Polybrominated diphenyl ethers and their hydroxylated and methoxylated derivatives in seafood obtained from Puget Sound, WA

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Word Count: 2,663

Abstract

Synthetic polybrominated diphenyl ethers (PBDEs) are ubiquitous environmental contaminants and known to occur in most food items. Consumer fish products have been identified as having some of the highest PBDE levels found in USA food sources. Natural formation of hydroxylated (OH-) and methoxylated (MeO-) PBDEs are also known to occur in simple marine organisms, which may be bioaccumulated by seafood. In this study, we report findings of an initial survey of PBDE, OH-PBDE and MeO-PBDE content in common seafood items available to residents living in the Puget Sound region of Washington State. Seafood samples were either purchased from local grocery stores or caught off the coast of SE Alaska and in Puget Sound. The edible portions of the seafood were analyzed, which for finfish was white muscle (skinless fillets) and for shellfish, either the entire soft tissue (bivalves) or processed meat (calamari, shrimp and scallops). Results indicated that finfish typically had higher levels of PBDEs compared to shellfish with BDE-47 and BDE-99 as the most common congeners detected. Among shellfish, bivalves (clams and mussels) were notable for having much higher levels of OH- and MeO-PBDEs compared to other types of seafood with 6'-OH-BDE-47 and 2'-MeO-BDE-68 being the more common OH- and MeO- congeners, respectively. Based on our results and recent updates to daily fish consumption rates, estimated intake rates for Washington State residents will be between 34-644 ng PBDEs / day, depending on species consumed. For the OH- and MeO- forms, daily exposure is much more variable but typically would range between 15-90 ng/day for most seafood types. If shellfish are primarily consumed, OH-PBDE intake could be as high as 350 ng/day. These daily intake rates for PBDEs are higher than most dietary intake rates calculated for populations in other world regions.

1. Introduction

Synthetic polybrominated diphenyl ethers (PBDEs) were widely used as flame retardants in a variety of consumer products and have become ubiquitous environmental contaminants (de Wit 2004). The use of PBDEs was gradually banned in the USA, beginning with penta-PBDE formulations in 2005 and then deca-PBDE in 2013 (US EPA 2014). However, it is recognized that many PBDE containing products are still in use or have been recently discarded, which is expected to cause continued environmental release. This is consistent with monitoring of PBDEs in aquatic environments such as the Northeast Pacific, which indicates a trend of relatively constant levels of PBDEs in finfish (Ikonomou et al. 2011). Worldwide monitoring of PBDEs in human blood suggests that USA exposures are much higher than in the rest of the world (Fromme et al. 2016). Human exposure to PBDEs primarily occurs through ingestion of contaminated food and contact with indoor dust (Frederiksen et al. 2009). Consumer fish products have been identified as having some of the highest PBDE levels found in USA food (Schecter et al. 2010).

It is well-established that natural formation of PBDE derivatives occurs in marine organisms such as algae and bacteria (Vetter 2006). For example, Kuniyoshi et al. (1985) reported the occurrence of two forms of PBDE derivatives in marine green algae in Japan that were hydroxylated (OH-) and methoxylated (O-methyl; referred to as MeO- henceforth). Recently, Agarwal et al. (2014) demonstrated the formation of OH- and MeO-PBDEs by marine bacteria associated with marine sponges. Similar compounds have been reported in many marine organisms including shellfish (Löfstrand et al. 2011) and finfish (Dahlberg et al. 2016b). Additionally, high levels of these compounds have also been found in marine mammals,

22 including seals and whales (Vetter et al. 2001, 2002; Pettersson et al. 2004; Montie et al. 2010).
23 There have been no reports of industrial production of OH- and MeO-PBDEs, which strongly
24 suggests their presence in marine shellfish and finfish is due to bioaccumulation of naturally
25 occurring compounds and/or biotransformation of natural and anthropogenic PBDEs (Haglund et
26 al. 1997; Marsh et al. 2004; Wan et al., 2009). Thus, it is increasingly recognized that some OH-
27 and MeO-PBDEs produced by simple marine organisms are bioaccumulated by marine shell-
28 and finfish that are important human food sources.
29 Persuasive evidence indicates that OH-PBDEs are the most potent forms of PBDE at
30 disturbing thyroid homeostasis and are hypothesized to be potent agents of neurological effects
31 (Dingemans et al. 2011; Kitamura et al. 2008). Additionally, OH-PBDE derivatives have been
32 shown to be inhibitors of enzymes involved in steroidogenesis and may have anti-androgen
33 effects (Canton et al. 2006; Canton et al. 2007). A recent review has concluded that reported
34 blood levels of hydroxylated PBDEs and other halogenated phenolic contaminants in humans are
35 within concentration ranges that impact thyroid homeostasis and produce neurological effects
36 (Montaño et al. 2013). Furthermore, demethylation of a MeO-PBDE has recently been
37 demonstrated in several vertebrate species (Wan et al. 2009; Wen et al. 2015) indicating that
38 MeO-PBDEs may also be a source of the more toxicologically active forms of PBDEs. These
39 concerns make it important to better understand the extent of human exposure to PBDEs and the
40 associated OH- and MeO- derivatives and whether seafood consumption is a significant source
41 of these contaminants.
42 In this study, we report findings of an initial survey of PBDE, OH-PBDE and MeO-PBDE
43 content in commonly consumed seafood items. Seafood samples were purchased from local
44 grocery stores in the Puget Sound region of Washington, USA. Additional finfish samples

caught off the coast of Alaska and in Puget Sound were included in this survey. To our knowledge, this is the first report of OH-PBDE and MeO-PBDEs in seafood from Puget Sound.

2. Materials and methods

2.1 Sample Collection

All shellfish samples were purchased raw from local grocery stores in Sequim and Seattle, WA, USA between October 2015 and January 2016. Package labeling for bivalves (live manilla clams, *Venerupis philippinarum* and blue mussels, *Mytilus edulis*) indicated they were either wild harvested from Sequim, WA, USA or from regional aquaculture farms denoted by: Dabob Bay, WA, USA (Dab); Hammersley Inlet, WA, USA (Ham); Whidbey Island, WA, USA (Whidbey); unspecified Puget Sound (P.S.) or British Columbia, CA (B.C.) locations. The shrimp samples were labeled gulf coast shrimp and appeared to be white shrimp (*Litopenaeus setiferus*) from USA. The calamari and scallops were unspecified species and labeled as aquacultured in China.

Fish samples were obtained by several methods. Rainbow trout (*Oncorynchus mykiss*), canned albacore tuna (*Thunnus alalunga*) and fish sticks were purchased from Sequim, WA, USA area grocery stores. Package labeling for the trout samples indicated the fish were steelhead, an anadromous form of rainbow trout that was commercially caught from the lower Columbia River, WA, USA after re-entry from the ocean. The canned tuna labeling indicated it had been caught and processed in Thailand. Package labeling on the fish sticks indicated Alaskan pollock (*Gadus chalcogrammus*) was used as the fish source and had been previously cooked. We also collected coho salmon (*Oncorynchus kisutch*), sablefish (*Anoplopoma fimbria*) and

67 rockfish (*Sebastes sp.*) by recreational fishing near Ketchikan, AK, USA. English sole
68 (*Pleuronectes vetulus*) were collected by stern trawler near the central Seattle waterfront, Seattle,
69 WA, USA. Upon arrival at the lab, samples were stored at - 20°C until time of analysis.

70 *2.2 Chemicals*

71 Authentic standards of all target PBDEs, OH-PBDEs and MeO-PBDEs listed in Tables 1
72 and 2 were purchased from AccuStandard (New Haven, CT, USA) and Wellington Laboratories
73 Inc. (Guelph, ON, Canada). Additional chemicals used as surrogate standards were: 3,3′,4,4′-
74 tetrabromodiphenyl ether (BDE-77), 4-OH-2',3,3',4,5,5'-hexachlorobipheny (4-OH-PCB-159)
75 both purchased from AccuStandard and 2,2',4,4',6-pentabromo-6'-methoxy[$^{13}C_{12}$] diphenyl
76 ether (^{13}C-6-MeO-BDE-100), which was purchased from Wellington Laboratories Inc.
77 Diazomethane was prepared from N-methyl-N-nirosoguanidine, following Aldrich Technical
78 Information Bulletin Number AL-121 and purchased from Sigma Chemical (St. Louis, MO,
79 USA). All other chemicals were of reagent grade or better and were obtained from common
80 sources.

81 *2.3 Sample preparation and analysis*

82 All glassware was baked overnight at 460 °C before use. The procedures used for
83 extraction and measurement of parent PBDEs, OH-BDEs and MeO-BDEs were modified from
84 methods described by Hovander et al. (2002). All samples were initially homogenized in
85 deionized water (5:1, v/w) using a rotor/stator type tissue homogenizer (IKA T 25 Ultra-Turrax,
86 NC, USA). For all types of seafood, a composite sample was prepared from individuals of each
87 species/food type (n=5 for shellfish; n=4 for finfish; a total of 16 composite samples were
88 analyzed). For shellfish, the entire sample (excluding the shell from clams and mussels) was

89 homogenized. For freshly obtained fish, skinless fillets were prepared. The canned tuna was used
90 as purchased. For fish sticks, the outer breading was removed prior to homogenization. After
91 homogenization, 5 g of the homogenate was quantitatively transferred to a Pyrex tube and the
92 surrogate standards BDE-77, ^{13}C-6-MeO-BDE and 4-OH-PCB-159 were added. Next, 2 mL of 6
93 M HCl and 6 mL of 2-propanol was added, vortexed and the denatured homogenate stood at RT
94 for 10-min. The mixture was then extracted three times with hexane/methyl tert-butyl ether
95 (MTBE) (1:1 v/v). The solvent extracts were pooled, washed with 4 mL of a 1% KCl solution
96 then transferred to a new, pre-weighed pyrex tube. The combined solvent extracts were
97 evaporated under N_2 until dry and the residual weight was recorded for determination of sample
98 lipid content after an adjustment for sample dilution with water during homogenization. Next,
99 the sample was reconstituted in 2 mL of hexane and the phenolic fraction (containing the OH-
100 PBDEs) was separated from neutral compounds by adding 2 mL of 0.5 M KOH in 50% ethanol
101 (1:1 v/v). The aqueous layer, which contains phenolic compounds, was transferred to a new tube,
102 acidified with 2 M HCl and then extracted three times with hexane/MTBE (9:1 v/v). The
103 combined hexane/MTBE extracts, now containing the phenolic compounds, were dried over ~ 2
104 g of sodium sulfate for at least one hour, then volume reduced to 2 mL and mixed overnight with
105 200 µL of diazomethane for conversion to methyl derivatives. After derivatization, 1 mL of
106 concentrated sulfuric acid was added to the phenolic fraction to remove lipids.
107 The separate extracts containing either the neutral (parent PBDEs, MeO-PBDEs) or
108 derivatized phenolic compounds were further cleaned up using an acid silica gel column. The
109 columns were prepared with 2-3 g of acid silica, 1 g of neutral silica, and a top layer of sodium
110 sulfate. Prior to sample loading, the column was exhaustively rinsed with dichloromethane and
111 hexane. After loading, the samples were eluted from the column with 40 mL of dichloromethane.

112 Afterwards, the dichloromethane extract was evaporated and then solvent exchanged with
113 hexane and subsequently evaporated to approximately 100 μL in volume. The samples were
114 spiked with internal standard BDE-166 and stored at -20 ºC before being analyzed.
115 Samples were analyzed using an Agilent 7890B gas chromatograph (GC) connected to an
116 Agilent 5977A mass spectrometer (MS) operating in electron capture negative ionization (ECNI)
117 mode using selected ion monitoring (m/z 79 and 81). Methane was used as the reagent gas. The
118 GC column was a 30 m Agilent J&W HP5-MS Ultra Inert column (0.25 mm i.d., 0.25 μm film
119 thickness). The GC oven was programmed from 80°C (held for 2 min) to 200°C at 25°C/min,
120 200°C to 250°C at 2.5°C/min, 250°C to 300°C at 5°C/min followed by 300°C isotemp for 30
121 min. The GC injector and GC/MS interface were maintained at 285°C and 300°C, respectively.
122 The ion source temperature and the MS quad temperature were set to 200°C and 150°C,
123 respectively. Compound identification was performed using GC retention times by comparison
124 to the commercially available standards.
125
126 *2.4 Quality assurance/control*

127 In the finfish samples, recoveries for all the surrogate standards ranged from 94-147% for
128 BDE-77, 103-148% for ^{13}C-6-MeO-BDE-100 and 88-99% for 4-OH-PCB-159. In the shellfish
129 samples, recoveries were 94-148% for BDE-77, 94-115% for ^{13}C-6-MeO-BDE-100 and 69-98%
130 for 4-OH-PCB-159. Reported values for both finfish and shellfish samples were adjusted for
131 surrogate recoveries in each sample with BDE-77 used for PBDEs, ^{13}C-6-MeO-BDE-100 used
132 for MeO-PBDEs and 4-OH-PCB-159 used for OH-PBDEs. A procedural blank prepared with
133 deionized water was processed concurrently with each batch of six samples. Calibration curves
134 were made using authentic standards for each analyte. The limit of detection (LOD) was

135 determined for each congener and considered to be three times the background area observed in
136 procedural blanks. Only measurements above the LOD are reported. The LODs for most PBDEs
137 ranged from 1.5 - 5.1 pg/g (ww) with BDE-47, 99 and 183 being higher at 16, 18 and 44 pg/g
138 respectively. The LODs for OH- and MeO-PBDEs ranged from 1.4 - 9.3 pg/g (ww) with 2-MeO-
139 BDE-68 and 6-MeO-BDE-85 being higher at 28 and 35 pg/g respectively.

140 **3. Results**

141 Tables 1 and 2 summarize measurements made in the finfish and shellfish samples.
142 Values shown in the Tables are on wet weight basis but also include total lipid content for each
143 sample composite. Lipid normalized values are summarized in Figure 1.
144
145 *3.1 Finfish*
146 PBDEs were detected in all samples with BDE-47 as the most abundant congener,
147 accounting for 50% or more of the total PBDE content. The PBDE congener pattern in finfishes
148 (BDE-47>99>100>> other BDEs; Table 1) is consistent with the relative congener abundance in
149 DE-71, a widely used commercial PBDE product that was phased out in 2005 (La Guardia et al.
150 2006). The highest concentration of PBDEs were measured in English sole, sablefish and trout,
151 largely due to much higher levels of BDE-47. The PBDE content of the remaining samples were
152 less than 1,000 pg/g (w.w). OH-PBDEs were detected in all samples except for the trout sample.
153 Total concentrations of OH-PBDEs were low, at or near 100 pg/g (w.w) with no single congener
154 predominating. MeO-PBDEs were also lower than PBDEs but detected in all samples, although
155 values were more variable among the different samples, 6-MeO-BDE-47 was the predominant
156 congener in most samples.
157

158 *3.2 Shellfish*

159 PBDEs were detected in all samples with BDE-47 and BDE-99 as the most abundant
160 congeners, accounting for 60% or more of the total PBDE content. The highest concentration of
161 PBDEs was measured in the calamari sample, which had relatively high levels of both BDE-47
162 and BDE-99. In general, the PBDE content of most shellfish samples was less than levels
163 observed in finfish samples. OH-PBDEs were detected in all samples and were typically higher
164 than observed in finfish samples. The PBDE congener profile was similar to finfish although
165 concentrations of BDE-47 and 99 were similar in shellfish. However, total concentrations of OH-
166 PBDEs were highly variable among the samples, ranging from low levels near 100 pg/g to over
167 2,000 pg/g (w.w.). The highest levels were observed in bivalves. No single OH-PBDE congener
168 predominated, except for 6-OH-BDE-47, which accounted for 63% of the total in Dabob Bay
169 clams. MeO-PBDEs were also detected in all samples and tended to be more abundant in
170 shellfish than in the finfish samples. The two most abundant MeO-PBDE congeners were 6'-
171 MeO-BDE-47 and 2'-MeO-BDE-68. The total concentration of MeO-PBDEs also displayed
172 relatively high variability among shellfish, with bivalves consistently having the highest levels.
173

174 **4. Discussion**

175 This study is an initial survey of PBDE, OH-PBDE and MeO-PBDE levels in diverse
176 types of seafood that are available to residents living in the Puget Sound region of Washington
177 State. We included both locally captured and non-local seafood as both are commonly found in
178 regional grocery stores. Alaskan origin seafood comprises much of the seafood sold in the USA
179 (NMFS 2016), which is why we also included several types of seafood harvested in Alaska. We
180 also focused on the edible portions of the seafood, which for finfish was primarily white muscle

(fillets) and for shellfish, either the entire soft tissue (bivalves) or processed meat (calamari, shrimp and scallops). Differences in these tissue types, associated lipid content and other established variables known to influence accumulation such as age likely explain some of the differences observed in the results. Most distinctive was the pattern of PBDE accumulation observed in all bivalve samples, regardless of origin. This was characterized as having much higher levels of OH- and MeO-PBDEs relative to PBDE levels. All other seafood samples had the opposite pattern where PBDE levels were much higher than other forms. The lipid-normalized values also show the same pattern (summarized in Figure 1), suggesting bivalves do tend to have higher levels of OH- and MeO-PBDEs than other types of seafood.

Our results for bivalve shellfish are consistent with findings from Europe and the Baltic region, where mussels (*M. edulis*) also have much higher levels of OH- and MeO-PBDEs compared to PBDEs (Löfstrand et al. 2011; Dahlberg et al. 2016a). These studies have also observed that 6'-MeO-BDE-47 and 2'-MeO-BDE-68 are the most abundant MeO-PBDEs in mussels, similar to our results for bivalves. Another interesting observation made from studies in the Baltic region is the identification of a seasonal pattern in accumulation with highest levels of OH- and MeO-PBDEs occurring in the summer and lowest in winter (Löfstrand et al. 2011; Dahlberg et al. 2016a). The summer peak levels coincide with high levels of OH- and MeO-PBDEs measured in filamentous algae (Dahlgren et al. 2015; Lindqvist et al. 2017), leading to the hypothesis that algal formation and seasonal abundance regulate bivalve concentrations (Löfstrand et al. 2011). Our samples were obtained in the fall and winter, suggesting higher levels could be occurring during the summer and warrants further investigation.

With regard to our finfish analysis, lipid normalized levels of all PBDEs were either comparable or within the range of recent reports in fish from European regions (Haglund et al.

204 2010*;* Dahlberg et al. 2016b). However, fish from some regions such as Korea, can show a
205 different pattern with OH- and MeO-PBDEs being more abundant (Kim et al. 2015). Regional
206 differences in the accumulation pattern of PBDEs likely reflects dietary habits among fishes but
207 also exposure to anthropogenic sources. The latter is probably the reason for the relatively high
208 levels of PBDEs we observed in the English sole sample. These fish were collected near the
209 Seattle waterfront, a location with an established history of elevated PBDE pollution relative to
210 other regions in Puget Sound (West et al. 2017). Another factor that could influence PBDE levels
211 is biotransformation. Several in vitro and in vivo studies in mammals have demonstrated the
212 formation of OH-PBDEs after PBDE exposure (Erratico et al. 2011, 2012; Li et al. 2017). Fish
213 do not appear capable of hydroxylating PBDEs to detectable levels, but demethylation of MeO-
214 PBDEs has been established in several fishes (Wan et al. 2009; Liu et al. 2015). Therefore, MeO-
215 PBDEs may also be a source of OH-PBDEs in fish. These conclusions are also supported by a
216 recent study in a Baltic Sea food chain where MeO-PBDEs appeared to decline at higher trophic
217 levels relative to OH-PBDEs, apparently due to increased biotransformation of MeO-PBDEs in
218 some fishes (Dahlgren et al. 2016). In our finfish analysis, both OH- and MeO-PBDEs were
219 relatively low in concentration, which would indicate fish used in this study were not highly
220 exposed to these types of PBDEs.
221 Human exposure to the various types of PBDEs from consumption of seafood clearly
222 depends on both the type and quantity consumed. Cooking could alter PBDE content, however, a
223 recent study indicated little change in PBDE levels in finfish after cooking (Bendig et al. 2013).
224 Thus, our measurements done primarily in raw seafood appear to be relevant for human
225 exposure. A recent US EPA update to the fish consumption rate for residents in the State of
226 Washington puts average consumption at 175 g/day (US EPA 2016). Based on our

227 measurements shown in Table 1, this translates to 34-644 ng PBDEs/day ingested, depending on
228 species consumed. For the OH- and MeO- forms, daily exposure is more variable, but typically
229 would range between 15-90 ng /day for most types of seafood. Most notably, if a similar quantity
230 of shellfish were consumed, OH-PBDE intake could be as high as 350 ng/day. These daily intake
231 rates for PBDEs are higher than most dietary intake rates calculated for other worldwide
232 populations (assuming 70 kg average body weight; reviewed in Fromme et al. 2016). Less data
233 for comparison is available for the OH- and MeO-PBDEs, but our results suggest daily intake is
234 similar to estimates made for Hong Kong residents (Wang et al. 2011) or higher if bivalve
235 consumption is included. These findings highlight the need for expanded monitoring of all forms
236 of PBDEs in seafood sold in the USA.
237

Acknowledgments

We thank Dr. Jim West of WDFW for providing English sole. This work was supported in part by NIOSH Grant *1R21OH010259-01A1.* Ms. Cade received support from the U.S. Department of Energy, Office of Science and Office of Workforce Development for Teachers and Scientists (WDTS) under the Science Undergraduate Laboratory Internship (SULI) program.

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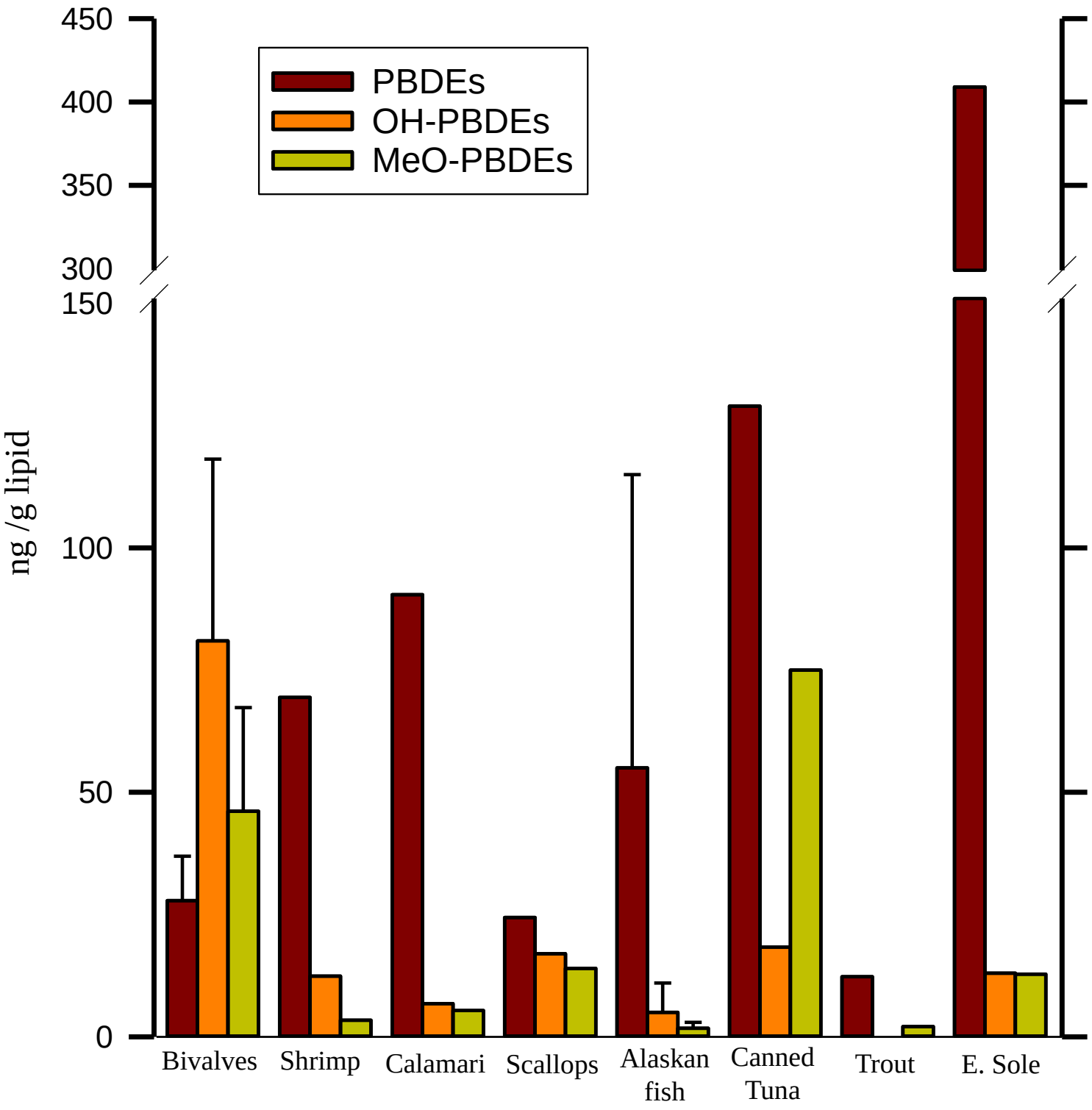


Figure 1. Lipid normalized total PBDE, OH-PBDEs and MeO-PBDEs in different types of seafood. Bivalves (clams and mussels) and Alaskan origin fish (Coho, sablefish, rockfish and fish sticks) were separately combined and the mean +/- SD shown. All values are from Tables 1 and 2, adjusted for lipid content listed for each sample on the Tables.

Table 1. Summary of measured PBDE/OH-PBDE/MeO-PBDEs congeners in skinless fish fillets or processed fish purchased at Puget Sound area markets or field collected (pg/g wet weight).

	English Sole[1]	Trout	Canned Tuna	Fish Sticks (Pollock)	Sablefish[1]	Coho Salmon[1]	Rockfish[1]
Total Lipid	0.9%	10.6%	0.6%	4.4%	2.5%	10.1%	0.5%
BDE-17	56.2	86.4			103.2	60.4	
BDE-28	27.4		18.3	9.1			
BDE-47	2,104	644.2	431.6	143.5	699.8	390.5	248.0
BDE-100	454.1	132.1	74.5	37.5	179.8	61.8	90.7
BDE-99	815.7	442.4	199.0	248.0	532.4	213.9	373.1
BDE-154	133.5		13.9				
BDE-153	89.4		17.3				
∑ PBDEs	3,680	1,305	754.6	438.1	1,515	726.5	711.8
2'OH-BDE-28	23.9					36.4	
4-OH-BDE-42	35.1						
6-OH-BDE-47	13.2						
2'-OH-BDE-68			23.7	11.8			16.5
2'-OH-BDE-75			29.9	25.6	29.6		28.7
5'-OH-BDE-100				17.2			
4'-OH-BDE-103	31.3		23.4	36.1	38.5		29.0
6'-OH-BDE-99	13.6		21.4				
3-OH-BDE-100			11.3				
∑OH-PBDEs	117.1		109.8	90.6	68.1	36.4	74.2
4'-MeO-BDE-17					7.7		
2'-MeO-BDE-28	6.91				12.3	6.60	
6-MeO-BDE-47	37.8	177.3	351.6	10.1	52.9	75.6	
3-MeO-BDE-47						9.11	
5-MeO-BDE-47	32.5						
4'-MeO-BDE-103		22.5	53.4	17.9		20.1	
6'-MeO-BDE-99	16.5		43.8		10.6		
3-MeO-BDE-100	21.5	21.8		2.71	7.2		7.60
∑ MeO-PBDEs	115.2	222.6	448.7	30.6	90.6	111.4	7.60

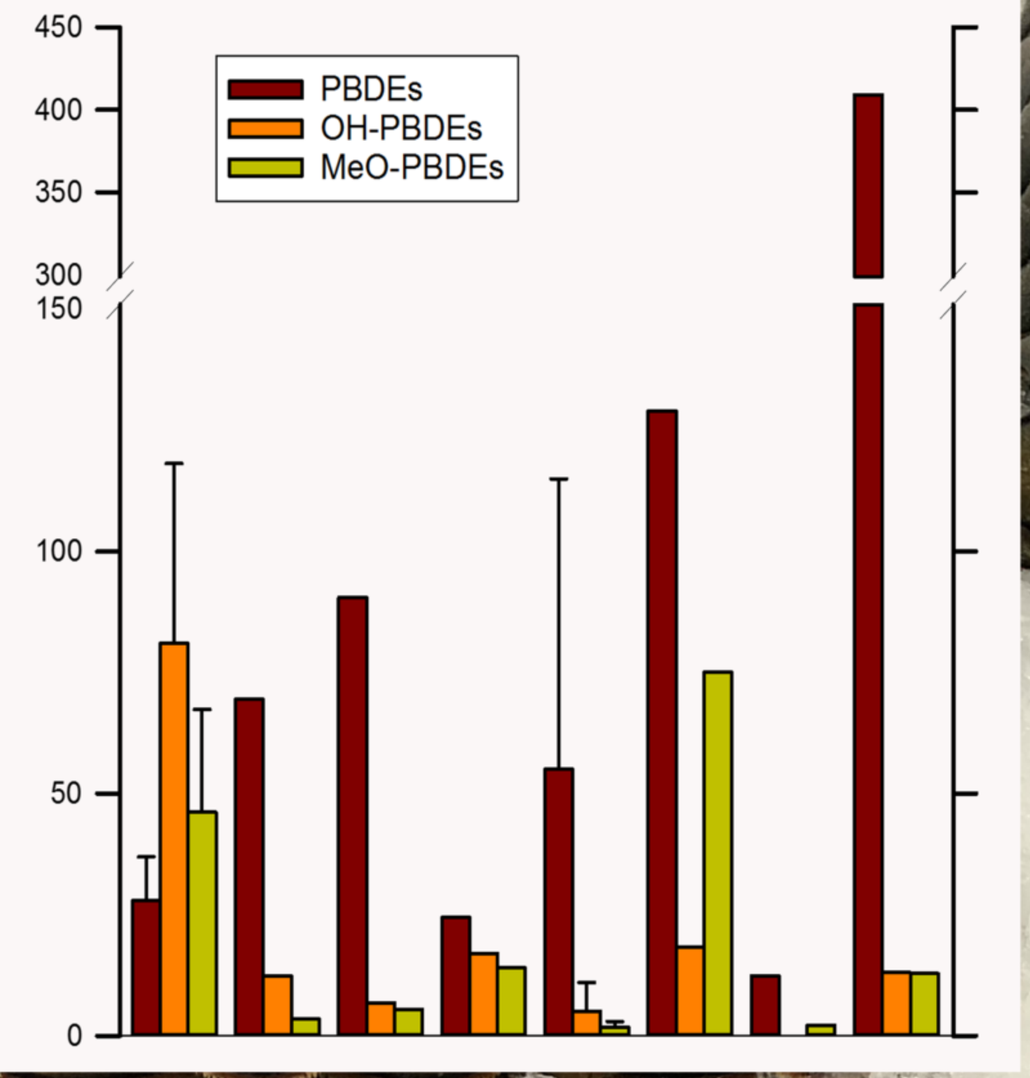
Note: The OH, MeO-PBDEs in **bold** are confirmed to be naturally produced (Wiseman et al. 2011). BDE-66, 71, 138,183, 4'-OH-BDE-17, 3-OH-BDE-47, 5-OH-BDE-47, 4'-OH-BDE-49, 6-OH-BDE-85, 5'-OH-BDE-99, 4'-OH-BDE-101, 4-MeO-BDE-42, 3-MeO-BDE-47, 4'-MeO-BDE-49, 2'-MeO-BDE-68, 2'-MeO-BDE-75, 6-MeO-BDE-85 and 5'-MeO-BDE-99 were not detected above the LOD in any samples.

[1]These samples were collected in the field.

Table 2. Summary of measured PBDE/OH-PBDE/MeO-PBDEs congeners in shellfish purchased at Puget Sound area markets (pg/g wet weight).

	Dab. clams	Ham. clams	Sequim clams	B.C. clams	P.S. clams	Whidbey mussels	Shrimp	Calamari	Scallops
Total Lipid	1.5%	1.4%	0.9%	0.5%	1.1%	2.9%	0.7%	1.3%	0.8%
BDE-28	52.6	73.6	28.8						
BDE-47	138.0	120.9	102.6	81.3	134.3	155.8	270.0	702.3	31.4
BDE-100	54.9	40.5	21.1	10.9	29.8	64.8	41.6	98.8	
BDE-99	245.4	93.2	135.4	99.4	71.4	159.8	165.6	281.8	132.2
BDE-154	30.6	12.4	6.61		7.6	8.81		17.0	31.9
BDE-153		12.9				8.9	8.80	74.3	
∑ PBDEs	521.5	353.5	294.6	191.5	243.2	398.1	486.1	1,174	195.5
4'-OH-BDE-17	85.9	55.8	28.2	25.6	22.5	451.2	27.5		
2'-OH-BDE-28	133.5	155.9	137.0	278.8	123.7				82.4
4-OH-BDE-42				101.7	109.6				
6-OH-BDE-47	1,261	217.0	151.4	23.2	105.8	117.0			
3-OH-BDE-47	99.0	44.7	40.5	32.8		341.5			
2'-OH-BDE-68		62.3		94.1	225.4	226.7		18.8	
2'-OH-BDE-75						140.8			
6-OH-BDE-85	157.8	54.3	52.4	19.7	91.9			26.0	53.9
5'-OH-BDE-100	202.7	49.9	51.0	16.1		416.2			
4'-OH-BDE-103				17.9	18.3		59.6	44.1	
6'-OH-BDE-99	28.3				13.4	113.5			
3-OH-BDE-100	36.7	30.8			45.8				
∑OH-PBDEs	2,005	671	461	610	756.5	1,806	87.1	88.9	136.3
4'-MeO-BDE-17		45.8	21.6	14.6		12.7			
2'-MeO-BDE-28	42.9		10.6	20.7	29.1	26.9			
4-MeO-BDE-42									
6-MeO-BDE-47	393.9	161.3	142.3		44.3	445.9		33.0	31.2
3-MeO-BDE-47	43.6					37.4			25.1
5-MeO-BDE-47	41.2		6.40						12.0
2'-MeO-BDE-68		760.6	367.6		381.2	871.3			
6-MeO-BDE-99	20.9			14.4	18.4			12.9	
3'-MeOBDE-100									13.7
5'-MeO-BDE-100			13.8		30.1	45.5			
4'-MeO-BDE-103	27.7	20.0				11.1	23.8	23.8	30.2
∑ MeO-PBDEs	570.3	987.7	562.3	49.6	503.1	1,451	23.8	69.7	112.2

Note: The OH, MeO-PBDEs in **bold** are confirmed to be naturally produced (Wiseman et al. 2011). BDE-17, 66, 71, 138, 183, 5'-OH-BDE-47, 4'-OH-BDE-49, 5'-OH-BDE-99, 4'-OH-BDE-101, 4-MeO-BDE-42, 4-MeO-BDE-49, 2'-MeO-BDE-75, 6-MeO-BDE-85, 6'-MeO-BDE-99, 3-MeO-BDE-100, 5'-MeO-BDE-99 and 4'-MeO-BDE-101 were not detected above the LOD in any samples.


PBDEs
OH-PBDEs
MeO-PBDEs
450
400
350
300
150
100
50
0