# NATURAL AND ANTHROPOGENIC HALOGENATED COMPOUNDS IN DOLPHIN BLUBBER AND BRAIN FROM SOUTHWEST MEDITERRANEAN SEA

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## Introduction

More than 5000 halogenated natural products (HNPs) with different structures and thus belonging to different families have been described through the years. It is believed that some species of sponges and algae are the main producers of these compounds and are responsible for their presence in the environment. To date, these compounds are mainly considered a marine problem, since they have only been found scarcely in species with a terrestrial diet. By contrast, different HNPs have been detected in marine species such as marine mammals, fish, birds and mollusks. This means that these compounds have bioaccumulation capacity, and should be included when studying bioaccumulation behaviors of other halogenated compounds that occur in the environment. However, the number of studies is still limited and they often include a small number of samples.

Some examples of these HNPs are (1*R*,2*S*,4*R*,5*R*,1'*E*)-2-bromo-1-bromomethyl-1,4-dichloro-5-(2'-chloroethenyl)-5-methylcyclohexane (MHC-1), 2,7-dibromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene (TriBHD), 2,5,7-tribromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene (TetraBHD), methoxylated PBDEs (MeO-BDEs), 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Q1) and other 1-methyl-1,2´-bipyrroles related with Q1 but with different halogenation pattern which include substitution of chlorine by bromine . These compounds are $BrCl_6$-MBP, $Br_2Cl_5$-MBP and so on, until the $Br_7$-MBP[1]. It is believed that their presence in the marine environments it not due to a wide-range transport but that they are produced worldwide by different marine organisms. However, the number of studies is scarce.

On the other hand, flame retardants (FRs) have been used for decades in order to reduce the flammability of a wide range of materials such as textiles, plastics, wood or electronic furniture. Halogenated flame retardants (HFRs) are a group of halogenated compounds which proved to be effective in the inhibition of the combustion process. One of the most widely used HFRs were Polybrominated diphenyl ethers (PBDEs). Due to their proved bioaccumulation, biomagnification and wide-range transport, and also to their toxicity, technical penta- and octa-BDE mixtures were banned in 2004, while the production of deca-BDE is being stopped. PBDEs have been found in several environmental and biological samples from all over the world. Nowadays, in response to the legal restrictions over them, other brominated flame retardants have appeared as an alternative, i.e. decabromodiphenyl ethane (DBDPE), pentabromoethyl benzene (PBEB) or hexabromobenzene (HBB) Halogenated norbornene derivatives are a family of chlorinated FRs used for many years but not detected in the environment until 2006. These are Dechlorane 602 (Dec 602), Dechlorane 603 (Dec 603), Dechlorane 604 (Dec 604), and Dechlorane Plus (DP). DP exists in commercial products as two isomers: *syn*- and *anti*-DP. Since its first determination in 2006, DP has been found in environmental matrices such as sediment or water, and also in biological matrices such as fish, eggs, dolphin liver or blood. Reported levels show that both DP isomers have bioaccumulation capacity and might biomagnify among the trophic chain. Dec 602, Dec 603 and Dec 604 are supposed to have similar environmental behaviour and toxicological properties than DP but it has not been reported yet[2].

In this work, we determined several natural and anthropogenic halogenated compounds in two different tissues (blubber and brain) in stranded dolphins from the Mediterranean Sea. The aim of the study was to compare HNPs and anthropogenic burdens and study possible tissue-specific accumulation patterns.

## Materials and methods

Sampling

Blubber and brain from 26 stranded individuals were collected between 2004 and 2011 among the Alboran Sea (Southwest Mediterranean Sea). Pairs of samples of five different species were obtained: Ten samples of short-beaked common dolphin (*Delphinus delphis)*, eleven samples of striped dolphin (*Stenella coureloalba)*, three samples of long-finned pilot whale (*Globicephala melas*), one sample of Risso's dolphin (*Grampus griseus)* and one sample of common bottlenose dolphin (*Tursiops truncatus)*. Samples were kept at -20 ºC until the analysis.

Analysis

Sample extraction and purification was based on previous works[3]. Samples were weighted and spiked with internal standards ($^{13}$C-PBDEs and $^{13}$C-syn-DP) prior to the extraction by pressurized liquid extraction (PLE). Lipid content was determined gravimetrically and then acid treatment with sulphuric acid and cleanup with solid phase extraction (SPE) with Al-N were used to purify the extracts. Perdeuterated α-l,2,3,4,5,6-hexachlorocyclohexane (α-PDHCH) was added prior to the instrumental determination.

HNPs were analysed using a gas chromatograph in combination with electron-capture negative-ion mass spectrometry (GC/ECNI-MS) using an Agilent 7890/5975C system in combination with an HP 7673 automatic injector (Agilent Technologies, Waldbronn, Germany) operated in electron-capture negative ion mode. The transfer line temperature was 300 ºC and ion source and quadrupole temperatures were both set at 150 ºC. GC oven was programmed as follows: 60 ºC held for 2 min, the ramped at 10 ºC/min to 300 ºC and held for 24 min for a total run time of 50 min. Selective ion monitoring (SIM) was used for the detection and quantification of the different compounds using the two most abundant ions of the molecular ion for each compound. Quantification was done using a response factor to the internal standard α-PDHCH obtained in a standard mixture. MBPs related to Q1 were quantified using the response factor obtained for Q1 since there were no available standards of these compounds[1].

PBDEs, emerging BFRs (HBB, PBEB and DBDPE) and halogenated norbornene derivatives were analyzed by an Agilent Technologies 7890A GC system coupled to 7000A GC/MS Triple Quadrupole, working in electron ionization (EI) and ECNI modes, respectively. Thus, two different injections were made. The elution program for PBDEs started at a temperature of 140 ºC, was held for 2 min and then ramped to 325 ºC at 10 ºC/min and held for 10 min. The injector and source temperatures were set at 280 ºC and 250 ºC respectively. In order to enhance the sensitivity, selected reaction monitoring (SRM) mode was applied monitoring the two most intense transitions from the EI spectra. Halogenated norbornene derivatives were analyzed working in ECNI using $CH_4$ as reagent gas. Temperature program started at 80 ºC, was held for 2 min and then ramped to 300 ºC in 10 ºC/min. Final temperature was maintained for 10 min. Source temperature was set at 175 ºC and electron energy and emission current were set at 200 eV and 150 eV, respectively. In order to enhance the sensitivity and selectivity, SRM mode was applied as well[3].

**Results and discussion**

Several HNPs and anthropogenic compounds were detected in all the samples. HNPs detected included MHC-1, TriBHD, TetraBHD, Q1, several mixed halogenated congeners of Q1, 2'-MeO-BDE-68 and 6-MeO-BDE-47. Moreover, among anthropogenic compounds detected there were several PBDEs (BDE-28, BDE-47, BDE-100, BDE-99, BDE-153 and BDE-154) and some halogenated norbornenes (Dec-602, Dec 603, *syn*-DP and *anti*-DP). Total natural and anthropogenic burden was different for blubber and brain in the five species studied (Table 1). The most contaminated species was the *G. griseus* with a total burden of 7490 ng/g lw, divided into a contamination of 7330 ng/g lw in blubber and a contamination of 155 ng/g lw in brain. *S. coureloalba* was the second one, with a total burden of 5730 ng/g lw divided in 4450 ng/g lw and 1280 ng/g lw in blubber and brain, respectively. The following one was *D. delphis*, with a total burden of 4960 ng/g lw with a contribution of 4290 ng/g lw in blubber and 673 ng/g lw in brain. The following species was *T. truncatus*, with a burden of 3170 ng/g lw in blubber and 866 ng/g lw in brain for a total burden of 4030 ng/g lw. Finally, the less contaminated species was *G. melas*, with a total burden of 2390 ng/g lw, while burdens in blubber and brain were 1950 and 439 respectively. As can be seen, concentrations were higher in blubber than in brain for all the species.

Table1. Mean values (expressed in ng/g lw) of HNPs and anthropogenic compounds in each dolphin species analysed.

| | *D. delphis* | | *S. coeruleoalba* | | *G. melas* | | *G. griseus* | | *T. truncatus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | blubber | brain | blubber | brain | blubber | brain | blubber | brain | blubber | brain |
| ΣHNPs | 3200 | 358 | 3410 | 726 | 1410 | 279 | 6400 | 127 | 2290 | 393 |
| Σanthropogenic | 1090 | 316 | 1040 | 552 | 537 | 160 | 931 | 27.9 | 877 | 474 |
| ΣHNPs+anthr. | 4290 | 674 | 4450 | 1280 | 1950 | 439 | 7330 | 155 | 3170 | 867 |
| Σblubber+brain | 4960 | | 5730 | | 2390 | | 7490 | | 4030 | |

Furthermore, differences in the ΣHNP and Σanthropogenic levels in the same tissue were also observed (Figure 1). For four of the five species studied the ratio between blubber and brain concentrations was higher for HNPs, ranging from 21 to 126, than for anthropogenic compounds, were ratios ranged from 20 to 83. For *G. melas*, ∑HNPs and ∑anthropogenic ratio were similar (20 and 21). These could indicate that HNPs are most likely accumulated in blubber than in brain rather than the anthropogenic FRs. However, tissue-specific accumulation has been observed for some compounds. Regarding HNPs, TetraBHD was one of the most abundant compounds in brain while it was barely found in blubber. Similarly, 2'-MeO-BDE-68 was not detected in brain while it was detected in many blubber samples. This behaviour was similar in all the species and could suggest a tissue-specific accumulation of these compounds. Regarding anthropogenic FRs, HBB was detected in brain but not in blubber.

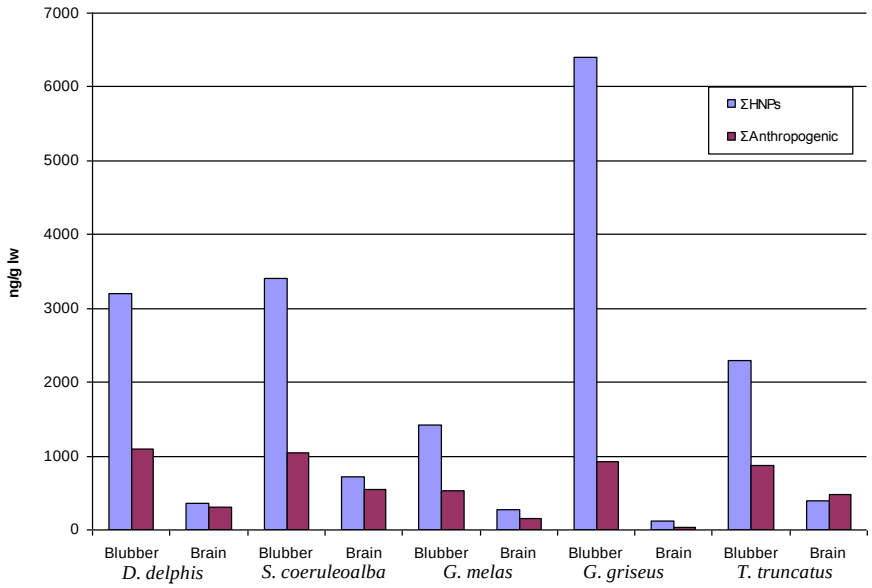


Figure 1. Distribution of HNPs and anthropogenic contaminants between blubber and brain in the five dolphin species analysed.

Q1 and 6-MeO-BDE-47 were the most abundant HNPs in all the samples. Q1 levels ranged from nd to 4107 ng/g lw and from nd to 412 ng/g lw in blubber and brain, respectively, for *D. delphis*. For *S. coureloalba* Q1 concentrations ranged from nd to 2485 ng/g lw in blubber and from nd to 1551 ng/g lw in brain. For *G. melas* values in brain and blubber ranged from 437 to 505 ng/g lw and from nd to 341 ng/g lw, respectively. For *T. truncatus* and *G. griseus*, as said above, only one sample was available. Q1 in blubber and brain of *T. truncatus* was 3584 ng/g lw and 45 ng/g lw, respectively, while in *G. griseus* was 699 ng/g lw and 96 ng/g lw, respectively. Levels of 6-MeO-BDE-47 were in the same order than Q1 levels.

Despite the fact that Q1 has been considerably studied, its related congeners (MBPs) were described recently and there is not much information about them. In this study, $BrCl_6$-MBP, $Br_2Cl_5$-MBP, $Br_3Cl_4$-MBP and $Br_4Cl_3$-MBP were found both in blubber and brain in the different studied species, while $Br_5Cl_2$-MBP, $Br_6Cl$-MBP and $Br_7$-MBP were only found occasionally in blubber. This might mean that their presence in brain was too low to

be detected rather than a tissue-specific accumulation. Despite the fact that Q1 was more abundant than MBPs, representing about the 50-60% of the total contribution, levels found for MBPs were higher than those previously described in cetaceans from Australia, where the contribution of the other congeners of Q1 was up to 27%. In this study the contribution of $BrCl_6$ and $Br_2Cl_5$ was specially relevant, with about 25-30% to the total concentration of Q1 and related congeners (Figure 2). These could mean that these compounds were more abundant in the Alboran Sea than elsewhere. In this study, five $BrCl_6$ MBPs, four $Br_2Cl_5$-MBPs, three $Br_3Cl_4$-MBPs, two $Br_4Cl_3$-MBPs, two $Br_5Cl_2$-MBPs, two $Br_6$-Cl-MBPs, and the $Br_7$-MBP, were dected at different levels of concentration depending on the species. However, the abundance mainly followed the same trend in all species and tissues: Q1 > $BrCl_6$-MBP > $Br_2Cl_5$-MBP > $Br_3Cl_4$-MBPs > $Br_4Cl_3$-MBPs > $Br_5Cl_2$-MBPs > $Br_6$-Cl-MBPs > $Br_7$-MBP.

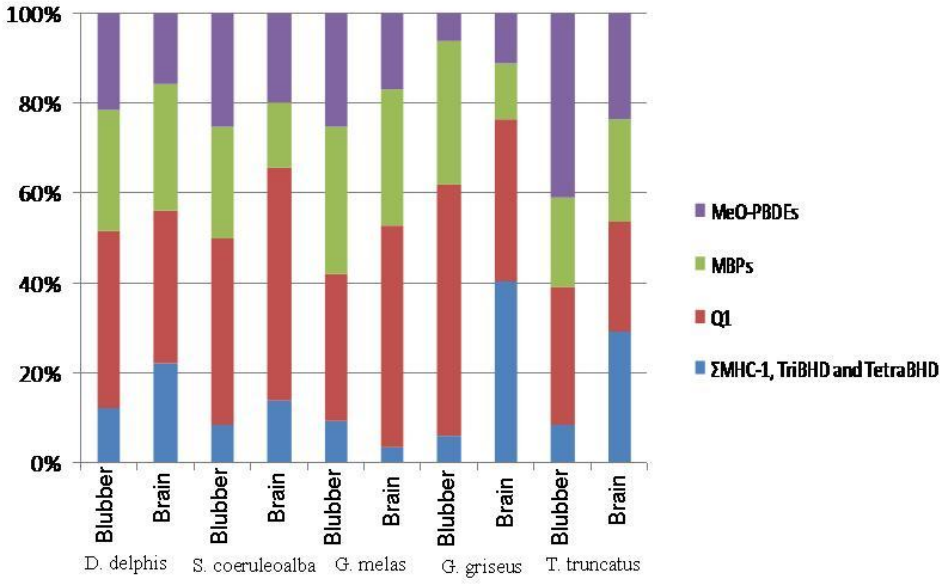


Figure 2. Percentage contribution of each family of HNPs included in this study to the total HNP burden.

To conclude, this study suggests that more attention should be paid to HNPs and to Q1 and related congeners in particular, since their levels were always higher than the anthropogenic compounds. Despite it has been described in other studies as well, the difference between the number of studies focusing only on anthropogenic compounds and the studies focusing in both families is still very high. Furthermore, some compounds seem to show a tissue-specific accumulation, which should be studied deeply in the future.

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**References**
1. W. Vetter, S. Gaul, D. Olbrich, C. Gaus, Chemosphere 66 (2007) 2011.
2. M. Alaee, P. Arias, A. Sjödin, Å. Bergman, Environment International 29 (2003) 683.
3. E. Barón, E. Eljarrat, D. Barceló, Journal of Chromatography A 1248 (2012) 154.