EVALUATION OF PBDEs, IN STRIPED DOLPHIN (*Stenella coeruleoalba*) FROM THE MEDITERRANEAN SEA

Begoña Jiménez[1], Enrique Blázquez[1], Belen Gómara[1], Daniela Bucalossi[2], Silvia Maltese[2], Letizia Marsili[2], Mehran Alaee[3], Cristina Fossi[2]
[1] Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC. Juan de la Cierva 3, 28006 Madrid, SPAIN. [2] Department of Environmental Science, University of Siena. Via Mattioli 4, 53100 Siena, ITALY. [3]National Water Research Institute, Aquatic Ecosystem Protection Research Branch, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, Canada, L7R 4A6.

Abstract
Polybrominated diphenyl ethers (PBDEs) are a major family of brominated flame retardants which are lipophilic, persistent, and toxic to both fauna and humans. The highest levels of PBDEs have been found in the top of the marine food web. Many cetacean species, in particular odontocetes, are top predators in the marine food chain and therefore accumulate high levels of lipophilic compounds such as halogenated hydrocarbons. Today there is a growing concern about the accumulation of brominated organic compounds in the marine food web. The aim of this study was to investigate the levels of PBDEs in specimens of striped dolphin (*Stenella coeruleoalba*) sampled with the non-lethal method of biopsy in the Mediterranean Sea at different anthropogenic impact: the Strait of Gibraltar, the Pelagos Sanctuary (Ligurian Sea) and the west Ionian Sea (Sicily). Total PBDEs found in this study were in the range 12-2.7 ppm (ug/g fresh weight), being samples from Pelagos Sanctuary the highest contaminated compared to specimens from the Strait of Gibraltar. BDE-47, BDE-100, BDE-99 and BDE-153, were the most abundant congeners. Total PBDE levels found in this study are similar to total PCB levels found in the same specimens.

Introduction
Polybrominated diphenyl ethers (PBDEs) are a major family of brominated flame retardants which are lipophilic, persistent, and toxic to both fauna and humans. Levels of these ubiquitous contaminants are increasing in the environment and this raises concern that wildlife may suffer a variety of toxic effects, with consequences for their populations. The highest levels of PBDEs have been found in the top of the marine food chain[1]. Many cetacean species, in particular odontocetes, are top predators in the marine food web and therefore accumulate high levels of lipophilic compounds such as halogenated hydrocarbons. Many studies indicate the possibility of using these animals as bioindicators of environmental contamination of relatively limited areas. Today there is a growing concern about the accumulation of brominated organic compounds in the marine food web. Studies by Fossi et al.[2, 3] suggest that cetacean skin biopsies are a powerful non-invasive tool for assessing ecotoxicological risk to Mediterranean marine mammals species. The aim of this study was to investigate PBDE levels in specimens of striped dolphin (*Stenella coeruleoalba*) sampled with the non-lethal method of biopsy from different Mediterranean areas.

Materials and methods
Sampling and Study area
In this research, we used a non-lethal approach (skin biopsy) to investigate the toxicological status of striped dolphin (*Stenella coeruleoalba*) from the Mediterranean Sea. Three different areas located at the Mediterranean Sea were selected in this study: Gibraltar Straits, Pelagos Sanctuary (Ligurian Sea) and the west Ionian Sea (Sicily). Two hundred biopsy samples of striped dolphins were collected using a biopsy pole in the Mediterranean Sea in summer 2006. In order to investigate the presence of PBDEs in the species, eighteen biopsy samples (six per sampling area) were selected for the present study

Residue analysis
Sample treatment consisted basically in Soxhlet extraction with n-hexane, followed by sulphuric acid clean-up and Florisil chromatography as described in detail by Marsili et al.[4]

Fifteen PBDEs, including from tri- to deca- substituted congeners (Nos. 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197, and 209), were determined using a 6890N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) working in the electron capture negative ionization mode (ECNI). Standards and samples were injected in hot splitless mode (300ºC, 1 uL; splitless time 2.0 min). A low bleed GC capillary column DB-5MS (15 m, 0.2 mm i.d., 0.2 µm film thickness) purchased from J&W Scientific (USA) was used for separation. The column temperature was programmed as follows: 110ºC (1.5 min) at 30ºC/min to 200ºC, then at 5ºC/min to 275ºC, then at 40ºC/min to 300ºC (10 min), and then at 10ºC/min to 310ºC (2 min). Helium was used as the carrier gas at a constant flow rate of 1.5 ml/min. The temperature of the transfer line was set at 310ºC and the source and quadrupole temperatures were fixed at 150 ºC. The identification of target compounds was based on detection, at the corresponding retention time, of the *m/z* 79 and 81 (corresponding to bromine atoms) plus two more ions corresponding to the cluster of $[M\text{-}H_xBr_y]^-$ which are specific and characteristic of each congener.
For higher brominated compounds, we also used for confirmation purposes High resolution GC/MS. Analyses of PBDEs were carried out on a Micromass AutoSpec Ultima mass spectrometer connected to a Hewlett-Packard 6890 GC equipped with a CTC A200s autosampler. The GC injection port was configured for 1 uL splitless injections, at constant temperature of 275 ^oC. Separation was accomplished using a 15m X 0.25 mm X 0.25 µm Restek Rt_x5 capillary column. The GC column was maintained at 110^oC for 0.5 min, then ramped at 10 ^oC/min to 300^oC , held for 20 minutes, further ramped at 20^oC/min to 310^oC and held there for 5 minutes. Total run time was 45 minutes. Sample ionization was performed by electron ionization (EI) at an electron voltage ranging from 30 to 40 eV depending on the optimization parameters of the instrument. Source temperature was 270 ^oC and the resolving power of the analyzer was 10,000. The mass spectrometer was operated in SIM mode using a total of 8 descriptors to analyse the lower PBDE congeners, and single descriptor for higher brominated PBDEs.

Results and discussion
Table 1 shows average values found in each sampling area for every congener analysed. It is remarkable that the lowest concentrations of total PBDEs (as the sum of 15 congeners) were found in specimens from the Strait of Gibraltar, while levels found in the other areas are quite similar and one order of magnitude higher compared to biopsies from the Strait of Gibraltar.

Table 1. Average PBDE concentrations (ng/g, on a wet weight basis) in skin biopsies from striped dolphin at the three sampling areas selected at the Mediterranean Sea.

PBDE	Ionian Sea, Sicily	Strait of Gibraltar	Pelagos Sanctuary
17	< L.O.D	< L.O.D	< L.O.D
28	83	< L.O.D	7
47	6545	1754	6715
66	187	25	238
100	2506	504	2302
99	2195	319	2241
85	< L.O.D	< L.O.D	< L.O.D
154	< L.O.D	< L.O.D	< L.O.D
153	518	83	987
184	< L.O.D	< L.O.D	< L.O.D
183	36	9	69
191	< L.O.D	< L.O.D	< L.O.D
197	< L.O.D	< L.O.D	< L.O.D
196	< L.O.D	< L.O.D	< L.O.D
209	< L.O.D	< L.O.D	< L.O.D
TOTAL	**12,071**	**2,694**	**12,560**

BDE-47, BDE-100, BDE-99 and BDE-153, were found as the most abundant in all the samples studied. A similar PBDE distribution was observed in the different areas studied. Regarding the congener profiles, it can be observed in figure 1 that BDE-47 was the most abundant in all the sampling locations with percentage contributions over 50%. BDE-100 and BDE-99 showed similar percentage contributions with a 20% contribution each one. BDE-153 had the lowest contribution in all the sampling locations, being lower than 10%. It is also remarkable that higher brominated compounds were not found in any of the samples studied.

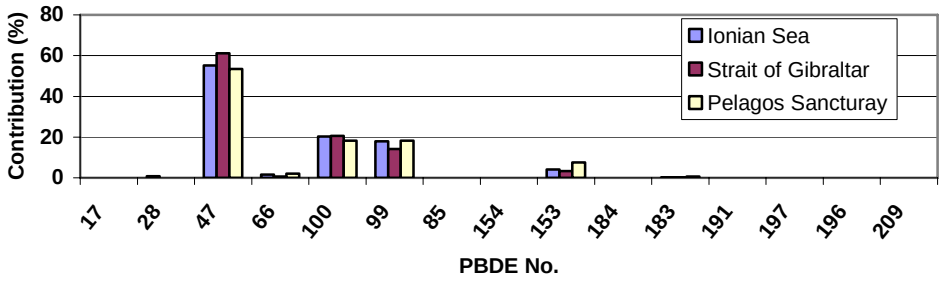

Figure 1. Percentages of contribution of the 15 congeners of BDE to the total BDEs in each sampling area.

To our knowledge, this constitutes the first study dealing with PBDEs in skin biopsies from dolphins inhabiting the Mediterranean Sea. Studies by Tuerk et al.[5] in blubber samples from two dolphin species (*L. acutus* and *S. brenadensis*) stranded on the West Atlantic, found the highest total PBDE (sum 47, 99, 100, 153 and 154) concentrations in juvenile *L. acutus*, being mean concentrations 2.41 ug/g (wet mass). Mean concentrations of PBDEs in the blubber of bottlenose dolphins and striped dolphins from the Florida Coast were 1190 +/- 1580 and 660 ng/g, lipid wt, respectively[6]. Tetra-BDE 47 was the major congener detected in dolphin samples, followed by BDE-99, BDE-153, BDE-100, and BDE-154. With the exception of BDE-154, results were similar to data found in our study. Levels found in this study are slightly higher, compared with the reported data, especially at the Pelagos Sanctuary in Italy, although being a protected area for marine mammals. In addition, PBDE concentrations found in this study are at the same order of magnitude than total PCBs found in cetaceans from the Mediterranean Sea[4]. Our finding agrees with conclusions raised by Rayne et al.[7], concluding that PBDEs must be considered as one of the potentially dominant organohalogen compounds and particularly in the marine environment. Preliminary toxicological studies with this species suggest that PBDEs induce toxic responses in fibroblast cell cultures[8]. In consequence, this important class of emerging pollutants should be regarded as a potential element of concern.

Acknowledgements
Enrique Blázquez and Belén Gómara were supported by I3P Program from the European Community.

References
1. Law R.J., Alaee M., Allchin C.R., Boon J.P., Lebeuf M., Lepom P. and Stern G.A. *Environ. Int.* 2003; 29:757.
2. Fossi MC, Marsili L, Neri G, Casini S, Bearzi G, Politi E, Zanardelli M, Panigada S. Mar Environ Res. 2000; 50:517.
3. Fossi MC, Marsili L, Lauriano G, Fortuna C, Canese S, Ancora S, Leonzio C, Romeo T, Merino R, Abad E, Jiménez B. *Mar Environ Res.* 2004; 58:269.
4. Marsili L., Fossi M.C., Notarbartolo di Sciara G., Zanardelli M., Nani B., Panicada S., Focardi S. *Chemosphere* 1988; 37: 1501.
5. Tuerk K.J.S., Kucklick J.R., Becker P.R., Stapleton H.M., Baker J.E. *Environ. Sci. Technol.* 2005; 39: 692.
6. Johnson-Restrepo B., Kannan K., Addink R., Adams D.H. *Environ Sci Technol.* 2005; 21: 8243.
7. Rayne S., Ikonomu M.G., Ross P.S., Ellis, G.M., Barrett-Lennard L.G. *Environ Sci Technol.* 2004; 38: 4293.
8. Marsili L., Fossi M.C., Jiménez B., Bucalossi D., Porcelloni S., Casini S. *Marine Environmental Res.* 2006; 62, 57.