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Contemporary ^{14}C radiocarbon levels of oxygenated polybrominated diphenyl ethers (O-PBDEs) isolated in sponge–cyanobacteria associations

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Abstract

Considerable debate surrounds the sources of oxygenated polybrominated diphenyl ethers (O-PBDEs) in wildlife as to whether they are naturally produced or result from anthropogenic industrial activities. Natural radiocarbon (^{14}C) abundance has proven to be a powerful tool to address this problem as recently biosynthesized compounds contain contemporary (i.e. modern) amounts of atmospheric radiocarbon; whereas industrial chemicals, mostly produced from fossil fuels, contain no detectable ^{14}C . However, few compounds isolated from organisms have been analyzed for their radiocarbon content. To provide a baseline, we analyzed the ^{14}C content of four O-PBDEs. These compounds, 6-OH-BDE47, 2'-OH-BDE68, 2',6-diOH-BDE159, and a recently identified compound, 2'-MeO-6-OH-BDE120, were isolated from the tropical marine sponges *Dysidea granulosa* and *Lendenfeldia dendyi*. The modern radiocarbon content of their chemical structures (i.e. diphenyl ethers, $\text{C}_{12}\text{H}_{22}\text{O}$) indicates that they are naturally produced. This adds to a growing baseline on, at least, the sources of these unusual compounds.

Keywords

O-PBDEs; Radiocarbon; Accelerator mass spectrometry; Sponges; Mariana Islands; Pacific Ocean

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One particular group of halogenated organic compounds (HOCs) are the polybrominated diphenyl ethers (PBDEs), a class of industrially produced, brominated flame retardants, that are ubiquitous in the environment (Hassanin et al., 2005; Hale et al., 2008; Gao et al., 2009; Shaw and Kannan, 2009; de Wit et al., 2010). In addition, many derivatives of PBDEs, such as hydroxylated (OH-PBDEs) and methoxylated (MeO-PBDEs) polybrominated diphenyl ethers, have been found in wildlife (Haglund et al., 1997; Verreault et al., 2005; Letcher et al., 2010) as well as in humans (Athanasiadou et al., 2008; Sudaryanto et al., 2008; Lacorte and Ikonomidou, 2009). We refer to these compounds collectively as oxygenated polybrominated diphenyl ethers (O-PBDEs). These chemicals and their parent PBDE compounds are present as contaminant residues that may exhibit different toxicological effects (Brouwer et al., 1998; Harju et al., 2007; Dingemans et al., 2008; Boxtel et al., 2008; Cantón et al., 2008; Ucan-Marín et al., 2010). Several experimental studies have shown the bioaccumulation of higher levels of O-PBDEs than their parent compounds in marine wildlife, along with a limited animal metabolic capacity to produce some of these O-PBDE metabolites at different trophic levels (Wan et al., 2009; Letcher et al., 2009). Recently, these two groups of O-PBDEs have also been determined in surface waters, rain, snow (Ueno et al., 2008), rivers and coastal waters (Vetter et al., 2009), as well as in seafood (Covaci et al., 2007). However, the environmental behaviour of the O-PBDEs, their routes into the environment, their toxic effects on exposed organisms, as well as whether some are metabolites or by-products of synthetic PBDEs or naturally produced HOCs remains uncertain (Kelly et al., 2008; Wan et al., 2009).

Since some marine organisms (e.g. sponge–cyanobacteria associations), produce similar O-PBDE compounds (Faulkner et al., 1994; Handayani et al., 1997; Hanif et al., 2007), it has been shown or suggested that OH-PBDE and MeO-PBDE compounds are biosynthesized from natural sources, which could then bioaccumulate in exposed organisms. The bioaccumulation in one stranded whale (*Mesoplodon mirus*) of two naturally produced MeO-PBDEs (i.e. 6-MeO-BDE47 and 2'-MeO-BDE68) was confirmed by measuring their natural radiocarbon content (Teuten et al., 2005; Teuten and Reddy, 2007). This approach has proven to be a useful tool for determining the sources of HOCs (Reddy et al., 2002, 2004; Teuten et al., 2005).

To provide additional baseline data on the radiocarbon content of O-PBDEs, and therefore their sources, we analyzed four compounds that were isolated from the tropical marine sponges *Dysidea granulosa* and *Lendenfeldia dendyi* (Fig. 1). These compounds, 6-OH-BDE47, 2'-OH-BDE68, 2',6-diOH-BDE159 and a recently identified compound, 2'-MeO-6-OH-BDE120, were measured for their natural radiocarbon content by accelerator mass spectrometry (AMS).

The sponges *D. granulosa* and *L. dendyi* were collected from Akino reef, Saipan in May 2005 (15° 12' 43" N; 145° 41' 48" E) at a depth of up to 15 m [they represent a depth gradient of 1 m (sample AS-I-48B = compound I) and 15 m (AS-I-55B = II)] and Papua New Guinea in June 1998 (11° 02' 51" S; 152° 28' 72" E) at a depth of 7 m [MR-LD-1 = III and MR-LD-2 = IV], respectively. A voucher specimen of the sample *D. granulosa* (SA20503037) was deposited at the Ocean Biotechnology Center and Repository, National Oceanographic and Atmospheric Agency (NOAA), Oxford, MS and a voucher specimen of

the sponge *L. dendyi* (Coll. No. C018887) was deposited at Smithsonian Institute, Washington DC. The isolation and extraction of the compounds were as follows; the freeze-dried, powdered sponge samples were extracted with 1:1 dichloromethane/ methanol and the resulting crude extract was fractionated by vacuum liquid chromatography on a silica gel column using *n*-hexane/ethyl acetate gradient mixtures as eluents. The fractions were analyzed by nuclear magnetic resonance (NMR) and then repeatedly purified either by column chromatography or by normal phase high pressure liquid chromatography (HPLC) to yield pure compounds I (4.4 g), II (30.0 mg), III (350.0 mg) and IV (304.0 mg) for each sample processed. HPLC was performed on a Waters 2695 model system (Phenomenex, Si, 5 μ m, 250 $\times$ 10 mm column). ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) and 2D-NMR spectra were recorded using the residual solvent signal as an internal standard on a Varian AS-400 and Bruker Advance DRX-400 spectrometers. Infrared (IR) spectra were recorded on an ATI Mattson Genesis series Fourier transform infrared (FTIR) spectrometer. High-resolution mass spectra were recorded via direct injection into a Bruker Magnex BioAPEX 30es ion cyclotron resonance Fourier transform mass spectrometer (HR-FT-MS). Melting points were determined on a Thomas Hoover capillary melting point apparatus. The structures of PBDEs were identified by comparison of NMR and mass spectral data to that reported in the literature (Carté and Faulkner, 1981; Hanif et al., 2007).

The molecular radiocarbon measurements (^{14}C) were performed as described previously (Reddy et al., 2002). Briefly, these analyses encompass three steps: (1) combustion of the organic carbon into carbon dioxide, (2) reduction of the carbon dioxide into graphite and (3) analysis of the graphite by AMS. About 10% of the carbon dioxide was reserved for $\delta^{13}\text{C}$ analysis by isotope ratio mass spectrometry (IRMS). The ^{14}C radiocarbon measurements were done at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at Woods Hole Oceanographic Institution, Woods Hole, MA (McNichol et al., 1994). All ^{14}C measurements were normalized to $\delta^{13}\text{C}$ values of -25‰ and expressed as ^{14}C values. The latter term is the per mille (‰) deviation from the international standard for ^{14}C dating, Standard Reference Material 4990B “Oxalic Acid” (Stuvier and Polach, 1977). In this context, fossil carbon has a ^{14}C of -1000‰ (i.e. ^{14}C -free), while values $>0\text{‰}$ reflect modern levels of ^{14}C radiocarbon (i.e. corresponding to the natural background in addition to post nuclear bomb tests ^{14}C radiocarbon inputs from the 1950s and 1960s). Routine precision for $\delta^{13}\text{C}$ and ^{14}C measurements are ~ 0.1 and 10%, respectively.

The structures of the isolated compounds (Fig. 1) were identified as two OH-PBDEs, I = 6-OH-BDE47 (2-(2',4'-dibromophenoxy)-3, 5-dibromophenol); II = 2'-OH-BDE68 (2-(2',4'-dibromophenoxy)-4,6-dibromophenol), a diOH-PBDE; III = 2',6-diOH-BDE159 (2,3, 4,5-tetrabromo-6-(3',5'-dibromo-2'-hydroxyphenoxy)phenol) and a OH-/MeO-PBDE IV = 2'-MeO-6-OH-BDE120 (2,3,5-tribromo-6-(3',5'-dibromo-2'-methoxyphenoxy)phenol). All the compounds in this study correlated with the structure classification of sponge-derived oxygenated polyhalogenated diphenyl ethers (O-PHDEs), according to the core formula and the substitution pattern classes, described recently by Calcul et al. (2009). Thus, compounds I and II correspond to class I (i.e. one OH- or MeO-substitution in the *ortho* position; C_{12}O_2) and were found in *D. granulosa*, while compounds III and IV corresponding to class II-1 (i.e. one OH- or MeO-substitution in the *ortho* position in each ring; C_{12}O_3) were found in *L. dendyi*. Similar structures based on diphenyl ether molecular skeletons (i.e. $\text{C}_{12}\text{H}_{22}\text{O}$) have

been previously identified in other species of marine sponges. The OH-PBDE compounds (structures I and II) are the most frequent isolated structures in the *Dysidea* sp. collected in different geographical areas along the tropical Pacific Ocean, while the diOH-PBDE compound (structure III), has been found so far, in *D. granulosa* and *Dysidea dendyi*. Table 1 reviews the tropical sponge species and the collection areas from the literature where these compounds have also been found. The OH- or MeO-functional group substitution in the *ortho* position of the diphenyl ether backbone is often found in compounds isolated from marine species and has helped to elucidate the origin of the PBDE metabolites observed at different trophic level organisms (Marsh et al., 2004). To date, more than 40 O-PHDEs are known to be derived from sponge–cyanobacteria associations (Zhang et al., 2008; Calcul et al., 2009), exhibiting bromine or chlorine atoms or both. However, the fourth compound isolated reported here, 2'-MeO-6-OH-BDE120, to our knowledge has not been previously described in the literature, although closer bromine substitution patterns were found in similar hydroxyl-methoxy-diphenyl ether structures isolated from marine sponges (Fu et al., 1995; Utkina et al., 2001; Oda et al., 2005).

The ^{14}C measurements along with the $\delta^{13}\text{C}$ values are shown in Table 2. The ^{14}C values for the four isolated compounds were within the range -6.6% to 21% , which indicate contemporary (or modern) levels of radiocarbon. These radiocarbon levels point to recently assimilated carbon used during the biosynthesis of these chemicals; however, this range found is lower than compared with previous ^{14}C radiocarbon studies of HOCs. For example, Teuten et al. (2005) obtained values of $+103\%$ and $+119\%$ for the methoxylated analogue compounds 6-MeO-BDE47 and 2'-MeO-BDE68, respectively, accumulated in True's beaked whale (*M. mirus*) blubber that was found dead on the Northwest Atlantic coast, while the values found for the hydroxylated compounds identified in this study, 6-OH-BDE47 and 2'-OH-BDE68 were $+21\%$ and $+17.2\%$, respectively (Table 2). Clearly, they are not from industrial sources as they would have ^{14}C values of -1000% (Reddy et al., 2002, 2004). Previously, Reddy et al. (2002) reported a value of $^{14}\text{C} = +73\%$ for a structurally similar compound (i.e. 2',6-diMeO-BDE68) isolated from the sponge *Phyllospongia foliascens*, collected in Palau (western Pacific, 134°E). This could clearly indicate geographical and temporal ^{14}C variations in the dissolved inorganic carbon composition between these locations (i.e. coral reefs) in the Pacific. It is worth noting that large scale circulation and ^{14}C distributions occur in the Pacific Ocean, which show dropping trends of post-bomb (after the 1950s and 1960s) radiocarbon levels in some oceanic locations (Druffel, 2002). Moreover, sessile sponge–cyanobacteria symbionts are exposed through their life cycle to the local hydrodynamics, quite opposite to open-ocean planktonic life cycle organisms exposed to the surface waters, which are highly enriched with post-bomb testing ^{14}C (Teuten et al., 2005).

Marine pharmacological research has provided a large library of bioactive chemicals from marine sponges, such as polyhalogenated diphenyl ethers (PHDE) compounds (Faulkner et al., 1994; Unson et al., 1994); among other classes of compounds, commonly, sesquiterpenes (Alvi et al., 1992) and polychlorinated peptide derivatives (Sauléau et al., 2005). As far as we know, there has not been a review of PHDE compounds. Some of these compounds have shown antibacterial and cytotoxic activity, such as the congener 2'-OH-BDE68 studied in *D. granulosa* (Shridhar et al., 2009). Most studies indicate the symbiont

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Kelly et al., 2008; Wan et al., 2009; Letcher et al., 2009, 2010). The 3-OHBDE47, 4'-OH-BDE49 and 6-OH-BDE47 were detected in plasma samples from glaucous gulls (*Larus hyperboreus*), although 4'-OHBDE49 and 4-OH-BDE42 were the only OH-PBDEs congeners quantifiable in polar bears (*Ursus maritimus*) according to Verreault et al. (2005). In opposition, the study conducted by Kelly et al. (2008) reported an undetectable occurrence of OH-PBDEs in samples of blood, muscle, and/or liver of fish and marine wildlife in specimens from the Arctic, with the exception of beluga whale blubber and milk samples, although at very low concentrations. However, elevated concentrations were found in other Arctic organisms for MeO-PBDEs with the highest concentrations of 6-MeO-BDE47 and 2'-MeO-BDE68 in beluga whales. Weijs et al. (2009) found also the bioaccumulation of 6-MeO-BDE47 and 2'-MeO-BDE68 in seals in the southern North Sea. The differences in biomagnification processes were explained in terms of differences in metabolic breakdown for the two species of harbour seals (*Phoca vitulina*) and harbour porpoises (*Phocoena phocoena*). According to Letcher et al. (2009), who reports a comparative study for East Greenland ringed seal (*Pusa hispida*) blubber to polar bear (*U. maritimus*) tissues (adipose, liver and brain), for diverse congeners of persistent chlorinated and brominated contaminants and metabolic by-products, PBDEs, MeO-PBDEs and OH-PBDEs can bioaccumulate but do not appear to be biotransformed or are biotransformation products produced in the polar bear. These authors found the 6-OH-BDE47 congener bioaccumulated in seals, but unlike OH-PCBs metabolites, OH-PBDEs in the bear tissues appear to be mainly bioaccumulated from the seal blubber, due to the low PBDEs oxidative metabolism exhibited from *in vitro* assays using polar bear (*U. maritimus*) hepatic microsomes (Letcher et al., 2009). Wan et al. (2009) pointed to the formation of OH-PBDEs from MeO-PBDEs rather than directly from industrial PBDEs metabolism. Another explanation has also been suggested to link the occurrence of O-PBDE compounds in wildlife with environmentally transformed synthetic PBDEs rather than direct animal metabolism, through routes, such as sewage effluent discharges (Hua et al., 2005; Ueno et al., 2008).

Currently, these MeO-PBDEs compounds, which are now frequently measured in marine wildlife, could be considered to originate from a natural sources due to the lack of evidences of synthesis through animal metabolism (e.g. *in vivo* studies) as well as higher concentrations in wildlife relative to PBDEs (Letcher et al., 2009, 2010). In contrast, the OH-PBDEs would originate both from anthropogenic sources *via* animal metabolism and natural sources as confirmed by the radiocarbon data in this study. Investigations in the Great Barrier Reef in Australia (Vetter et al., 2009) showed the occurrence of dissolved concentrations in seawater of natural halogenated organic compounds (e.g. 6-MeO-BDE47; 2'-MeO-BDE68; 2',6-diMeO-BDE68). The occurrence of the same compounds in sponges, dolphins and marine mammals in Queensland/ Australia (Vetter et al., 2001, 2002; Agrawal and Bowden, 2005), indicate that the bioaccumulation process in the marine food web might start in the same geographical area, perhaps limiting the extent of the bioaccumulation potential through the oceanic dispersion of these natural HOCs. Yet, the sponges containing cyanobacteria associations represent about 100 species in 29 families which is a large pool of O-PBDEs producing organisms mainly located in the tropical ocean. Recently, an *Oscillatoria*-type symbiont has been found in a sponge in the west Atlantic Ocean (Diaz et

al., 2007), which exhibits higher rates of photosynthetic production than *O. spongelliae*, and would constitute an advantage over other reef organisms, as well as a potential PBDE compounds producer. Nevertheless, these chemical compounds released in tropical areas are likely to be also exposed to transformation by abiotic processes in the environment. If so, rather than global distributions of OPBDEs from tropical areas (e.g. produced by sponge–cyanobacteria associations), there would be unidentified marine species producing naturally O-PBDEs compounds, which could be bioaccumulated afterwards in higher trophic levels in the marine food web. Therefore, the $^{14}\text{C} > 100\%$ values found in the bioaccumulated MeOPBDEs in a whale (*M. mirus*) in the North Atlantic Ocean (Teuten et al., 2005) or identified O-PBDEs in other marine organisms (Marsh et al., 2005; Haraguchi et al., 2009) in the Pacific Ocean (offshore Japan), could correspond to a marine microorganisms whose life cycle occurs in the surface of the ocean as a planktonic organisms (i.e. planktonic cyanobacteria) with higher turnover rates, thus being highly enriched in post-bomb ^{14}C from surface waters, rather than bioaccumulated sponge–cyanobacteria metabolites originated in the tropical ocean.

The existence of potentially different sources of O-PBDEs in the environment (i.e. synthetic, natural and transformed), without regard to methylation, debromination, dechlorination or phototransformation processes (Steen et al., 2009), challenges the issue of source and the potential risks to exposed wildlife. Therefore, elucidation of sources is a key to understanding the environmental occurrence, transformation processes, transport processes and bioaccumulation of O-PBDEs. The molecular level radiocarbon technique allows discriminating between these origins. The baseline data presented in this study provides evidence of natural production in sponge–bacteria associations.

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Fig. 1.

Structures of compounds isolated in this study. I = 6-OH-BDE47 (2-(2',4'-dibromophenoxy)-3,5-dibromophenol); II = 2'-OH-BDE68 (2-(2',4'-dibromophenoxy)-4,6-dibromophenol); III = 2',6-diOH-BDE159 (2,3,4,5-tetrabromo-6-(3',5'-dibromo-2'-hydroxyphenoxy)phenol) and IV = 2'-MeO-6-OH-BDE120 (2,3,5-tribromo-6-(3',5'-dibromo-2'-methoxyphenoxy)phenol). See also Table 1 for equivalent nomenclature.

Table 1

Tropical marine sponge species known to contain the oxygenated polybrominated diphenyl ethers isolated in this study.

Compound ^a	Sponge specie	Location	References
I. 6-OH-2,2',4,4'-tetraBDE (6-OH-BDE47)	<i>Dysidea granulosa</i>	Saipan	This study; Becerro and Paul (2004)
	<i>Dysidea herbacea</i> and <i>Dysidea dendyi</i>	Papua New Guinea/Fiji	Calcul et al. (2009)
	<i>Dysidea dendyi</i>	–	Xu et al. (2005)
	<i>Dysidea</i> sp.	Indo-Pacific	Fu et al. (1995) and Zhang et al. (2008)
II. 2'-OH-2,3',4,5'-tetraBDE (2'-OH-BDE68)	<i>Dysidea granulosa</i>	Saipan	This study; Becerro and Paul (2004) and Shridhar et al. (2009)
	–	–	–
	<i>Dysidea herbacea</i> and <i>Dysidea dendyi</i>	Papua New Guinea/Fiji	Calcul et al. (2009)
	<i>Dysidea dendyi</i> (<i>Phyllospongia dendyi</i>)	–	Liu et al. (2004) and Xu et al. (2005)
	<i>Dysidea</i> sp.	Indo-Pacific	Fu et al. (1995) and Zhang et al. (2008)
	<i>Dysidea herbacea</i>	Indonesia	Handayani et al. (1997), Carté and Faulkner (1981) and Faulkner et al. (1994)
	<i>Phyllospongia foliascens</i>	Republic of Palau	Reddy et al. (2002) and Carté and Faulkner (1981)
III. 2',6-diOH-2,3,3',4,5,5'-hexaBDE (2',6-diOH-BDE159)	<i>Lendenfeldia dendyi</i>	Papua New Guinea	This study
	<i>Dysidea</i> (<i>Lamellodysidea</i>) <i>herbacea</i>	–	Hanif et al. (2007)
	<i>Dysidea herbacea</i> and <i>Dysidea granulosa</i>	Papua New Guinea/Fiji	Calcul et al. (2009)
	<i>Dysidea</i> sp.	Indo-Pacific (Fiji)	Fu et al. (1995)
	<i>Dysidea dendyi</i>	–	Utkina et al. (2001)
IV. 2'-MeO-6-OH-2,3',4,5,5'-pentaBDE (2'-MeO-6-OH-BDE120)	<i>Lendenfeldia dendyi</i>	Papua New Guinea	This study

^aFull chemical name according Maervoet et al. (2004) (in brackets corresponds to BZ (Ballschmitter and Zell, 1980) or IUPAC-accepted BZ numbering rules).

Table 2

Radiocarbon measurements for the compounds isolated from marine sponges in the western Pacific Ocean. See Table 1 for equivalent nomenclature.

Compound chemical name	Specimen	^{14}C (per mil)	$\delta^{13}\text{C}$ (per mil)	NOSAMS accession No.
I. 6-OH-2,2',4,4'-tetraBDE	<i>Dysidea granulosa</i> ^a	21	−35.3	OS-62355
II. 2'-OH-2,3',4,5'-tetraBDE	<i>Dysidea granulosa</i> ^a	17.2	−35.2	OS-62354
III. 2',6-diOH-2,3,3',4,5,5'-hexaBDE	<i>Lendenfeldia dendyi</i> ^b	−6.6	−34.7	OS-62536
IV. 2'-MeO-6-OH-2,3',4,5,5'-pentaBDE	<i>Lendenfeldia dendyi</i> ^b	12.7	−36.2	OS-62353
2',6-diMeO-2,3',4,5'-tetraBDE	<i>Phyllospongia foliascens</i> ^c	73.2	−35.9	OS-30008

Note: $\delta^{13}\text{C}$ values are listed only for information purposes. The isolation process was undertaken without any precautions for possible fractionation of the stable carbon isotope ratios $^{13}\text{C}/^{12}\text{C}$.

^aCollected in Saipan in 2005.

^bCollected in Papua New Guinea in 1998.

^cCollected in Koror, Republic of Palau (collected several decades ago; data from Reddy et al., 2002).