Compound-specific bromine isotope compositions of one natural and six industrially synthesised organobromine substances

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Environmental context. Brominated organic compounds of both natural and anthropogenic origin are commonly found in the environment. Bromine has two stable isotopes and the isotopic composition of brominated compounds may vary depending on production pathways and degradation processes. These variations are a result of isotope fractionation effects, when heavy isotopes react slower than lighter isotopes. We apply compound-specific bromine isotope analysis to industrial brominated organic compounds, and one naturally produced analogue, to test the feasibility of the technique to investigate the source and environmental fate of these compounds.

Abstract. The stable bromine isotopic composition ($\delta^{81}$Br) was determined for six industrially synthesised brominated organic compounds (BOCs) and one natural BOC by gas-chromatography multi-collector inductively coupled plasma mass spectrometry (GC-mcICP-MS). The $\delta^{81}$Br compositions of brominated benzenes, phenols (both natural and industrial), anisoles, and naphthalenes were constrained with the standard differential measurement approach using as reference a monobromobenzene sample with an independently determined $\delta^{81}$Br value ($+0.39\%_{\text{v.}}$. Standard Mean Ocean Bromide, SMOB). The $\delta^{81}$Br values for the industrial BOCs ranged from $+4.3$ to $+0.4\%_{\text{v.}}$. The average $\delta^{81}$Br value for the natural compound (2,4-dibromophenol) was $0.2 \pm 1.6\%_{\text{s.d.}}$, and for the identical industrial compound (2,4-dibromophenol) $-1.1 \pm 0.9\%_{\text{s.d.}}$, with a statistically significant difference of $1.4\%$ ($P < 0.05$). The $\delta^{81}$Br of four out of six industrial compounds was found to be significantly different from that of the natural sample. These novel results establish the bromine isotopic variability among the industrially produced BOCs in relation to a natural sample.

Additional keywords: brominated organic compounds (BOCs), compound-specific isotope analysis (CSIA), gas-chromatography multi-collector inductively coupled plasma mass spectrometry (GC-mcICP-MS), pollutants.

Introduction

Brominated organic compounds (BOCs) are found worldwide in various matrices and are known to have both natural and anthropogenic sources. More than 1600 organobromine compounds are naturally produced. For example, mono-, di- and tribrominated phenols are excreted by marine organisms such as algae, polychaetes, and hemichordates. BOCs produced in situ are a consistent feature of pristine marine soft-bottom habitats, and their spatial and temporal abundance correlates with the abundance of infauna that produces these metabolites. The physiological and ecological function of BOCs may be to deter feeding, hinder or inhibit settlement of competitors, and regulate internal salt or hydrogen peroxide levels. Natural BOCs can be formed via photochemical, abiotic, Fe-mediated and enzymatic reactions. Many anthropogenic BOCs are (or have until recently been) produced in large quantities for uses such as disinfectants, flame retardants, pesticides and gasoline additives. Industrial BOCs are mostly produced by bromination of hydrocarbon precursors using bromine gas and by addition reactions of already brominated precursors. These compounds exhibit persistence in the environment, long-range transport and possibly toxic effects in humans and biota. There is thus a need to comprehend the environmental dynamics of BOCs, and knowledge of the relative magnitude of natural and industrial sources will be an important contribution to that end.

Here we apply compound-specific bromine isotope analysis (CSIA-Br) to investigate the plausibility of apportioning the aforementioned natural and anthropogenic sources, as a baseline for further CSIA-Br studies of the environmental fate of BOCs. Similar to other stable isotope systems, it is reasonable to expect that different synthetic pathways in some cases give rise to
distinguishable bromine isotope effects and thus imprint distinct and potentially diagnostic $^{81}\text{Br}$ values. Methods have recently been developed to allow environmental applications of CSIA-Br\cite{14-16} by measuring the ratio of the two stable bromine isotopes ($^{79}\text{Br}$ and $^{81}\text{Br}$; average $^{81}\text{Br}/^{79}\text{Br} = 0.97277$) using gas-chromatography multi-collector inductively coupled plasma mass spectrometry (GC-mcICP-MS). The composition of bromine isotopes in a sample is usually expressed in $^{81}\text{Br}$-Br notation – the permille deviation of the ratio $^{81}\text{Br}/^{79}\text{Br}$ in the sample ($R_{\text{Sample}}$) relative to the corresponding ratio ($R_{\text{Reference}}$) in the reference isotope effect of CSIA-Br\cite{14–16} by measuring the ratio of the two stable bromine isotopes in a sample is usually expressed in $^{81}\text{Br}$-Br notation – the permille deviation of the ratio $^{81}\text{Br}/^{79}\text{Br}$ in the sample ($R_{\text{Sample}}$) relative to the corresponding ratio ($R_{\text{Reference}}$) in the reference material (standard mean ocean bromide, SMOB):

$$^{81}\text{Br} = \frac{R_{\text{Sample}}}{R_{\text{Sample}} - 1} \times 1000$$

(1)

The isotopic composition of organic compounds can vary because of mass-dependent isotope fractionation where one isotope may react faster or be incorporated preferentially in one or several phases.\cite{17} Kinetic isotope effects, where the lighter isotope usually reacts faster than the heavier isotope, are generally more pronounced than equilibrium isotope effects and arise due to slight differences in the chemical bond energy of the isotopes.\cite{18} The isotopic difference in bond energy, and thus reaction rate, is correlated with the relative mass difference between the isotopes. Thus, the isotopes of lighter elements are more likely to exhibit strong isotope fractionation than those of heavier elements. For instance, Reddy et al.\cite{19} found a Cl isotope effect of 11\% depletion in $^{37}\text{Cl}$ relative to the chloride reagent stock during the enzymatic chlorination of organic substrates via haloperoxidase; the effect for Br is expected to be less (e.g. $\sim 4\%$).\cite{14} Very few data of $^{81}\text{Br}$-BOC have been reported in the literature. Nevertheless, this methodology has the potential to yield important information about sources as well as degradation and other environmental-fate processes of BOCs in analogy to that of chlorinated organic compounds.\cite{20-24}

The aim of this study was to investigate the stable bromine isotope composition of industrial BOCs by GC-mcICP-MS. This work provides the necessary baseline for forthcoming attempts towards source and fate studies of anthropogenic and naturally produced BOCs based on their bromine isotope composition.

### Experimental

#### Chemicals

The analytical standard solutions were prepared in n-hexane from the following pure reagents, purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany (the –SA suffix refers to Sigma Aldrich): monobromobenzene (MBB–SA); 1,4-dibromobenzene (DBB–SA); 1,3,5-tribromobenzene (TBB–SA); 2,4-dibromoanisole (ANI–SA); 4-bromophenol (BPH–SA); 2,4-dibromophenol (DBPH–SA); and 2-bromonaphthalene (NAPH–SA). Isolation of the natural product 2,4-dibromophenol (DBPH–WH) from gut-voided Saccoglossus bromphenollosus, collected at Lowes Cove (Maine, USA), was performed at the Woods Hole (WH) Oceanographic Institution (Table 1) as detailed in the previous work on these samples.\cite{2}

#### Derivatisation of bromophenols

Brominated phenols were derivatised by silylation before injection using bis(trimethylsilyl)-trifluoroacetamide (BSTFA). Briefly, the brominated phenols were dried under a gentle stream of $\text{N}_2$, redissolved with 200 $\mu$L of BSTFA and placed in an oven at 90°C for 1 h. Next 500 $\mu$L of n-hexane and 500 $\mu$L of acetonitrile were added, the samples were mixed and the acetonitrile was removed. This step was repeated twice. The n-hexane fraction was transferred to a graded vial and concentrated to the original volume under a gentle stream of nitrogen. The chosen derivatisation reaction does not affect the bonds to the bromine substituents and is therefore unlikely to cause any bromine isotope fractionation. Any such effects would be secondary and of no significance to the obtained isotope data.

#### Instrumental analysis

A Hewlett-Packard 5890 Series II GC (Agilent, Santa Clara, CA, USA) with a split/splitless injector was coupled to an Isosrobe ICP-mcMS (GVS Instruments, Manchester, UK, formerly Micromass), which features a hexapole collision cell and multi-collector detection system. Gas-chromatographic separation was performed on a 30-m mega-bore column to facilitate the analysis of large samples (Factor Four VF-5 ms: 0.53-mm internal diameter, 0.5-μm film thickness, 5% phenyl–95% dimethylpolysilsxiane, Varian Inc., Walnut Creek, CA, USA) with the following temperature programming: 90°C, hold 1.5 min, 15°C min$^{-1}$ to 270°C, hold 7 min. The injector was operated at 240°C with a splitless time of 1.5 min. Helium was used as carrier-gas at a flow of 6 mL min$^{-1}$. A deactivated fused silica (FS) capillary (0.25-mm internal diameter) without stationary phase was joined to the analytical column with a standard press-fit union (Siltek Press-Tight, Scantec Lab AB, Sweden) and used for the final length of the transfer line. A detailed description of the instrument set-up is given in Holmstrand et al.\cite{15}

#### Quality control

Signal intensities in the range 2–8 V were obtained from injecting a sample amount equivalent of 30–150 ng of Br (Table 1). This variation in sensitivity depends on the precise position of the capillary within the torch injector tube and on the exact torch position relative to the MS cone aperture. Another source of variation could be due to differences in the ionisation yield of the target compounds in the plasma. Initial tests where an independently determined monobromobenzene (MBB) isotope standard (see below) was measured over several days showed variations of 1.16\% ($\sigma$, $n = 75$) in the obtained $^{81}\text{Br}$ values.\cite{2} The target BOCs were therefore co-injected with the monooaromatic MBB, which was used as a near-authentic reference material in order to reduce the effect of the instrument drift using the standard bracketing procedure. The MBB isotope standard brackets ($^{81}\text{Br}$ of $-0.39\%$ v. SMOB) were subsequently used for the calculation of the $^{81}\text{Br}$ for all compounds as detailed by Holmstrand et al.\cite{15} Multiple injections of samples together with standard brackets were performed for each sample in every measurements campaign ($n = 3$ to $5$ sample injections). The same sets of samples were analysed during the two measurement campaigns and derivatisation of phenolic compounds was performed immediately before injection.

#### Mass spectrometry peak integration

The mass spectrometry data files were analysed using a Matlab script (version 7.9.0, The MathWorks, Natick, MA, USA). The position of the data peaks were manually selected using an interactive plot. A non-peak region was used to fit a second degree polynomial to account for baseline drift. The baseline corrected data was subsequently used for peak integration. The peak integration was made using a cumulative addition of the intensities in the region of $-200$ data points to $+300$ data points relative to the peak position (a data range corresponding to 85 s).

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Table 1. Description, chemical structures, delta bromine values ($\delta^{81}$Br) and related statistical data of the analysed compounds

–SA, suffix refers to Sigma Aldrich; –WH, suffix refers to natural sample collected by Woods Hole Oceanographic Institution. ALL–SA, total delta values of industrial compounds. $P$ values $< 0.05$ denote a statistically significant difference.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Chemical formula</th>
<th>Molecular weight (MW)</th>
<th>Amount injected (ng Br)</th>
<th>$\delta^{81}$Br</th>
<th>Mean (n) (s.d.)</th>
<th>Variance (s.e.)</th>
<th>Student’s t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromobenzene (MBB–SA)</td>
<td></td>
<td>Br$_2$C$_6$H$_6$</td>
<td>157.02</td>
<td>60</td>
<td>-2.25, 0.07, -1.41, -0.60, -0.33, -0.24, 0.11, 1.01</td>
<td>-0.45 (8) (0.99)</td>
<td>0.99 (0.35)</td>
<td>No, $t = -0.993$</td>
</tr>
<tr>
<td>4-Bromophenol (BPH–SA)</td>
<td></td>
<td>Br$_2$C$_6$H$_4$OH</td>
<td>173.01</td>
<td>67–86</td>
<td>-0.96, -0.28, 1.17, 2.19, 2.38, -1.12, -1.72</td>
<td>0.23 (7) (1.66)</td>
<td>2.78 (0.63)</td>
<td></td>
</tr>
<tr>
<td>2,4-Dibromophenol (DBPH–WH)$^a$</td>
<td></td>
<td>Br$_3$C$_6$H$_3$OH</td>
<td>251.90</td>
<td>52–79</td>
<td>-2.34, -1.08, -1.29, -0.62, 0.53, -2.42, -0.99, -2.37, -1.74, -0.59, -0.97, -0.77</td>
<td>-1.12 (13) (0.91)</td>
<td>0.84 (0.25)</td>
<td>Yes, $t = -2.381$ ($P &lt; 0.02$)</td>
</tr>
<tr>
<td>2,4-Dibromophenol (DBPH–SA)</td>
<td></td>
<td>Br$_3$C$_6$H$_3$OH</td>
<td>251.90</td>
<td>54–80</td>
<td>-2.85, -1.35, -5.35, -0.75, -0.70, -0.94</td>
<td>-1.66 (6) (1.82)</td>
<td>3.32 (0.74)</td>
<td>No, $t = -1.999$</td>
</tr>
<tr>
<td>2,4-Dibromoanisole (ANI–SA)</td>
<td></td>
<td>Br$_3$C$_6$H$_3$OCH$_3$</td>
<td>265.93</td>
<td>50–76</td>
<td>-0.85, -1.35, -5.35, -0.75, -1.66 (6) (1.82)</td>
<td>-1.91 (6) (1.45)</td>
<td>2.12 (0.59)</td>
<td>Yes, $t = -2.454$ ($P &lt; 0.03$)</td>
</tr>
<tr>
<td>1,4-Dibromobenzene (DBB–SA)</td>
<td></td>
<td>Br$_4$C$_6$H$_4$</td>
<td>235.90</td>
<td>104–156</td>
<td>-1.87, -1.59, -0.61, -3.82, -2.74, -2.46, -2.34</td>
<td>-1.91 (6) (1.45)</td>
<td>2.41 (1.63)</td>
<td>Yes, $t = -2.454$ ($P &lt; 0.03$)</td>
</tr>
<tr>
<td>1,3,5-Tribromobenzene (TBB–SA)</td>
<td></td>
<td>Br$_5$C$_6$H$_3$</td>
<td>314.80</td>
<td>60–90</td>
<td>-4.53, -4.44, -3.90, -4.29 (3) (0.34)</td>
<td>-4.29 (3) (0.34)</td>
<td>0.11 (0.19)</td>
<td>Yes, $t = -4.511$ ($P &lt; 0.001$)</td>
</tr>
<tr>
<td>2-Bromonaphthalene (NAPH–SA), ALL–SA</td>
<td></td>
<td>Br$_5$C$_9$H$_7$</td>
<td>207.05</td>
<td>35–54</td>
<td>-2.86, -3.06, -1.03, -1.98, -2.31 (6) (0.73), -2.59, -2.31</td>
<td>-2.31 (6) (0.73), -1.58 (42) (1.44)</td>
<td>0.54 (0.30), 2.09 (0.22)</td>
<td>Yes, $t = -3.44$ ($P &lt; 0.005$), yes, $t = -3.014$ ($P &lt; 0.004$)</td>
</tr>
</tbody>
</table>

$^a$Natural sample.
the ratios of the calculated integrals for two different isotopes that are used to calculate the δ values, it is the relative values of the integral (rather than the absolute) that are most important. As the cumulative integration scales directly with the intensity of the data, this integration method provides an accurate estimate of the intensity ratio. Both peaks \( m/z \) 79 and 81 were integrated over the same time period, no significant time difference was found for the start, centroid or end of the \( m/z \) 79 and 81 peaks.

**Data analysis**

Standard descriptive statistics were calculated for δ\(^{81}\)Br values for the industrial and natural compounds analysed. Student’s \( t \)-test was used to examine δ\(^{81}\)Br differences between natural and industrial BOCs. Statistical significance was considered at \( P < 0.05 \) (two sided). Statistical analysis was performed with the SPSS version 15.0 statistical program (SPSS Inc., Chicago, IL, USA).

**Results and discussion**

δ\(^{81}\)Br values of industrial BOCs

The average δ\(^{81}\)Br value for the industrial compounds ranged from \(-4.3\) to \(-0.4\)‰ (1 s.d.) (Table 2, Fig. 1). This places initial constraints on the extent of the Br isotopic variability among the industrially produced BOCs in view of the scarce compound-specific δ\(^{81}\)Br data reported in the literature.

Sylva et al.\(^{14}\) analysed industrial monobromobenzene (MBB) and 1,3,5-tribromobenzene (TBB), using dibromobenzene with an unknown δ\(^{81}\)Br as the isotopic anchor point. Although not able to report results as δ\(^{81}\)Br values relative to SMOB, that study demonstrated that the difference in the bromine isotope composition relative to a dibromobenzene was 0.24 ± 0.24‰ for MBB and 0.25 ± 0.30‰ for TBB in a single measurement campaign, indicating a very small isotopic variability among these industrially produced compounds. Gelman et al.\(^{16}\) analysed 79Br/81Br ratios of industrial BOCs and found a difference of \(\sim 2\)‰ between dibromoethane and other brominated aromatic compounds (toluene, benzene and phenol). They related this variation to different synthetic routes used in the industrial production of the compounds. Another study performed by Holmstrand et al.\(^{15}\) analysed larger BOCs such as polybrominated diphenyl ethers (PBDEs) in the technical flame retardant mixture Bromkal 70–5DE. That study, similar to the present investigation, employed standard bracketing with MBB and found δ\(^{81}\)Br values for the Bromkal components BDE#47, #99 and #100 of \(-0.6\) to \(+1.6\)%.

**Table 2. Delta bromine values (δ\(^{81}\)Br), standard deviation (s.d.) and number of replicates (\(n\)) of the analysed compounds**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Campaign 1</th>
<th>Campaign 2</th>
<th>Complete data set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\delta^{81})Br</td>
<td>s.d.</td>
<td>(n)</td>
</tr>
<tr>
<td>DBPH–WH</td>
<td>0.9</td>
<td>1.5</td>
<td>5</td>
</tr>
<tr>
<td>DBPH–SA</td>
<td>(-0.9)</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>BPH–SA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DBB–SA</td>
<td>(-0.9)</td>
<td>1.3</td>
<td>3</td>
</tr>
<tr>
<td>TBB–SA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ANI–SA</td>
<td>(-1.1)</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>NAPH–SA</td>
<td>2.3</td>
<td>1.1</td>
<td>3</td>
</tr>
</tbody>
</table>

**Fig. 1.** δ\(^{81}\)Br values (mean and s.d.) for natural and industrial brominated organic compounds. Asterisks denote statistical significant difference with the natural compound (Student’s \(t\)-test, \(P < 0.05\)).
study also analysed the difference in $^81\text{Br}$ for a BDE#47 and a methoxy BDE#47, both extracted from the blubber of a stranded whale and found it to be just 0.3 ± 0.7‰. Thus, putatively different natural and anthropogenic sources of those particular compounds could not be distinguished with current $^81\text{Br}$ techniques. The present study found slightly negative $^81\text{Br}$ values for all the industrial compounds (mean value of $-1.58 \pm 1.44\%$) (Table 1). The variability among the industrial BOCs may be related to differences in starting materials, synthesis routes, purification or storage.

$^81\text{Br}$ values of natural v. industrial products

Student's $t$-test was used to examine differences between the $^81\text{Br}$ for the natural and the different industrial compounds, using a statistical significance of $P < 0.05$ (two sided). The average $^81\text{Br}$ for the natural compound ($\text{DBPH–WH}$) was $0.2 \pm 1.6\%$ (1 s.d.) and for the identical industrial compound ($\text{DBPH–SA}$) $-1.1 \pm 0.8\%$ (1 s.d.). The difference between the natural product ($\text{DBPH–WH}$), and the identical industrial compound ($\text{DBPH–SA}$) was $1.4\%$ and statistically significant at $P < 0.05$ (Accessory publication, Table A1). Furthermore, the $^81\text{Br}$ of the natural compound is significantly different from the mean $^81\text{Br}$ of the industrial compounds (Accessory publication, Table A1). However, the $^81\text{Br}$ of the natural compound is not significantly different from that of compounds ANI–SA and BPH–SA when tested statistically on an individual basis. Thus, the present data set reveals an overlap in the $^81\text{Br}$ of industrial and natural compounds. Even if a higher measurement precision might facilitate the resolution of the $^81\text{Br}$ values of the two groups (i.e. natural and anthropogenic sources), the dynamic range appears too small to be of practical use for source apportionment in this source spectrum.

A perspective on this difference can be had by comparison with the aforementioned experiment of enzymatic chlorination of monoaromatic compounds where the end product was depleted in $^37\text{Cl}$ by some $-11\%$ relative to the starting material. The isotopic effect for bromine in the analogous chemical reaction has been estimated to be one-third of that for chlorine.[23] As our brominated phenol ($\text{DBPH–WH}$) is believed to displace chlorine gas and can be extracted as bromine gas, considering the $^81\text{Br}$ of the industrial mixtures of polybrominated dibenzo-p-dioxins and dibenzofurans relative to the starting material.

Conclusions

The herein studied set of seven industrially produced brominated organic compounds (BOCs) exhibited a small variability in their $^81\text{Br}$ values, falling in the range $-4.3$ to $-0.4\%$ (average: $-1.6 \pm 1.4\%$, 1 s.d.) relative to standard mean ocean bromine. A statistically significant difference ($P < 0.05$) of $1.4\%$ was found between a natural sample of dibromophenol and its industrial counterpart of identical chemical structure. It was also found that the range in $^81\text{Br}$ of the six analysed industrial compounds overlaps to some extent with the $^81\text{Br}$ of the natural sample. This study provides a baseline for future work employing $^81\text{Br}$-BOCs to study the sources and degradation of BOCs in the environment. It is conceivable that ambient microbial and photochemical degradation of brominated flame retardants, for example, induces a Br isotopic effect so that bromine isotopic analysis can be used to assess the extent of environmental degradation of such chemicals, in analogy to the known isotopic fractionation for chlorine in organochlorine compounds.

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