

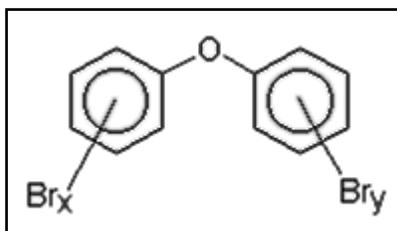
## **Report for 2004NJ75B: Fate of Brominated Flame Retardants in New Jersey Wastewater Treatment Facilities**

- Conference Proceedings:
  - Fennell, D.E.; F. Liu; M. Giacalone. 2004. Dehalogenation of polyhalogenated aromatics in cultures and enrichments. Proceedings of the American Chemical Society, Division of Environmental Chemistry, 228 th ACS National Meeting, Philadelphia, PA, August 22-26, 2004.
  - Zarnadze, A.; L. Totten; D.E. Fennell, D.E.; M. Giacalone; and U. Krogmann. 2004. PBDEs in the NY/NJ Harbor estuary. Proceedings of the American Chemical Society, Division of Environmental Chemistry, Poster presentation at the 228th ACS National Meeting, Philadelphia, PA, August 22-26, 2004.
- Other Publications:
  - Fennell, D.E.; F. Liu; E.-K. Son; A. Zarnadze; U. Krogmann; L.Totten. 2005. Biotransformation of halogenated contaminants in sludges and enrichments from municipal anaerobic digesters. Submitted to the SETAC North America 26th Annual Meeting, Baltimore, MD, November 13 17, 2005.

Report Follows

## Problem and Research Objectives

Wastewater treatment facilities (WWTF) are called upon to prevent macropollutants such as organic compounds measured as biochemical oxygen demand, nitrogen and phosphorous from entering aquatic systems. Increasingly they are also expected to remove trace persistent, bioaccumulative and toxic chemicals (PBTs). This class of chemicals includes emerging pollutants such as the brominated flame retardants, polybrominated diphenyl ethers (PBDEs) (Betts, 2002) (Figure 1).



**Figure 1. Chemical structure of polybrominated diphenyl ether (PBDE). The compound may contain up to 10 bromine substituents.**

WWTF are the first line of defense in preventing contamination of aquatic systems by these compounds. WWTF influents commonly contain a multitude of PBTs, however little is known about their fate in the facilities. Like polychlorinated biphenyls (PCBs) and chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDD/Fs), PBDEs are hydrophobic, associated with organic matter, and are resistant to aerobic degradation. Thus these compounds tend to accumulate in wastewater treatment sludges (*Hale et al., 2001; NRC, 2002; Litten et al., 2003*). After these wastewater treatment sludges are treated to reduce pathogens and to stabilize them, in New Jersey, 41.3% are beneficially used in-state (e.g., in agriculture, in top soils distributed to the public and in landfill covers), and 30.9% are used beneficially out-of-state (*NJDEP, 2003*). Sludge treatment processes used in New Jersey include anaerobic digestion, aerobic digestion, lime stabilization, advanced alkaline stabilization, composting, pelletization and wet air oxidation. Little is known how the different sludge treatment processes affect the fate of PBDEs. Few data are available on concentrations of PBDEs in sewage, sludges and biosolids (treated sewage sludge) however, those that are available suggest a significant presence (*Hale et al., 2001; Litten et al., 2003*). We intend to document the presence and level of PBDEs in New Jersey sewage, sludges and biosolids from selected WWTF.

Anaerobic bacterial dehalogenation has been shown to be an effective method of removal or detoxification of halogenated environmental pollutants in groundwater, soils and sediments. Exploitation of dehalogenating bacteria for detoxification during anaerobic digestion of municipal wastewater treatment sludges may also be possible. We

will determine whether environmentally relevant congeners of PBDEs are transformed or detoxified during one sludge treatment process—anaerobic digestion.

The specific objectives of the project are:

- (1) Document the presence and level of PBDEs in New Jersey sewage, sludges and biosolids at selected WWTF.
- (2) Document the ability of anaerobic digestion to dehalogenate/detoxify selected environmentally relevant congeners of PBDEs.
- (3) Prepare a full proposal to the EPA and/or the National Science Foundation for a broader assessment of the life cycle of halogenated PBTs, including PBDEs in the wastewater treatment process from influent to final disposal.

## Methodology

**Analysis of PBDEs in Sewage, Sludge and Biosolids.** Sewage influent, finished effluent, anaerobic digester sludge, and processed biosolids were obtained from four regional wastewater treatment facilities. The participating facilities treat various combinations of domestic and industrial wastewater with differing potentials for PBDE content. The samples were collected by WWTF personnel using in-place compositing protocols specific to each facility. Samples were collected over (at most) a 24-hour period and were stored at 4°C until pickup by Rutgers personnel. Samples were then stored at 4°C until processing. A detailed sample processing and extraction protocol (see description of the newly developed methods in **Principal Findings and Significance** section) was developed based on published methods for determination of PDBEs in sewage, sludges and biosolids (Hale *et al.*, 2001; Hyotylainen and Hartonen, 2002).

**Dehalogenation of Spiked PBDE in Anaerobic Digester Sludges.** We monitored the transformation of PBDEs in batch studies of digester sludge from the NJ wastewater treatment facilities and in a highly enriched mixed culture originally started from, digester sludge from the municipal anaerobic digester in Ithaca NY and containing a known dehalogenating bacterium, *Dehalococcoides ethenogenes* strain 195 (Fennell *et al.*, 2004). DecaBDE, one of the most commonly used PBDE formulations was used to spike sludge inoculated enrichments at levels slightly elevated over those that have been observed in sludges (Hale *et al.*, 2001; Litten *et al.*, 2003) to allow observation of activity.

*Culture set up.* Both the sludge inoculated bottles and the *D. ethenogenes*-containing culture were set up for deca-BDE transformation using methods described previously for polychlorinated dibenzo-p-dioxins and furans (Vargas *et al.*, 2001). Briefly, for sludges, triplicate sterile 160 mL serum bottles containing about 0.5 g of dry, ground, sterile sludge were prepared for each treatment. A 0.07 mL volume of toluene stock solution containing 2660 µmol/L of decaBDE (0.5 µmol deca-BDE) was added to each bottle in order to completely coat the sediment. For *D. ethenogenes*-containing

culture triplicate sterile 50 mL serum bottles containing about 0.25 g of fine dry, sterile sediment were prepared for each treatment. A 0.5 mL volume of stock solution containing 1000  $\mu\text{mol/L}$  of decaBDE (0.5  $\mu\text{mol}$  deca-BDE) was added to each bottle in order to completely coat the sediment. All bottles were purged overnight with sterile anoxic nitrogen to remove the toluene carrier.

After bottle preparation, the NJ sludge enrichments were developed by diluting 10 mL sludge into 90 mL (i.e., 10% v/v dilution) of an anaerobic minimal salts medium (Fennell *et al.*, 2004) into the bottles. The highly enriched mixed culture containing *D. ethenogenes* strain 195, was grown at 25°C as described previously (Fennell *et al.*, 2004) on PCE and butyric acid. The mixed culture contained, through stoichiometric estimation based upon chloride release and the hydraulic (solids) retention time, approximately 16  $\mu\text{g}$  *D. ethenogenes* protein/mL (Fennell *et al.*, 2004a) or about  $10^8$  cells/mL. The culture (35 mL) was added to the prepared 50 mL serum bottles. In addition to decaBDE, some bottle sets were amended with alternate halogenated compounds tetrachloroethene (PCE) or 1,2,3,4-tetrachlorobenzene (TeCB). A mixture of organic acids and yeast extract were added periodically as a carbon/energy/hydrogen source. **Table 1** shows the bottle set up protocol for assessing decaBDE transformation.

**Table 1. Enrichment protocol for assaying PBDE biotransformation by anaerobic enrichments.**

Bottle Set	Treatments			
	Dehalococcoides-containing mixed culture			
	Electron Donors ( $\mu\text{M}$ )	Other Electron Acceptors ( $\mu\text{M}$ )	Trace Nutrients	deca-BDE ( $\mu\text{M}$ )
1 (killed)	None	None	None	13
2	Butyrate (440)	None	Yeast extract	13
3 (plus co-substrate)	Butyrate (440)	PCE (110)	Yeast extract	13
	NJ anaerobic digester sludges (10 % v/v)			
1 (killed)	None	None	None	5
2	Lactate (100)/ Butyrate (100)	None	Yeast extract	5
3 (plus co-substrate)	Lactate (100)/ Butyrate (100)	PCE (25)	Yeast extract	5
4 (plus co-substrate)	Lactate (100)/ Butyrate (100)	1,2,3,4-TeCB (25)	Yeast extract	5

*Culture sampling.* 1- or 2-mL samples of well-mixed bottle contents were withdrawn periodically using a sterile, anoxic glass syringe for PBDE analysis. 0.1 ml samples of gas headspace were withdrawn from the bottles periodically and analyzed for methane production and transformation of alternate halogenated compounds PCE and TeCB to monitor health of the enrichments.

*Molecular analysis.* NJ anaerobic digester sludges were examined for the presence of *Dehalococcoides* using polymerase chain reaction (PCR) analysis. Briefly, 1 mL of sludge was centrifuged at 15,000 g and the supernatant was discarded. Microbial community DNA was extracted from the pellet using a soil DNA extraction kit (MoBio Laboratories, Inc.). The community DNA was subjected to PCR using universal bacterial PCR primers (Ahn *et al.*, 2003; Fennell *et al.*, 2004b) and PCR primers specific for the 16S rRNA gene of *Dehalococcoides* (Maymo-Gattel *et al.*, 1997). The PCR product obtained using the universal bacterial 16S rRNA primers was subjected to re-amplification using the *Dehalococcoides* specific primer in a nested PCR procedure (Fennell *et al.*, 2001).

## **Principal Findings and Significance (Progress Report)**

The on-going project seeks to document both presence of PBDEs in wastewater and sludges, but also to document transformation capacity of anaerobic microbial communities. Primary results to date are development of a sampling, extraction and analysis protocol and initial results for microbial enrichments.

*Sample and extraction protocols.* Development of standard operating protocols (SOPs) for extraction and analysis of PBDEs has been completed. We modified published and in-house methods to create PBDE extraction and analysis SOPs tailored to microbial enrichments and WWTF samples. The draft SOPs are shown in **Figure 2**. Analysis of samples is on-going and will be reported at the conclusion of the project.

*Microbial enrichments.* We examined the biotransformation of halogenated pollutants by microorganisms in anaerobic digester sludges and microbial enrichments from sludges. An anaerobic digester mixed culture enrichment containing *D. ethenogenes* strain 195, did not dehalogenate decaBDE over a six month incubation period. In a related NJWRRI-funded project, the organism did not dechlorinate octachlorodibenz-*p*-dioxin however; it did dehalogenate a hexachlorodibenzofuran congener to penta- and tetra dibenzofuran daughter products. The tests with NJ sludges have been on-going for about six months. Tetrachloroethene added to the sludges was dehalogenated to trichloroethene and dichloroethene with no formation of vinyl chloride or ethene. On-going experiments are being analyzed to determine whether decaBDE is being dehalogenated by these sludges.

*Molecular analysis.* *Dehalococcoides*-like bacteria were not detected by direct or nested PCR analysis of community DNA using *Dehalococcoides*-specific primers. The observed dechlorination pattern with PCE (trichloroethene and dichloroethene with no formation of vinyl chloride or ethene) suggests the presence of dehalogenating bacteria other than *Dehalococcoides*.

*Discussion.* Several dehalogenating bacterial isolates were originally obtained from anaerobic digesters. Our results to date suggest that one—*D. ethenogenes* strain 195—does not dechlorinate deca-BDE to any detectable extent in a 3 month incubation period. Although *Dehalococcoides* sp. was not detected in the four NJ WWTF sludges, initial activity on PCE suggests the presence of other dehalogenating strains. Results from on-going microbial dehalogenation tests and examination of PBDEs in WWTF samples will be reported at the conclusion of the project.

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## Figure 2. PBDEs (and PCBs) Wastewater Treatment Plant Draft Sample SOP (working draft).

### PBDEs (and PCBs) Wastewater Treatment Plant Sample SOP

#### A. Important Notes:

- All procedures should be performed under low light. Wrap vials, flasks or separatory funnels in aluminum foil whenever possible.
- All glassware should be baked at 450°C overnight.
- All other surfaces that come into contact with the sample should be rinsed with either hexane or dichloromethane just prior to performing the procedure.

#### B. Wastewater Effluent Samples

##### 1. Preparation:

- 1) Rinse separatory funnels with dichloromethane (DCM, methylene chloride) and let dry under hood. Bake at 450°C overnight.
- 2) Bake sodium sulfate (450°C for 4 hours).
- 3) Open each triplicate I-Chem Jar and remove a well-mixed sample (100 to 150 mL) for determination of Total Suspended Solids and Volatile Suspended Solids according to Standard Methods 2540D and 2540E, respectively. [Note, volume for solids measurements is estimate, it may need to be adjusted to achieve 2.5 to 200 mg dried residue.]

##### 2. Extraction:

- 1) Add surrogate standard <sup>13</sup>C 183 PBDE (total PBDE measured previously in US effluent is approximately 29,023 pg/L ± 1490 (North, 2004), thus we should have about 18 ng PBDEs in the total sample (800 mLs) to be extracted) directly to sample jar using protocol in **Table 1**.
- 2) Add PCB surrogates using protocol in **Table 1** directly to sample jar.
- 3) Transfer entire remaining sample (e.g., 950 mL – 150 mL = 800 mL) to a 2-L separatory funnel. [Note, give the values expected from North (2004) we should have about 18 ng PBDEs in the total sample to be extracted].
- 4) Add the same volume of dichloromethane (DCM) as sample to the empty sample jar to rinse, and then pour into the separatory funnel.
- 5) Shake funnel every few minutes for ½ hr.
- 6) Collect extracted aqueous fraction and discard.
- 7) Add 60 mL of milliQ water saturated with NaCl to separatory funnel and back extract to remove polar compounds from the DCM—shake every few minutes for ½ hr.
- 8) Add 1 gram of sodium sulfate to empty rotovap flask (round bottom flask).
- 9) Discard aqueous fraction from the separatory funnel. It is better to remove all water (and possibly lose a small amount of DCM) than have residual water in the sample.
- 10) Remove DCM fraction to the rotovap flask containing the sodium sulfate.
- 11) Rinse separatory funnel 3 times with a small amount of hexane and allow all three washings to go into the rotovap flask with the extract and sodium sulfate.

##### 3. Rotovap (Follow the Totten Lab Rotovap SOP in the PCBs SOPs document, note rotovap flask = round bottom flask):

- 1) Rinse empty rotovap flasks with hexane.
- 2) Carefully pour extract and hexane rinse from original rotovap flask from extraction into new rotovap flask. If Teflon beads and sodium sulfate are present in flask, do not let them reach the new flask during pour.
- 3) Rinse original rotovap flask 3 times with a small amount of hexane and transfer all three washings into the new rotovap flask (again do not let sodium sulfate or Teflon beads reach the new flask).
- 4) Rotovap DCM extract to about 5 mL. (see SOP for rotovapping).
- 5) Transfer remaining extract (now mostly hexane) into a 12-mL amber glass vial.
- 6) Rinse rotovap flask 3 times with hexane and add all three washings to the 12-mL glass amber vial containing the extract.
- 7) Blow down the extract in the amber vial under nitrogen to about 1 mL.
- 8) Store in vial in -20°C freezer until cleanup.

##### 4. Cleanup:

- 1) Perform clean up in accordance with PCBs SOPs on “Alumina Cleanup”
- 2) Collect Fraction 1 (hexane, about 12 mLs) separately from Fraction 2 (hexane:DCM, about 12 mLs) in amber glass vials of 25 to 50 mL volume (covered with foil and then capped). [Fraction 1 contains PCBs and PBDEs, Fraction 2 contains PBDEs].
- 3) **DO NOT** blow down the fractions.
- 4) Store at -20°C until analysis.

##### 5. GC/MS analysis:

- 1) Spike Fraction 1 and Fraction 2 amber collection vials (containing about 12 mLs) with PBDE internal standard solution according to **Table 1**. [We will decide what to do about PCBs in these samples later.]
- 2) Transfer about 0.5 mL of each fraction to separate GC vials for analysis for PBDEs (GC-MS).

### PBDEs (and PCBs) Wastewater Treatment Plant Sample SOP (Continued)

#### C. Wastewater Influent

##### 1. Preparation:

- 1) Rinse separatory funnels with DCM and let dry under hood. Bake at 450°C overnight.
- 2) Rinse filter apparatus with DCM and let dry under hood. Bake glass parts at 450°C ?? overnight (not sure what the stoppers on the bottom portion of the filter apparatus can withstand).
- 3) Remove a well-mixed sample (15 to 20 mL) from each jar and determine Total Suspended Solids and Volatile Suspended Solids according to Standard Methods 2540D and 2540E, respectively. [Note, volume for solids measurements is estimate, it may need to be adjusted to achieve 2.5 to 200 mg dried residue.]
- 4) Bake glass fiber filters for collection of solids samples for subsequent extraction at 450°C overnight.

##### 2. Collection of Solid/Liquid Fractions:

- 1) For each triplicate jar, remove triplicate 20 mL samples and filter through a 0.7 µm, 47 mm glass fiber filters, let air pull through for a little while to dry off excess water. [Note: total PBDEs previously measured in US influent was 640 ng/L (North, 2004) thus triplicate samples of 20 mL should yield about 40 ng PBDEs]. Filter the triplicate volumes into the same flask.
- 2) Wrap triplicate filters in foil together and freeze at -20°C until extraction.
- 3) Save filtrate and carry through liquid-liquid extraction as described for effluent samples (See **Section B** and **Section C.5** below).

##### 3. Extraction of Solids Captured on Filters:

- 1) Shove triplicate filters into Soxhlet apparatus.
- 2) Place a scoop of sodium sulfate and a scoop of Teflon chips in the bottom of each round bottom (rotovap) flask.
- 3) Add surrogate standard <sup>13</sup>C 183 PBDE to the Soxhlet according to the protocol shown in **Table 1** (total PBDEs previously measured in US influent was 637 ng/L (North, 2004), thus we expect a mass of PBDE of roughly 40 ng in our sample).
- 4) Add PCB surrogates (PCB solution containing PCBs 23, 66, and 165) directly to Soxhlet according to **Table 1**.
- 5) Soxhlet extract with dichloromethane (DCM) for 24 hrs (see PCBs SOPs for Soxhlet protocols).

##### 4. Rotovap:

- 1) Rinse empty rotovap flasks with hexane.
- 2) Carefully pour extract and hexane rinse from rotovap flask from Soxhlet extraction into new rotovap flask. If Teflon beads and sodium sulfate are present in flask, do not let them reach the new flask during pour.
- 3) Rinse original rotovap flask 3 times with hexane and transfer all three washings into the new rotovap flask.
- 4) Rotovap DCM extract to about 5 mL. (see PCBs SOPs for rotovapping).
- 5) Transfer remaining extract (now mostly hexane) into a 12-mL amber glass vial.
- 6) Rinse rotovap flask 3 times with hexane and add all three washings to 12-mL amber vial containing the extract.
- 7) Blow down the extract in the amber vial under nitrogen to about 1 mL.
- 8) Store in vial in -20°C freezer until cleanup.

##### 5. Cleanup:

- 1) Perform clean up in accordance with PCBs SOPs on “Alumina Cleanup”
- 2) Collect Fraction 1 (hexane) separately from Fraction 2 (hexane:DCM) in amber glass 25 to 50 mL amber vials (covered with foil and then capped). [Fraction 1 contains PCBs and PBDEs, Fraction 2 contains PBDEs].
- 3) **DO NOT** blow down the fractions.
- 4) Store at -20°C until analysis.

##### 5. GC/MS analysis:

- 1) Spike Fraction 1 and Fraction 2 collection vials (containing about 12 mLs each) with PBDE internal standard solution according to **Table 1**. [We will decide what to do about PCBs in these samples later.]
- 2) Transfer about 0.5 mL of each fraction to separate GC vials for analysis for PBDEs (GC-MS).

##### 7. Extraction of Liquid Fraction Generated by Filtration:

- 1) Add surrogate PBDE standard <sup>13</sup>C 183 PBDE and surrogate PCB standard (mass in filtrate would probably be about 1/100<sup>th</sup> of that in the total sample, see section C.3.3 above) to filtrate in filter flask according to **Table 1**.
- 2) Transfer entire sample (15 to 50 mL collected as filtrate during filtration of each of the influent aliquots) to a 500-L separatory funnel.
- 3) Add 50 mL dichloromethane (DCM) to the filter flask. Swish it around.
- 4) Transfer DCM from filter flask to separatory funnel and perform extraction and cleanup as described above for Wastewater Effluent in **Section B**. Add internal standard for PBDEs in the amount shown in **Table 1**.

## Figure 2. PBDEs (and PCBs) Wastewater Treatment Plant Draft Sample SOP (continued).

PBDEs (and PCBs) Wastewater Treatment Plant Sample SOP (Continued)				
<b>D. Sludges and Biosolids</b>				
1. Preparation: Make sure to do this under LOW LIGHT.				
<ol style="list-style-type: none"> <li>1) Rinse mortars and pestles with dichloromethane (DCM, methylene chloride) and let dry under hood. Bake at 450°C overnight.</li> <li>2) Remove triplicate well-mixed samples (25 to 50 g) and determine Total Solids and Volatile Solids according to Standard Methods 2540G.</li> <li>3) Weigh out ~5 g of biosolids into a mortar.</li> <li>4) Add <sup>13</sup>C BDE 183 as surrogate to the biosolids/sludge according to <b>Table 1</b> (total PBDEs previously measured in US sludges/biosolids was about 4000 µg/kg (North, 2004), thus we expect about 20 µg available for extraction in each sample).</li> <li>5) Add PCB surrogates according to <b>Table 1</b> (250 µL of PCB solution containing PCBs 23, 65, and 166) directly to biosolids/sludge.</li> <li>6) Add 5 g pre-baked sodium sulfate.</li> <li>7) Mix gently using a pestle until a powder.</li> </ol>				
2. Extraction of Dried Sludges/Biosolids:				
<ol style="list-style-type: none"> <li>1) Transfer sodium sulfate dried sample to Soxhlet apparatus.</li> <li>2) Place a scoop of sodium sulfate and a scoop of Teflon chips in the bottom of each round bottom rotovap flask.</li> <li>3) Soxhlet extract with dichloromethane (DCM) for 24 hrs (see PCBs SOPs for Soxhlet protocols).</li> </ol>				
3. Rotovap:				
<ol style="list-style-type: none"> <li>1) Rinse empty rotovap flasks with hexane.</li> <li>2) Carefully pour extract and hexane rinse from rotovap flask from Soxhlet extraction into new rotovap flask. If Teflon beads and sodium sulfate are present in flask, do not let them reach the new flask during pour.</li> <li>3) Rinse original rotovap flask 3 times with hexane and transfer all three washings into the new rotovap flask.</li> <li>4) Rotovap DCM extract to about 5 mL. (see PCBs SOPs for rotovapping).</li> <li>5) Transfer remaining extract (now mostly hexane) into a 12-mL amber glass vial.</li> <li>6) Rinse rotovap flask 3 times with hexane and add all three washings to 12-mL amber vial containing the extract.</li> <li>7) Blow down the extract in the amber vial under nitrogen to about 1 mL.</li> <li>8) Store in vial in -20°C freezer until cleanup.</li> </ol>				
4. Cleanup:				
<ol style="list-style-type: none"> <li>1) Label separate amber Fraction 1 and Fraction 2 vials for each sample. Add foil and cap to each vial. Get an empty tare weight for each vial.</li> <li>2) Perform clean up in accordance with PCBs SOPs on "Alumina Cleanup"</li> <li>3) Collect Fraction 1 (hexane) separately from Fraction 2 (hexane:DCM) in amber vials (covered with foil and then capped). [Fraction 1 contains PCBs and PBDEs, Fraction 2 contains PBDEs].</li> <li>4) After fraction collection, weigh each vial containing fraction plus foil and cap.</li> <li>5) <b>DO NOT</b> blow down the fractions.</li> <li>6) Store at -20°C until analysis.</li> </ol>				
5. GC/MS analysis:				
<ol style="list-style-type: none"> <li>1) Remove an aliquot of approximately 500 µL from each fraction collection vial using a pre-baked glass pipette and place each aliquot in a separate GC vial. Cap GC vial.</li> <li>2) Quickly recap Fraction 1 and Fraction 2 amber collection vials and reweigh.</li> <li>3) Calculate the percentage of the mass of analyte in the original fraction that the aliquot represents.</li> </ol>				
$\text{mass in aliquot\%} = \frac{\text{weight of fraction collection vial before aliquot removal} - \text{weight of fraction collection vial after aliquot removal}}{\text{weight of fraction collection vial before aliquot removal}} \times 100$				
<ol style="list-style-type: none"> <li>4) Add the PBDE internal standard solution to the GC Vial containing the aliquot according to <b>Table 1</b>. [We will decide what to do about PCBs in these samples later.]</li> </ol>				
<b>E. Laboratory Blanks</b>				
<ol style="list-style-type: none"> <li>1. Fill Amber I-Chem jars with 800 mL of milliQ water (same source of water as used for any handling of the samples or reagents), cap and shake a little.</li> <li>2. Carry through liquid-liquid extraction exactly as described for Wastewater Effluent (starting at <b>Section B.2</b>)</li> </ol>				
<b>F. Standard Additions Protocol</b>				
Table 1. Surrogate and Internal Standard Additions for PBDE and PCB Extractions from wastewater treatment plant effluent, influent and sludges/biosolids.				
Standards→	PBDE Surrogate Standard ( <sup>13</sup> C BDE 183)	PCB Surrogate Standard (PCBs 23, 66, 165)	PBDE Internal Standards (BDE 75)	*PCB Internal Standards (PCBs 30, 204)
<b>Standard Stock Concentration for Influent and Effluent Samples→</b>	<b>50 ng/mL</b>	<b>200 ng/mL</b>	<b>20 ng/mL</b>	<b>200 ng/mL</b>
<b>Samples</b>	<b>Volume</b>	<b>Volume</b>	<b>Volume</b>	<b>Volume</b>
Influent (residue on triplicate filters)	40 µL	10 µL	20 µL (F1, F2 no blowdown)	10 µL (F1)
Influent (filtrate collected from triplicate samples)	5 µL	5 µL	5 µL	5 µL
Effluent (liquid-liquid extraction)	40 µL	10 µL	20 µL (add to both F1 and F2 which have NOT been blown down)	10 µL (F1)
<b>Standard Stock Concentration for Sludges/Biosolids→</b>	<b>5 µg/mL</b>	<b>200 ng/mL</b>	<b>20 ng/mL</b>	<b>200 ng/mL</b>
<b>Samples</b>	<b>Volume</b>	<b>Volume</b>	<b>Volume</b>	<b>Volume</b>
Sludge/Biosolids	40 µL	250 µL	200 µL (add to an aliquot of F1 and F2 which have NOT been blown down)	250 µL (F1)
* To be determined				