SD)
OP	0.206 (1.9%)	1.46 (6.1%)	5.25 (2.9%)	7.49 (3.4%)
NP	5.38 (5.5%)	172 (4.1%)	820 (3%)	496 (6%)
NP1EO	0.722 (12%)	2.55 (13%)	81.7 (4%)	33.5 (12%)
NP2EO	nd	nd	25.3 (5%)	7.36 (32%)
Surrogate Recovery (Perinaphthenone)	85% (2.4%)	117% (2.4%)	89% (5.8%)	92% (5.4%)

Table 2. PBDEs ($\mu\text{g}/\text{kg}$ dry weight) in four biosolids.

PBDE	Compost (40 lb bag) n=3 $\mu\text{g}/\text{kg}$ (%SD)	Compost (bulk) n=3 $\mu\text{g}/\text{kg}$ (%SD)	Lime Stabilization n=3 $\mu\text{g}/\text{kg}$ (%SD)	Heat Treatment n=3 $\mu\text{g}/\text{kg}$ (%SD)
BDE-47	693 (1.4%)	1049 (2.1%)	499 (5.6%)	721 (2.6%)
BDE -100	85.0 (11%)	134 (1.4%)	70.8 (9%)	92.2 (7.2%)
BDE - 99	546 (4.1%)	851 (4.2%)	377 (2.7%)	523 (3.2%)
Surrogate Recovery (PCB-204)	119% (14%)	107% (4.6%)	93% (3.9%)	114% (5.8%)

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