

# Natural $^{14}\text{C}$ in *Saccoglossus bromophenolosus* compared to $^{14}\text{C}$ in surrounding sediments

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**ABSTRACT:** The natural radiocarbon ( $^{14}\text{C}$ ) content of whole, gut voided *Saccoglossus bromophenolosus* collected in Lowes Cove, Maine, USA, was compared with that of a non-voided worm, sectioned individuals, and the natural product 2,4-dibromophenol (2,4-DBP) isolated from *S. bromophenolosus*. In all cases, the  $^{14}\text{C}$  content was greater than that of the sediment from which the enteropneusts were collected. The  $^{14}\text{C}$  content of 2 polychaetes, *Glycera dibranchiata* and *Clymenella torquata*, also collected from Lowes Cove, were similarly enriched in  $^{14}\text{C}$  compared to the bulk sediment. These results show that all 3 species consumed recently fixed carbon that was much newer than organic carbon in the bulk sediment. The value (+10.4‰) obtained for 2,4-DBP isolated from *S. bromophenolosus* in this study differs from that reported in a previous study (–170‰). The discrepancy is attributed to methodological differences. The importance of selecting an appropriate method when isolating compounds for natural abundance  $^{14}\text{C}$  analysis is discussed.

**KEY WORDS:** Natural radiocarbon abundance · Intertidal · Benthos · Macrofauna · Halogenated organic compounds

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## INTRODUCTION

Marine organisms synthesize thousands of halogenated organic compounds (HOCs) and use these secondary metabolites to deter feeding, hinder or inhibit settlement of competitors, and perhaps regulate internal salt or hydrogen peroxide levels (Gribble 2000, 2003, Winterton 2000, Kicklighter et al. 2004). Generally, these compounds, with levels approaching as much as several percent of the total body mass, have been identified from analysis of grab samples of individual species. Hence, it has been relatively easy to match the naturally produced HOCs with a specific species. For example, 11 species of polychaetes surveyed in 2 temperate, marine sand flats contained many different brominated and chlorinated HOCs, including hydrocarbons, pyrroles and phenols (Fielman et al. 1999). Fielman et al. (1999) also found 2,6-dibromophenol in the mollusc *Terebra dislocata* and assumed that it arose from this species' diet of hemi-

chordate worms, which have been shown to produce this compound. Unlike the simple, 1 trophic-level transfer of 2,6-dibromophenol from the worm to the mollusc, several halogenated bipyrroles (Tittlemier et al. 1999, Vetter et al. 1999, 2000, Teuten et al. 2006), suspected to be naturally produced, have been detected in a wide range of trophic levels, including fishes, seabirds and marine mammals, as well as in human breast milk (Vetter et al. 2000, Tittlemier et al. 2002). These HOCs have no known natural source, yet none have been knowingly produced by the chemical industry.

To determine whether these suspected HOCs are actually natural and to attain some understanding of their carbon source, molecular-level  $^{14}\text{C}$  analysis has been employed. The basic premise is that synthetic HOCs (which are mainly derived from petrochemicals) do not contain any detectable  $^{14}\text{C}$  ( $\Delta^{14}\text{C} = -1000\text{‰}$ ), while natural products contain contemporary levels (Reddy et al. 2002, Teuten et al. 2005). An additional

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benefit of this approach is that the absolute amount of  $^{14}\text{C}$  in natural products may vary slightly depending on the type of organism that synthesized them, and also when and where the organism grew. To obtain some baseline data, Reddy et al. (2004) isolated 2,4-dibromophenol (2,4-DBP) and 2,3,4-tribromopyrrole (2,3,4-TBP) from *Saccoglossus bromophenolosus* and *S. kowalevskii*, respectively. These saccoglossoids were selected because they are easily sampled, and these compounds because significant research has been performed on their biosynthesis, chemical ecology and biogeochemistry (Higa et al. 1980, Fielman & Targett 1995, King et al. 1995, Woodin et al. 1997). Reddy et al. (2004) found that both compounds were natural as they contained detectable levels of  $^{14}\text{C}$  (and hence were not from a petrogenic source). The actual  $^{14}\text{C}$  values were more depleted ( $\Delta^{14}\text{C} = -170\text{‰}$  for 2,4-DBP and  $-130\text{‰}$  for 2,3,4-TBP) than recently fixed carbon, which for coastal and open ocean surface waters is  $>0\text{‰}$  (Fig. 1). Reddy et al. (2004) suggested that the saccoglossoids were preferentially using pre-aged organic carbon (OC) within the bulk sediment matter to biosynthesize the HOC skeletons. Unfortunately, Reddy et al. (2004) used literature values to estimate the  $^{14}\text{C}$  of content of the sediment OC and could only speculate on the carbon sources for these naturally produced HOCs. In addition, their speculation contrasts with the results of DeMaster et al. (2002), who showed that polychaetes in continental shelf surface sediments contained  $^{14}\text{C}$  that was significantly enriched relative to the bulk sediment OC in which they resided. In fact, the presence of nuclear bomb-derived  $^{14}\text{C}$  (Fig. 1) in the polychaetes indicated that recently fixed phytoplanktonic detritus was their major dietary carbon source.

To make a more direct comparison of the  $^{14}\text{C}$  content of relevant natural products and the food sources of their putative biological precursors, we measured the  $^{14}\text{C}$  content of *Saccoglossus bromophenolosus*, its halogenated metabolite 2,4-DBP and the OC from the surrounding sediment where the worms were collected. For reference, the  $^{14}\text{C}$  content of the polychaetes *Glycera dibranchiata* and *Clymenella torquata*, also collected in the same sediments, was analyzed.

## MATERIALS AND METHODS

**Sample collection.** *Saccoglossus bromophenolosus* were collected from the top 15 cm of sediment from Lowes Cove, Maine, USA, in July 2003. This location has been the site of numerous studies on *S. bromophenolosus* and their natural products (King et al. 1994, 1995, Giray & King 1997). Approximately 50 worms were preserved in jars containing methanol (~30 ml); 3 whole worms were also frozen directly with liquid

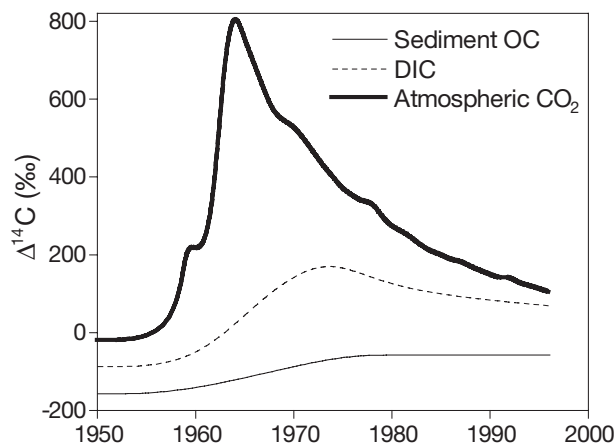


Fig. 1.  $^{14}\text{C}$  content of various carbon reservoirs for the period 1950 to 1996 in the Northern Hemisphere. Following above-ground testing of nuclear weapons beginning in 1954,  $^{14}\text{C}$  content of the atmosphere increased dramatically (known as the 'bomb spike'). Subsequent equilibration of the atmosphere with other carbon reservoirs has led to an increase in  $^{14}\text{C}$  in dissolved inorganic carbon (DIC) in upper layers of the oceans (0 to 100 m) and in organic carbon (OC) of the surface sediment (0 to 1 cm) (modified from Pearson 2000)

nitrogen. We collected 2 sediment cores by pushing polycarbonate tubes (50 cm length  $\times$  6.5 cm inner diameter, i.d.) into the sediment. During a second trip to Lowes Cove in May 2004, *S. bromophenolosus*, *Glycera dibranchiata* and *Clymenella torquata* (1 to 4 specimens of each) were collected in addition to 7 smaller sediment cores (18 cm length  $\times$  3 cm i.d.). All worms collected during the latter sampling were put into jars containing seawater for gut voiding. Flocculent material that was floating above 2 of the cores was collected by removing the water and suspended solids with a Pasteur pipette. The sediment cores and frozen *S. bromophenolosus* were stored at  $-40^\circ\text{C}$  until further analysis. The feeding habits of the worms and input of OC into the cove are assumed to be comparable for both sampling dates, as these were at a similar time of year.

**Extraction and analysis.** To determine the  $^{14}\text{C}$  content of *Saccoglossus bromophenolosus*, the frozen specimens were dried overnight at  $70^\circ\text{C}$  and then cooled in a desiccator (DeMaster et al. 2002). An identical procedure was used for analysis of the separated proboscis and trunk of 2 frozen individuals. Gut contents were voided by holding the worms overnight in seawater collected from Lowes Cove; the cleared worms were then frozen with liquid nitrogen and oven dried. The dried individuals were ground with a pestle and mortar and treated with 10% HCl to remove any inorganic carbon remaining in the gastrointestinal tract. The resulting OC was saved for  $^{14}\text{C}$  analysis (see

later subsection). An identical procedure was used to process *Glycera dibranchiata* and *Clymenella torquata*. Whole specimens were analyzed individually. For the sectioned enteropneusts, it was necessary to pool the trunks and proboscises of 2 individuals.

All worms preserved in methanol were sonicated for 40 min, and the methanol extract was decanted into a 250 ml separatory funnel. The residue was then washed 4 times with 15 ml aliquots of dichloromethane (DCM). DCM extracts were added to the methanol extract, and the combined organic extracts were washed first with 60 ml of distilled water and then 3 more times with 20 ml distilled water. The aqueous layers were back extracted with 15 ml DCM. The DCM layers were combined, rotary-evaporated to remove the solvent, and designated the total lipid extract (TLE). Approximately 4% of the TLE was set aside for  $^{14}\text{C}$  analysis (see later subsection). The remaining TLE was charged to a glass column (5 cm i.d., 10 cm in length) packed with fully activated silica gel (100 to 200 mesh) and eluted with a gradient of increasing DCM in hexane; 2,4-DBP eluted with 1% DCM in hexane and was identified by gas chromatography with mass spectrometric detection (GC-MS) and by comparison with commercially available 2,4-DBP (Sigma Aldrich). The purity of the isolated 2,4-DBP was determined by gas chromatography with flame ionization detection (GC-FID) and was 99%. All the isolated 2,4-DBP was saved for  $^{14}\text{C}$  analysis.

**Sediment cores.** Upon partial thawing, the July 2003 core was extruded and sliced at 2 cm resolution (0–2 to 12–14 cm). The outer 1 cm of each horizon was discarded to prevent smearing from between layers. The May 2004 core was extruded and sliced, discarding the outer 1 to 2 mm, at the following intervals: 1 mm (0 to 4 mm), 2 mm (0.4 to 1.0 cm), 1 cm (1 to 2 cm) and 2 cm (2 to 10 cm). Plant debris and stones were removed. Each sample was then homogenized, air-dried, treated with 10% HCl to remove inorganic carbon, and set aside for  $^{14}\text{C}$  analysis of the sediment OC. The floc from a May 2004 core was filtered, air dried and acid treated. The top 5 mm of a second core taken in May 2004 was sectioned and extracted into 50 ml methanol by sonication for 20 min. The methanol extract was added to 150 ml distilled water and then extracted into 50 ml DCM. The DCM was evaporated to obtain a TLE of the surface sediment. All sediment OC samples were used for  $^{14}\text{C}$  analysis.

**$^{14}\text{C}$  analysis.** The TLE and 2,4-DBP isolated from *Saccoglossus bromophenolosus*, the polychaetes, and each sediment horizon were analyzed for  $^{14}\text{C}$  content at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at Woods Hole Oceanographic Institution (McNichol et al. 1994). Details on preparation and analysis of each sample type

have been discussed previously (McNichol et al. 1994, Reddy et al. 2002, 2004). In this study, all  $^{14}\text{C}$  measurements have been normalized to  $\delta^{13}\text{C}$  values of  $-25\text{‰}$  and are expressed as  $\Delta^{14}\text{C}$  values. The latter term is the per mille (‰) deviation from the international standard for  $^{14}\text{C}$  dating, Standard Reference Material 4990B 'oxalic acid' (Stuiver & Polach 1977). In this context,  $\Delta^{14}\text{C}$  values of  $-1000\text{‰}$  reflect fossil carbon while  $\Delta^{14}\text{C}$  values  $>0\text{‰}$  indicate the presence of nuclear bomb-derived carbon (Fig. 1). Routine precision for sediment OC is generally  $<10\text{‰}$  (Schneider et al. 1995). Uncertainty is related to sample size, and is greater for smaller samples (Pearson et al. 1998). For sediment, animals and 2,4-DBP, the uncertainty in  $\Delta^{14}\text{C}$  was  $10\text{‰}$ , whereas the TLEs submitted as small samples ( $<300\text{ }\mu\text{g}$  of carbon) had uncertainties of  $20\text{‰}$ .

## RESULTS AND DISCUSSION

The  $^{14}\text{C}$  content of the 2,4-DBP isolated from *Saccoglossus bromophenolosus* ( $+10.4\text{‰}$ ) was comparable to bulk  $\Delta^{14}\text{C}$  values from analyses of whole individuals, which ranged from  $+4.2\text{‰}$  for a non-voided whole specimen to  $+32.8\text{‰}$  for the TLE (Table 1). These modern  $^{14}\text{C}$  values ( $\Delta^{14}\text{C} > 0\text{‰}$ , Fig. 1) are internally consistent, within the uncertainty of the measurements, and indicate that recently fixed photosynthate constitutes a major carbon source for *S. bromophenolosus*. To our knowledge, this is the first demonstration of an isolated natural product having the same  $^{14}\text{C}$  content as the organism that synthesized it, and will be of great importance for studies directed at determining the sources of naturally produced HOCs whose origin is unknown (Vetter et al. 2000, 2001, Reddy et al. 2004, Teuten et al. 2006).

In contrast to the modern values obtained for the worm, the OC in the sediment ranged from  $-75$  to  $-49\text{‰}$  for the 2003 core and from  $-92$  to  $-34\text{‰}$  for the high resolution core sampled 1 yr later (Table 2). This corresponds to an average age of 245 to 570 yr and 220 to 725 yr for the 2003 and 2004 cores, respectively, in terms of conventional  $^{14}\text{C}$  age (determined using the Libby half life and reported without reservoir corrections or calibration to calendar years). The variation in  $\Delta^{14}\text{C}$  with depth (Table 2) is probably a consequence of deposition of a complex mixture of organic matter that varies in age, including recent photosynthate and older, more refractory terrestrial carbon originating from the watershed of the Damariscotta River, which floods Lowes Cove. The dynamics of burial and degradation are also determined in part by sediment mixing through bioturbation (Rice 1986) and disturbances by commercial clam harvesting (a form of anthropogenic bioturbation). All the above processes can alter depth

Table 1. *Saccoglossus bromophenolosus*, *Glycera dibranchiata* and *Clymenella torquata*. Carbon isotope data ( $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$ ) for worms collected at Lowes Cove, Maine, USA. For  $\Delta^{14}\text{C}$ ,  $>0\text{‰}$  indicates modern  $^{14}\text{C}$  abundance;  $-1000\text{‰}$  is taken to represent samples whose  $^{14}\text{C}$  content was below detectable levels. NOSAMS Accession No.: identification provided for  $^{14}\text{C}$  analysis. TLE: total lipid extract; 2,4-DBP: 2,4-dibromophenol

Sample type	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	NOSAMS Accession No.
<b><i>S. bromophenolosus</i></b>			
Whole, not voided <sup>a</sup>	-18.2	+4.2	OS-41471
Proboscis, not voided <sup>b,c</sup>	-15.8	+19.7	OS-43700
Trunk, not voided <sup>b,c</sup>	-15.6	+11.3	OS-43701
Whole, voided <sup>b</sup>	-17.0	+25.6	OS-43702
TLE <sup>a</sup>	-19.2	+32.8	OS-43140
2,4-DBP (isolated from <i>S. bromophenolosus</i> ) <sup>a</sup>	-21.4	+10.4	OS-42445
2,4-DBP (purchased from Aldrich)	-26.8	-998	OS-37405
<b><i>G. dibranchiata</i></b>			
Whole, voided <sup>b</sup>	-13.8	+27.0	OS-43703
<b><i>C. torquata</i></b>			
Whole, voided <sup>b</sup>	-17.3	+38.6	OS-43704

<sup>a</sup>Samples collected in July 2003  
<sup>b</sup>Samples collected in May 2004  
<sup>c</sup>Trunks and proboscises from 2 individuals were combined

olina Mid-Atlantic Bight by DeMaster et al. (2002), who reported the presence of nuclear bomb-derived  $^{14}\text{C}$  in a variety of benthic fauna, including polychaetes ( $\Delta^{14}\text{C}$  ranging from +20 to +82‰). In contrast, surface sediment was characterized by pre-bomb levels of  $^{14}\text{C}$  ( $\Delta^{14}\text{C} < 0\text{‰}$ ). These results demonstrate unequivocally that, although *S. bromophenolosus* ingests surface sediment, it either selectively absorbs or selectively digests newer carbon pools containing recent photosynthate.

Variation in  $\Delta^{14}\text{C}$  values obtained for *Saccoglossus bromophenolosus* analyzed by slightly different procedures (Table 1) can be explained by the presence of recalcitrant organic components associated with the sediment matrix. The slight differences in  $\Delta^{14}\text{C}$  between voided *S. bromophenolosus* (+26‰), and an individual whose gut contains sediment (+4‰), probably

profiles and contribute to patchiness or variability in results.

Carey & Mayer (1990) showed that *Saccoglossus bromophenolosus* (formerly *S. kowalevskii*; King et al. 1994) in Lowes Cove feeds primarily on the upper 1 mm of sediment and probably selectively digests more recent OC pools. A high resolution (mm) analysis of the  $^{14}\text{C}$  content of sediment OC provided a detailed snapshot of carbon input and mixing dynamics (Table 2). However, surface  $\Delta^{14}\text{C}$  values remained substantially lower than the worm values. Similarly,  $\Delta^{14}\text{C}$  for the floc OC (-53‰) was comparable to average values for the 2003 and 2004 cores (-57 and -61‰, respectively), which suggests that bulk OC in surficial flocs originates primarily from resuspended sediment and does not contribute significantly to animal tissue carbon. Comparison of all the data in Tables 1 & 2 reveals that the  $^{14}\text{C}$  content of the sediment OC was lower and significantly different than the whole worms and their component parts for both years of sampling ( $p = 0.01$ ). Similar observations were made for the North Car-

Table 2. Mean ( $\pm$ SD) carbon isotope data ( $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$ ) and percent organic carbon (% OC) for sediment collected from Lowes Cove, Maine, USA; 2 replicates for each sediment horizon for % OC and  $\delta^{13}\text{C}$ . nd: not determined

Sample type Date	% OC	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	NOSAMS Accession No.	
<b>Sediment depth (cm)</b>					
23 Jul 03	0–2	0.82 $\pm$ 0.14	-21.3 $\pm$ 0.9	-49.4	OS-41464
	2–4	0.92 $\pm$ 0.14	-22.8 $\pm$ 0.6	-36.2	OS-41465
	4–6	0.77 $\pm$ 0.00	-20.9 $\pm$ 0.5	-63.9	OS-41466
	6–8	0.73 $\pm$ 0.01	-21.4 $\pm$ 0.2	-53.9	OS-41467
	8–10	0.76 $\pm$ 0.16	-21.9 $\pm$ 1.1	-57.1	OS-41468
	10–12	0.59 $\pm$ 0.00	-21.0 $\pm$ 0.5	-66.0	OS-41469
	12–14	0.47 $\pm$ 0.01	-21.2 $\pm$ 0.3	-74.7	OS-41470
28 May 04	0–0.1	nd	-21.0	-68.6	OS-43687
	0.1–0.2	nd	-20.7	-39.1	OS-43688
	0.2–0.3	nd	-21.0	-33.6	OS-43689
	0.3–0.4	nd	-20.8	-47.4	OS-43690
	0.4–0.6	nd	-21.2	-40.6	OS-43691
	0.6–0.8	nd	-20.6	-66.2	OS-43692
	0.8–1.0	nd	-20.8	-87.1	OS-43693
	1–2	nd	-21.6	-84.4	OS-43694
	2–4	nd	-21.5	-92.3	OS-43695
	4–6	nd	-21.3	-60.8	OS-43696
	6–8	nd	-21.8	-53.8	OS-43697
8–10	nd	-21.6	-61.8	OS-43698	
<b>Surface floc</b>					
28 May 04	nd	-21.4	-52.6	OS-43699	
<b>TLE (0–0.5 cm)</b>					
28 May 04	nd	-20.4	-0.5	OS-43700	

arise from the presence of older OC in the sediment in the gut contents. Similarly, DeMaster et al. (2003) recently observed a  $\Delta^{14}\text{C}$  enrichment of 50‰ in the tissues of a deposit-feeding holothurian compared to its gut contents. Although enrichment in the proboscis over that of the gut-containing trunk may be expected for *S. bromophenolosus*, the values in Table 1 are not significantly different within the uncertainty of the measurement ( $\pm 10\%$ ).

As a preliminary investigation into the usefulness of natural abundance of  $^{14}\text{C}$  in assessing the feeding habits of benthic invertebrates, we examined 2 other worms common to Lowes Cove: *Glycera dibranchiata*, a predatory polychaete, and *Clymenella torquata*, which feeds on subducted surface sediment (Dobbs & Whitlatch 1982, Levin et al. 1997), were collected within 10 m of the *Saccoglossus bromophenolosus* sampling site. The  $^{14}\text{C}$  contents of both were similar to the  $^{14}\text{C}$  contents of *S. bromophenolosus* (Table 1). Since comparable  $^{14}\text{C}$  data was obtained for both the 2003 and the 2004 cores (Table 2), despite their collection from slightly different locations, it has been assumed that the sediment  $^{14}\text{C}$  is consistent at the different worm sampling sites. The enrichment in  $^{14}\text{C}$  of *S. bromophenolosus* and *C. torquata* compared to the sediment illustrates that both worms are selectively assimilating newer carbon from the mixed pool of OC in the surface sediment. The  $^{14}\text{C}$  content of the predatory *G. dibranchiata* (which will be influenced by that of its prey) is also enriched in  $^{14}\text{C}$  compared to the sediment.

The use of sedimentary OC as a benthic faunal food source is determined by several factors, including binding of organics to mineral surfaces and hydrophobic associations that limit availability. The latter is exemplified by the enrichment in  $^{14}\text{C}$  of methanol soluble organic components (TLE) extracted from the upper 0.5 cm of the sediment ( $\Delta^{14}\text{C} = -0.5\%$ ) relative to bulk sediment OC ( $< -33\%$ ), which implies that older residual components are covalently bound or otherwise strongly adhering to the sediment matrix. The methanolic extract contains more highly mobile compounds, probably a variety of polar lipids and low molecular weight organics. The sediment TLE, however, contains less  $^{14}\text{C}$  than any of the voided worms, indicating that although this OC is labile, there is further selection of the compounds incorporated by the worms.

As discussed earlier, the 2,4-DBP isolated from *Saccoglossus bromophenolosus* contains nuclear bomb-derived carbon (Fig. 1), indicating that a modern carbon source ( $\Delta^{14}\text{C} > 0\%$ ) was used for its biosynthesis. Clearly this eliminates the possibility that the 2,4-DBP was synthesized from a pre-aged precursor. By contrast, industrially synthesized 2,4-DBP purchased from Aldrich is substantially depleted in  $^{14}\text{C}$  ( $\Delta^{14}\text{C} = -997\%$ ),

as expected for a compound synthesized from petrochemicals. The discrepancy between the 2,4-DBP  $^{14}\text{C}$  values in this study and that reported previously ( $-170\%$ , Reddy et al. 2004) is likely to reflect methodological differences in the 2 studies rather than differences in 2,4-DBP precursors. In both cases, 2,4-DBP was isolated from *S. bromophenolosus* from Lowes Cove during the spring or summer. In the present study, the 2,4-DBP was purified by silica gel column chromatography alone, whereas Reddy et al. (2004) used preparative reversed-phase high performance liquid chromatography (HPLC) to isolate 2,4-DBP. Solvent flow through the HPLC column results in a slow bleed of the  $\text{C}_{18}$  end-caps into the collected fractions. Depending on the polarity of the  $\text{C}_{18}$  functional group, the cleaved component may not have been detectable by gas chromatography, which was used to determine the purity and identity of the isolated 2,4-DBP. The presence of industrially synthesized alkyl end-caps, which are derived from petrochemicals containing no detectable  $^{14}\text{C}$ , would artificially 'age' a  $^{14}\text{C}$  measurement. For example, contamination of the 2,4-DBP sample by  $\text{C}_{18}$  alkyl chains present at 7% of the total sample mass would be sufficient to decrease our current  $\Delta^{14}\text{C}$  of 2,4-DBP by  $\sim 180\%$  to the value reported previously (Reddy et al. 2004). These results indicate that great care should be taken in the choice of methods used to isolate compounds subjected to  $^{14}\text{C}$  analysis.

In summary, we have shown that the  $^{14}\text{C}$  content of the natural product 2,4-DBP is consistent with that of the *Saccoglossus bromophenolosus* from which it was isolated. All worms investigated in this study contained nuclear bomb-derived carbon and were significantly enriched compared to the sediment from which they were collected, indicating that these worms are highly selective with respect to digestion and assimilation of recently photosynthesized organic compounds. These observations will be of great importance in the use of  $^{14}\text{C}$  for tracing the source of natural products accumulated in animal tissue and sediments.

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