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, $C_{12}H_{22}O$) 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

Research highlights

· O-PBDEs in the marine environment could have both natural and anthropogenic origin.

· Molecular-level 14C is measured by accelerated mass spectrometry.

· Industrial products, derived from fossil sources, are radiocarbon 14C-free.

· O-PBDEs compounds from marine sponges show modern levels of 14C.

· Some species could produce O-PBDEs rather than being biotransformation from industrial PBDEs.

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 Ikonomou, 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; Canton et al. 2008; Ucan-Marin 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, 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; Reddy et al., 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* (Figure 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 *Dysidea granulosa* and *Lendenfeldia 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 1m (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 *Dysidea 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 *Lendenfeldia dendyi* (Coll. No.C018887) was deposited at Smithsonian Institute, Washington D.C. 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, 5μm, 250x10mm column). ^{1}H-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 transforme 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 (Carte and Faulkner, 1981; Hanif et al., 2007).

The molecular radiocarbon measurements (Δ^{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 δ^{13}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 δ^{13}C values of -25‰ and expressed as Δ^{14}C values. The latter terms is the per mille (‰) deviation from the international standard for ^{14}C dating, Standard Reference Material 4990B “Oxalic Acid” (Stuiver and Polach, 1977).

In this context, fossil carbon has a $\Delta^{14}C$ of -1000‰ (i.e. ^{14}C-free), while values >0‰ 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 $\delta\ ^{13}C$ and $\Delta^{14}C$ measurements are ~0.1 and 10‰, respectively.

The structures of the isolated compounds (Figure 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 *Dysidea 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 *Lendenfeldia dendyi*. Similar structures based on diphenyl ether molecular skeletons (i.e. $C_{12}H_{22}O$) 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, whilst the diOH-PBDE compound (structure III), has been found so far, in *Dysidea 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 $\Delta^{14}C$ measurements along with the $\delta^{13}C$ values are shown in Table 2. The $\Delta^{14}C$ 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 (*Mesoplodon mirus*) blubber that was found dead on the Northwest Atlantic coast, whilst 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 $\Delta^{14}C$ values of -1000‰ (Reddy et al., 2002; Reddy et al. 2004). Previously, Reddy et al. (2002) reported a value of $\Delta^{14}C$ = +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 $\Delta^{14}C$ 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 $\Delta^{14}C$ distributions occur in the Pacific Ocean, which show dropping trends of post-bomb (after the 1950's and 1960's) 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, 1994; Unson et al., 1994); among other classes of compounds, commonly, sesquiterpenes (Alvi et al., 1992) and polychlorinated peptide derivatives (Sauleau 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 *Dysidea granulosa* (Shridhar et al. 2009). Most studies indicate the symbiont cyanobacteria, *Oscillatoria spongeliae,* as the responsible microorganism that produces O-PBDEs (Faulkner, 1994; Unson, 1994), although the mutual relationship between the sponge and the symbiont cyanobacteria, in relation to the production of chemical metabolites, is still not fully understood. The studies in *Dysidea granulosa* have investigated differences between the production of O-PBDEs and the environmental conditions, such as light or depth, showing a complex symbiotic relationship (Becerro and Paul, 2004), although chemical variations have also been shown to originate from the existence of closely related

cyanobacterial strains (Ridley et al., 2005). There is a positive agreement that these would act as chemical defenses against predation (Handayani et al., 1997). Different authors also have indicated the important role of the host-symbiont coevolution, to improve the adaptation to different habitats (i.e. intertidal), therefore, influencing to some extent the metabolite synthesis pathway (Steindler et al., 2002; Calcul et al, 2009). Some of these O-PBDEs compounds have also been found in other species. The metabolite 6-OH-BDE47 was found in an ascidian (*Didemnum sp.*) by Schumacher (1995), and the MeO-BDE68 has been found in green algae (*Cladophora fascicularis*) in the Pacific (Kuniyoshi et al., 1985). The structurally related analogues of the hydroxylated compounds found in this study, i.e., 6-MeO-BDE47 and 2'-MeO-BDE68, have also been isolated in several tropical marine sponges. Alternatively of the four compounds isolated in this study, the dihydroxylated and the hydroxy-methoxy compounds have never been reported in wildlife other than marine sponges or in the environment. However, similar chemical structures (e.g. diOH- and diMeO-containing) are often found in the marine environment and have been found in marine organisms as presumably naturally occurring HOCs (Marsh et al., 2005, Haraguchi et al., 2009). In contrast, the OH-PBDEs found in this study, 6-OH-BDE47 and 2'-OH-BDE68, as well as their MeO-containing analogues (i.e., 6-MeO-BDE47 and 2'-MeO-BDE68) have been frequently found in aquatic organisms and sourced to different origins. Kierkegaard et al. (2001) found six different OH-tetraBDEs in northen pike (*Esox lucius*) exposed to BDE-47, including the congener 6-OH-BDE47. The 6-OH-BDE47 was also found to be the highest congener retained along with nine other OH-PBDEs congeners in fish species in Detroit River (Valters et al., 2005), of the Great Lakes region, and these were suggested to have originated primarily from CYP enzyme-mediated oxidative metabolism of BDE-47, although MeO-BDEs were found

at very low concentrations. Furthermore, the production of hydroxylated metabolites from synthetic PBDEs has also been demonstrated in exposed organisms in several *in vivo* studies (Morck et al., 2003; Malmberg et al., 2005; Marsh et al., 2006), although these animal experiments have not shown the formation of their MeO-PBDE derivatives. On the contrary, the majority of the OH-PBDEs found in Baltic Sea Salmon (*Salmo salar*), were attributed to natural sources (Marsh et al., 2004), as most of the hydroxylated compounds identified exhibited the hydroxyl group in the *ortho* position on the diphenyl ether backbone rather than in the *meta* or *para* position, thus the latter metabolites would suggest PBDE metabolism. These structural trends for natural sources were recently supported by the occurrence of O-PBDEs in blue mussels (*Mytilus edulis*), red algae (*Ceramium tenuicorne*) and cyanobacteria (*Aphanizomenon flosaquae*) in the Baltic Sea (Malmvarn et al., 2005, 2008).

Observations in the Arctic wildlife (e.g. seals, porpoises, gulls, whales, polar bears) have provided the majority of data with regard the occurrence of O-PBDEs (Verreault et al., 2005;, Kelly et al. 2008; Wan et al., 2009; Letcher et al., 2009, 2010). The 3-OH-BDE47, 4'-OH-BDE49, and 6-OH-BDE47 were detected in plasma samples from glaucous gulls (*Larus hyperboreus*), although 4'-OH-BDE49 and 4-OH-BDE42 were the only OH-PBDEs congeners quantifiable in polar bears (*Ursus maritimus*) according 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 Letcher et al. (2009), who reports a comparative study for East Greenland ringed seal (*Pusa hispida*) blubber to polar bear (*Ursus 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 (*Ursus 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; Vetter et al., 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 *Oscillatoria spongeliae*, 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 O-PBDEs 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 $\Delta^{14}C>100‰$ values found in the bioaccumulated MeO-PBDEs in a whale (*Mesoplodon 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, 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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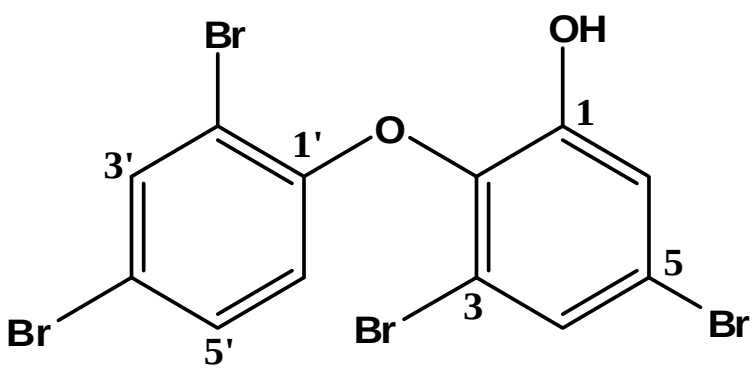
Figure 1. Structures of compounds isolated in this study. I = 6-OH-BDE47 (2-(2',4'-dibromophenoxy)-3,5-dibromophenol); II = 2'-OH-BDE68 (2-(2',4'-dibromophenoxy)-4,6-dibromophenol); III = 2',6-diOH-BDE159 (2,3,4,5-tetrabromo-6-(3',5'-dibromo-2'-hydroxyphenoxy)phenol) and IV = 2'-MeO-6-OH-BDE120 (2,3,5-tribromo-6-(3',5'-dibromo-2'-methoxyphenoxy)phenol). See also Table 1 for equivalent nomenclature.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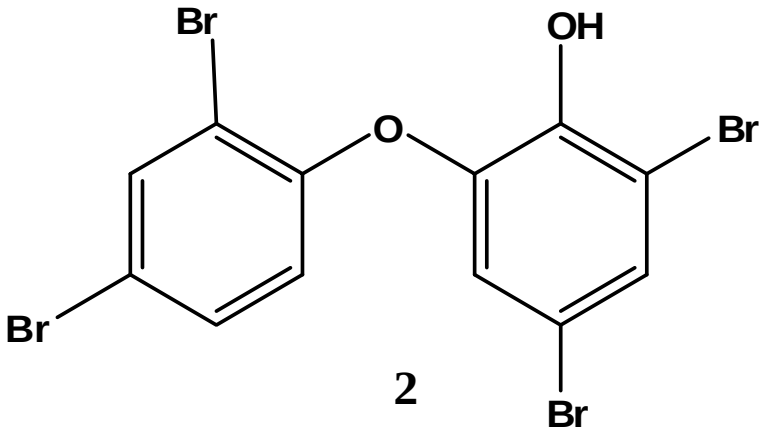
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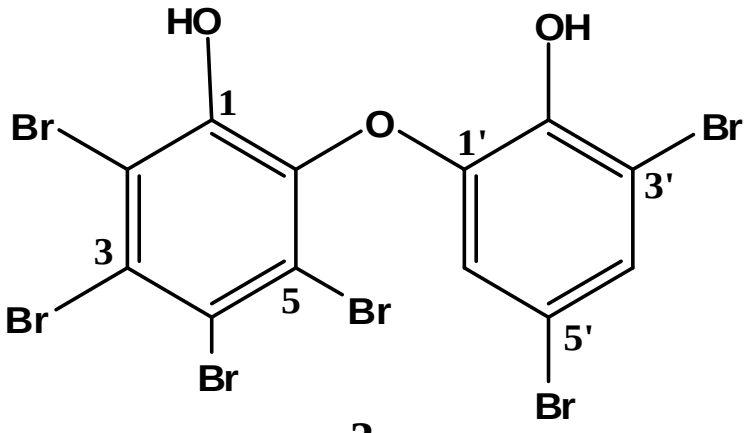
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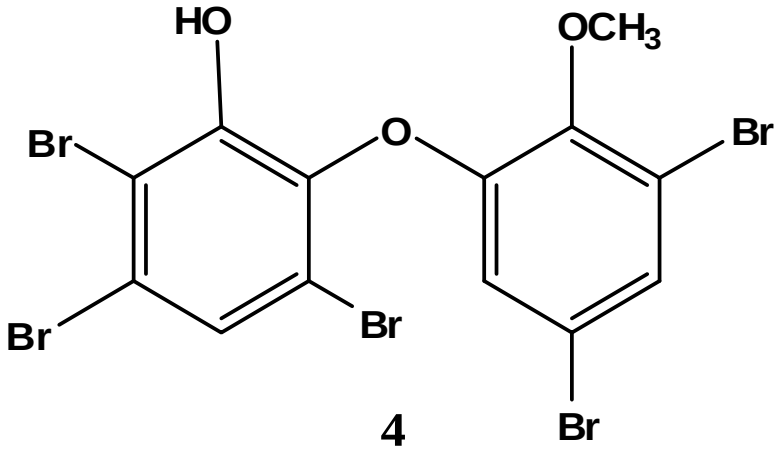
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1 Table 1. Tropical marine sponge species known to contain the oxygenated

2 polybrominated diphenyl ethers isolated in this study.

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

3 a= full chemical name according Maervoet et al., 2004 (in brackets corresponds to BZ (Ballschmiter

4 and Zell, 1980) or IUPAC-accepted BZ numbering rules).

1 Table 2. Radiocarbon measurements for the compounds isolated from marine sponges

2 in the western Pacific Ocean. See Table 1 for equivalent nomenclature.

Compound chemical name	Specimen	$\Delta^{14}C$ (per mil)	$\delta^{13}C$ (per mil)	NOSAMS accession no.
I. 6-OH-2,2',4,4'-tetraBDE	*Dysidea granulosa*[a]	21.0	-35.3	OS-62355
II. 2'-OH-2,3',4,5'-tetraBDE	*Dysidea granulosa*[a]	17.2	-35.2	OS-62354
III. 2',6-diOH-2,3,3',4,5,5'-hexaBDE	*Lendenfeldia dendyi*[b]	-6.6	-34.7	OS-62536
IV. 2'-MeO-6-OH-2,3',4,5,5'-pentaBDE	*Lendenfeldia dendyi*[b]	12.7	-36.2	OS-62353
2',6-diMeO-2,3',4,5'-tetraBDE	*Phillospongia foliascens*[c]	73.2	-35.9	OS-30008

3 a: collected in Saipan in 2005; b: collected in Papua New Guinea in 1998 and c: collected in Koror,

4 Republic of Palau (collected several decades ago; data from Reddy et al., 2002). Note: $\delta^{13}C$ values are

5 listed only for information purposes. The isolation process was undertaken without any precautions for

6 possible fractionation of the stable carbon isotope ratios $^{13}C/^{12}C$.

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