ANTHROPOGENIC AND NATURAL PERSISTENT, BIOACCUMULATIVE ORGANOHALOGEN COMPOUNDS IN DUGONGS (*DUGONG DUGON*) AND A BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) FROM AUSTRALIA

Walter Vetter[1], Elke Scholz[1], Bernd Luckas[1], Caroline Gaus[2], Jochen Müller[2], and David Haynes[3]

[1] Friedrich-Schiller-University Jena, Department of Food Chemistry, Dornburger Str. 25, D-07743 Jena, Germany
[2] National Research Centre for Environmental Toxicology, 39 Kessels Rd., Coopers Plains, Qld 4108, Australia
[3] Great Barrier Reef Marine Park Authority, PO Box 1379, Townsville, 4180, Australia

Introduction

Organohalogen compounds include a range of compounds which, due to a combination of physico chemical properties and toxicity have been described as a serious threat to the environment and thus a global ban on their manufacture is currently under discussion (http://www.unece.org/). To date the majority of studies on environmental concentrations of anthropogenic organohalogen compounds have been undertaken in the Northern Hemisphere. Despite the recognition that only limited data are available, it has been suggested that the levels of organohalogen compounds in the Southern Hemisphere are comparatively lower for a given environmental compartment and latitude[1,2]. Recent studies have also demonstrated the existence of unidentified sources of specific organohalogen compounds in the Southern Hemisphere[3]. Furthermore, together with compounds that have been primarily associated with anthropogenic sources, Q1 was detected in samples of marine mammals and birds from Africa and Antarctica[4,5,6]. Q1 has the molecular formula $C_9H_3Cl_7N_2$[7] and was identified as a bioaccumulative, persistent natural organochlorine that is most likely a heptachlorobipyrrol[6]. An initial comparison between marine mammal samples collected from the Southern and the Northern Hemisphere suggested that in contrast to many organohalogen compounds, Q1 concentrations are higher in samples from the Southern Hemisphere[8]. The aim of this study was to assess the distribution of anthropogenic organohalogens and Q1 in blubber of dugongs (*Dugong dugon*) and one bottlenose dolphin (*Tursiops truncatus*) from Australia.

Experimental Methods

Samples.

Blubber samples of 7 dugongs (*Dugong dugon*) found stranded along the coast of Queensland, Australia, were collected between 1996 and 1999. Dugongs are long-lived and feed almost exclusively on a number of seagrasses, especially species of the genera *Halodule* and *Halophila*[9]. Nearshore seagrass beds along the northeastern coast of Australia provide important habitat and feeding grounds for a significant proportion of existing world stocks of dugong[9]. Blubber was also available for analysis from an old (>20 y) bottlenose dolphin (*Tursiops truncatus*) found dead near Brisbane in 1996.

Sample clean-up. Approx. 1 g sample was spiked with the internal standard α-PDHCH. Combined microwave-assisted extraction and gel-permeation chromatography with ethyl

acetate/cyclohexane (1:1, v:v) was performed with an MLS 1200 system. Subsequently, adsorption chromatography on 3 g silica (deactivated with 30% water, w:w, in a 1 cm i. d. glass column) was carried out. The eluate was concentrated and the volume was adjusted to 1.0 mL. 800 µL were subjected to a PCB/CTT group separation[4].

Gas chromatography with electron capture detection. Sample extracts were analyzed on a Hewlett-Packard 5890 series II gas chromatograph equipped with two 50 m x 0.25 capillary columns (CP-Sil 2 and CP-Sil 8/C18) and two ECDs in parallel[4]. Q1 was quantified using the ECD response of trans-nonachlor[4,5].

Gas chromatography with electron capture negative ion mass spectrometry. A Hewlett-Packard 5890 series II/5989B GC/MS system was used. A ß-BSCD column (30 m length, 0.25 µm i. d., and 0.20 µm film thickness) consisting of 25% randomly *tert.*-butyldimethylsilylated ß-cyclodextrin diluted in PS086 (BGB Analytik, Adliswil, Switzerland) was installed in the GC oven. In the full scan mode m/z 50 through m/z 650 were monitored. Further parameters were published elsewhere[7]. In the SIM mode 6 m/z values were run in three time windows. Q1-selective m/z values were 384, 386, 388. m/z values 79, 81, 159,161, 516 and 530 were recorded for BC-1 and BC-2 (see below).

Results and Discussion

A range of organohalogen compounds investigated were detected in the dugong and the dolphin samples. Highest organochlorine levels were found in the dolphin sample for p,p′-DDE (4.8 µg/g). Furthermore, with a concentration of 1.9 µg/g, the natural compound Q1 was the second most abundant organohalogen detected. It is noteworthy that this is the highest concentration of Q1 determined to date in an environmental sample. PCB 153 (0.9 µg/g) and PCB 138 (0.55 µg/g) were also prominent in the sample. Furthermore, a series of unknown or less well described compounds including two brominated compounds (referred to as BC-1 and BC-2) were observable by extraxtring m/z 79 and m/z 81 during analysis using GC/ECNI-MS in the full scan mode (see **Figure 1** and **2a**).

Although it initially seemed that both signals originated from the enantiomers of a chiral compound, which were separated on the chiral stationary phase installed in the GC/MS. The second eluted compound additionally showed an intense fragment at m/z 159 (**Figure 2b**). A closer inspection of the ECNI-MS full scan mass spectrum revealed tetrabromo isotope patterns starting at m/z 512 and m/z 526, respectively (**Figure 3**). The molecular ion of BC-2 is that described for methoxy-tetrabromodiphenylethers that have been reported in blubber samples from the Baltic Sea[10] but, to our knowledge, not in any samples from the Southern Hemisphere. BC-1 showed similar fragmentation at high masses with a shift at 14 u to higher mass. This suggests an additional methylene group ($-CH_2-$) compared with BC-2.

BC-1 may be explained in three ways: (i) either by an additional methyl group (instead of H) on the diphenylether backbone of BC-2, (ii) an ethoxy group instead of the methoxy group on BC-2 or (iii) a dimethoxy-tetrabromobiphenyl. Note also a third brominated compound of the "BC-2" type at higher retention times (58.7 min, **Figure 2**).

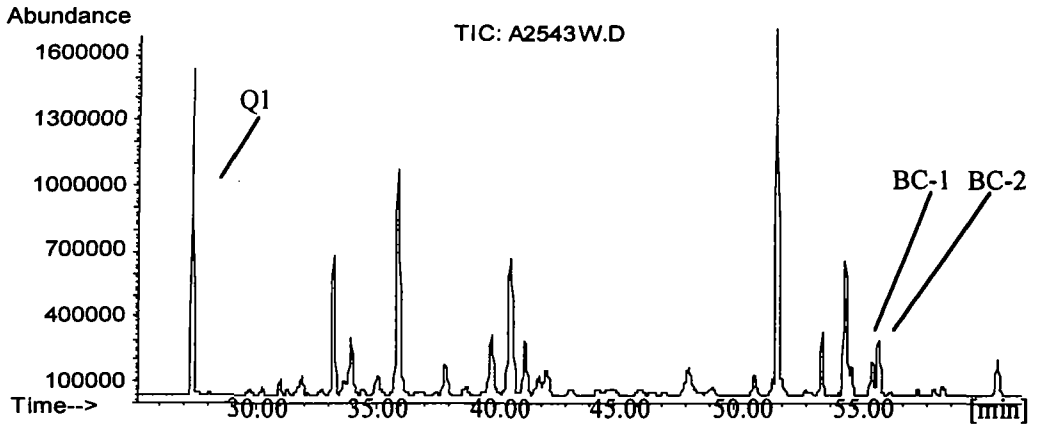

Figure 1: GC/ECNI-MS full scan chromatogram of the blubber of a bottlenose dolphin from Australia

Organohalogen levels in the dugong samples were considerably lower than in the dolphin. One adult female dugong showed significantly higher levels than the other 6 dugong samples whose levels were in the same range. Residues of DDT were dominated by p,p'-DDE (~85% of sum-DDT, levels ranging from 0.4-28.5 ng/g). PCB 153 and PCB 138 levels ranged from 2.1-11.9 ng/g and 1.9-6.3 ng/g.
Furthermore, at a concentration of 29 ng/g Q1 was the dominant component in the female adult dugong. Q1 was also an important component in the chromatograms of four other dugong samples (0.2-0.8 ng/g) while it was not detected or was below the limit of quantification in 2 samples. Traces of BC-1 and BC-2 were also detected in the female adult dugong sample.

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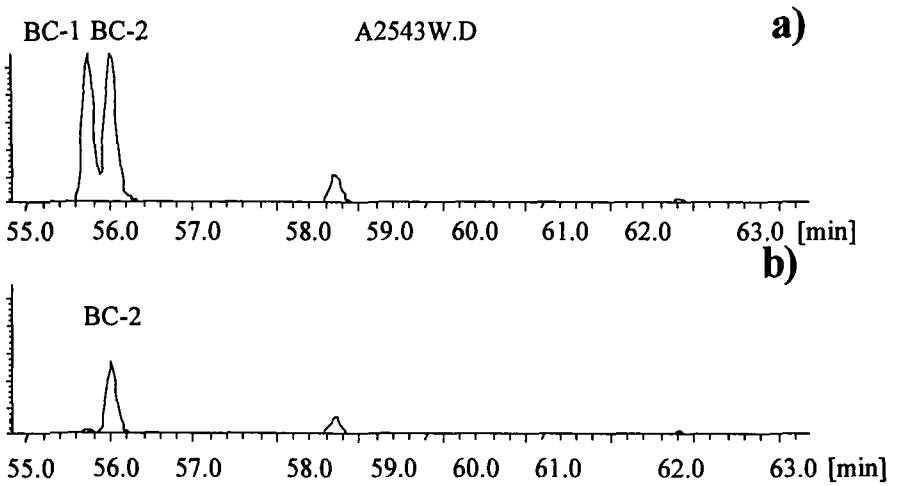

Figure 2: Br-selective SIM traces (a) m/z 79 and (b) m/z 161

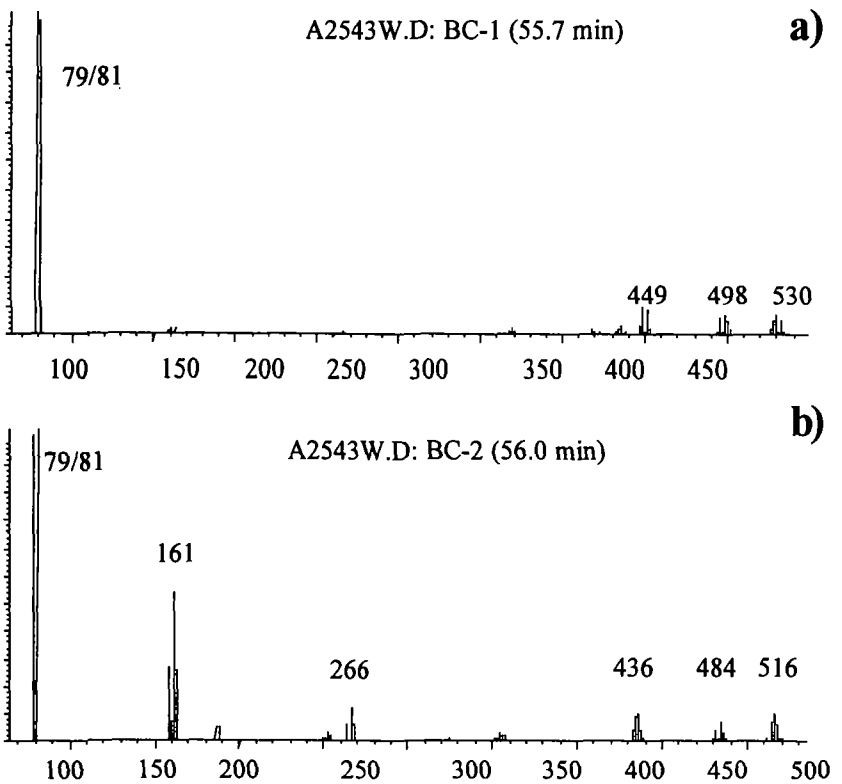

Figure 3: GC/ECNI-MS full scan mass spectra of brominated compounds (a) BC-1 and (b) BC-2. Labeled are the most abundant peaks in the Br-isotope patterns.