A COMPREHENSIVE NON-TARGETED SCREENING OF HALOGENATED ORGANIC COMPOUNDS IN DOLPHINS FROM BRAZIL

Alonso MB[1,2,3,4]*, Lailson-Brito J[2], Azevedo A[2], Santos-Neto E[2], Torres JPM[1], Malm O[1], Hoh E[3], Dodder N[4], Maruya K[4]

[1]Radioisotope Laboratory, Biophysics Institute, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho 273 CCS Bl.G, Cidade Universitária, Rio de Janeiro, Brazil; [2]Aquatic Mammal and Bioindicator Laboratory, School of Oceanography, Rio de Janeiro State University, R. São Francisco Xavier, 524, Maracanã, Rio de Janeiro, Brazil; [3]Graduate School of Public Health, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182, USA; [4]Southern California Coastal Water Research Project Authority, 3535 Harbor Boulevard, Suite 110, Costa Mesa, CA 92626, USA

Introduction

Typical environmental monitoring for persistent organic pollutants (POPs) reveals contamination by chemicals that are known in advance (e.g. PCBs, DDTs, chlordanes, PBDEs that are "routinely monitored"), but excludes new and unknown compounds that can bioaccumulate[1]. The lack of knowledge about compounds that exist in the environment and which are not included in monitoring studies includes a range of halogenated organic compounds (HOCs) that may cause potential impacts on human health and wildlife[2]. For many POPs, the original compound no longer plays the dominant role in the environment and their transformation products can become as or even more problematic[2]. Naturally-produced can also bioaccumulate, as is found in cetaceans where they have been reported at similar and/or in some cases higher tissue concentrations compared to POPs targeted in monitoring.[3]

Cetaceans have long been used as sentinel species for assessment of contamination by POPs. The bottlenose dolphin (*Tursiops truncatus*) is a cosmopolitan species, distributed worldwide and has site fidelity in coastal regions[4]. Due to its life history and presence in waters near densely populated areas, it is an excellent sentinel for the study of oceans and human health[5]. A number of recent studies have suggested endocrine disruption, neurotoxicity, immunosuppression and reproductive toxicity associated with HOCs in mammals[6]. The aim of this study was to identify and catalog bioaccumulative HOCs (both anthropogenic and natural) in *T. truncatus* stranded or incidentally caught along the Brazilian coast using a non-targeted analytical approach[1,2].

Materials and methods

Four blubber samples from fatally stranded male *T. truncatus* were collected from the Rio de Janeiro coast, Brazil, between 2004 to 2011. The study area is highly urbanized (metropolitan Rio de Janeiro) and has received discharges of industrial, domestic and agricultural waste effluents since the 1950's. This is one of the most contaminated areas in Latin America, and is considered a global hotspot for PCBs[7]. Only male dolphins were analyzed in this study in order to mitigate bias due to female dolphins transferring their contaminant loads to their progeny[8].

HOCs analysis was performed using previously described non-targeted analytical methods[1,2]. Blubber samples were extracted (3 - 5 g wet weight) by soxhlet (*Pyrex*, USA) with dichloromethane/hexane (1:1 v/v). The extracts were purified by gel permeation chromatography (2 cm i.d. x 22.5 cm, 24 g *S-X3 BioBeads, J2 Scientific*). The samples were injected in a splitless mode to LECO Pegasus 4D GC×GC/TOF-MS in electronic ionization (EI) mode. The instrumental parameters were previously optimized[1]. The isolated chromatographic peaks were examined for identification of their mass spectra.

Data analysis was conducted with LECO ChromaTOF software version 4.43.3. PCB congeners were excluded. For the first blubber sample analyzed (Sample #1), 150 peaks were identified as potential HOCs of interest at a signal-to-noise ratio (S/N) of 50 or higher, out of 7,763 total peaks. These HOCs peaks were used to create a reference data processing method that searched the remaining three samples for HOCs found in Sample #1 (S/N $\geq$ 50), with matching based on retention time, and spectral similarity. This resulted in a total of 170 unique HOCs across all four dolphin samples.

A procedural blank was analyzed along with the blubber samples and was processed with the final reference data processing method to ensure that no library compounds were present. Compound identifications were based on three mass spectral libraries: the 2011 NIST library and the other two from previously published studies by the co-authors conducted on northeastern Pacific and northwestern Atlantic dolphins[1,2]. Statistical analyses were performed and the significance level was defined at $p<0.05$. Non-parametrical tests were used since the data were found to have a non-normal distribution (Shapiro Wilk's W test).

Results and discussion

A total of 28,990 peaks were observed in the four samples, with a per sample mean of 7,248 ± 530. Of these, 501 were identified as HOCs, with a per sample mean of 125 ± 32. These univariate statistics indicated that the number of peaks as well as the number of HOCs detected and identified were relatively consistent across samples. A substantial number of detected HOCs (170) were considered unique (i.e., excluding the duplicate identification in multiple samples), which indicated a different regional profile or "fingerprint" was observed. Of this total, 88 were identified in all samples and suggest that these common HOCs were representative of part of the *T. truncatus* population that frequents the Rio de Janeiro coast. The number of typically monitored and non-monitored HOCs in the blubber of bottlenose dolphin males of Rio de Janeiro coast, Brazil is presented in **Table 1**.

Table 1. Halogenated organic compounds (HOCs) identified in the blubber of male bottlenose dolphin (*Tursiops truncatus*) of Rio de Janeiro coast, Brazil (n=4). The number of typically monitored and non-monitored HOCs and their sources.

Halogenated organic compounds	*typically monitored*	*not-monitored*	*Source*	***Total***
PBDEs	8	5	anthropogenic	**13**
DDTs	5	7	anthropogenic	**12**
OCPs (Organochlorinated Pesticides)	4	1	anthropogenic	**5**
Mirex	1	8	anthropogenic	**9**
MeO-BDE (Methoxy-PBDEs)	0	6	natural	**6**
MeO-PBB (Methoxy-PBBs)	0	1	natural	**1**
MBPs (Methyl bipyrroles)	0	6	natural	**6**
DMBPs (Dimethyl bipyrroles)	0	8	natural	**8**
TCMP (Tris(4−chlorophenyl)methane)	0	2	anthropogenic	**2**
Chlorinated-benzene	1	2	anthropogenic	**3**
Chloro/Bromo-Phenol	0	4	mixed	**4**
PCT (Polychlorinated terphenyl)	0	23	anthropogenic	**23**
PCS (Polychlorinated styrene)	0	2	anthropogenic	**2**
(MeO)-B/CDE (Methoxy Br/Cl diphenyl ether)	0	4	unknown	**4**
Brominated indole	0	2	natural	**2**
Brominated anisole	0	1	natural	**1**
Bromo-pyridinamine	0	1	unknown	**1**
Methylsulfonyl-PCB	0	4	anthropogenic	**4**
Unknown SWA (South Western Atlantic)	0	37	unknown	**37**
Unknown NWA (North Western Atlantic)	0	4	unknown	**4**
Unknown NEP (North Eastern Pacific)	0	11	unknown	**11**
Unknown both lib. (North Atlantic and Pacific)	0	12	unknown	**12**
Total number of compounds	**19**	**151**		**170**

The compounds were separated in 22 classes according to their chemical structure and original technical mixture (Table 1). The sources comprised anthropogenic (included the parent compound, their degradation or by-products and/or metabolites), naturally-occurring (synthesized mainly by bacteria, algae, organisms associated to sponges and another marine invertebrates) or mixed (anthropogenic and natural sources). In relation to HOCs typically monitored in environmental studies (n=19, Table 1 or 11% of the total), 151 compounds are not typically monitored, corresponding to 89% of the total (Table 2). Among those classified as not typically monitored, 54 are emerging anthropogenic HOCs that have not previously been reported in the Southern Hemisphere; 21 are naturally-produced (also that have not been reported in samples from Brazil); and 37 remain unknown. Gribble[9] cited that more than 4,700 naturally-produced HOCs have been discovered, mostly with chlorine and bromine, and this number is expected to increase due to new research. Most derived from marine environments are unique and synthesized by individual organisms.

Table 2. Percentages of HOCs identified in the blubber of bottlenose dolphin (*T. truncatus*) males of Rio de Janeiro coast, Brazil according to their sources.

Sources	%
Unknown	41
Anthropogenic	43
Natural	14
Mixed	2

In grouping the identified HOCs by source, 73 compounds (43%) were classified as anthropogenic; 24 compounds (14%) were of natural origin, including two previously undetected isomers of dimethyl bipyrroles (DMBP-Br_2Cl_4) and 2 new isomers of methyl bipyrrole (MBPs); 4 compounds (2%) have anthropogenic and natural sources; and 69 compounds (41%) remain unknown. Within the unknowns, 12 compounds were found in dolphins from NW Atlantic and NE Pacific, 11 were found in Pacific dolphins, 7 were found in the NW Atlantic and 37 are probably unique to the SW Atlantic (Figure 1).

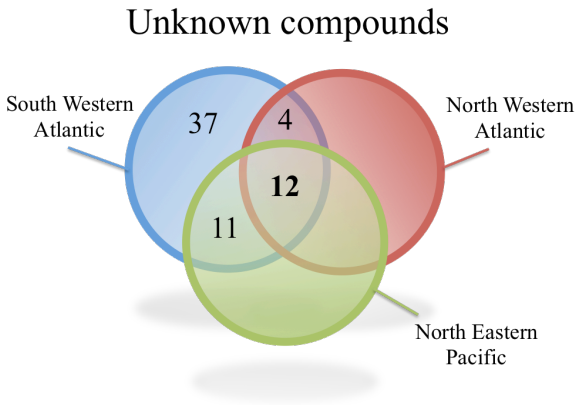

Figure 1. Number of HOCs of unknown structure and/or origin in blubber of male bottlenose dolphins (*T. truncatus*) from coastal Brazil, compared to those obtained from NW Atlantic and NE Pacific dolphins.

There were several classes of anthropogenic HOCs that are not typically monitored represented in the samples analyzed (Table 1). Examples were some PBDEs congeners (n=5), DDT-related and/or possibly new metabolites (n=7), chlordane possibly related to *trans*-nonachlor (n=1), Mirex-related compounds that could have originated from Dechlorane technical mixtures as well as Mirex metabolites (n=8), tri-(4-chlorophenyl)methane (TCPM) and a possible metabolites (n=2), chlorinated benzenes including a possible HCB metabolite (n=2), polychlorinated terphenyls (PCTs) (n=23), polychlorinated styrenes (PCS) (n=2) and methylsulfonyl-PCBs, including possible PCB metabolites or degradation products (n=4). These classes were also found in NE Pacific and NW Atlantic dolphins analyzed previously, showing their ubiquitous presence across three different major oceans[1,2].

For naturally-produced HOCs, we observed 6 isomers of methyl bipyrroles (MBP 7 Cl and MBP 6 Cl), 8 isomers of dimethyl bipyrroles (DMBPs), 6 isomers of methoxy-polybrominated diphenyl ethers (MeO-BDEs), 1 isomer of methoxy-polybrominated biphenyl (MeO-PBB), 2 brominated indoles, 1 brominated anisole, and 2 isomers of methoxy-polychlorinated/brominated diphenyl ethers (MeO-B/CDEs Br_3Cl). The Rio de Janeiro coast is a tropical marine area, with diverse coral and rocky reef fauna and flora, so that it is not surprising to observe a variety of naturally-produced HOCs in our samples. Ocean upwelling is also common in Rio de Janeiro, which can serve to transport HOCs from deeper waters into coastal ecosystems. This is especially important for investigating the occurrence of naturally-produced HOCs along the Brazilian coast.

This work applied non-targeted analysis of marine mammal tissues from the Southern hemisphere to, for the first time, catalog the occurrence of HOCs in blubber samples of *T. truncatus*

that frequent the Rio de Janeiro (Brazil) coast. A rigorous comparison of the HOC fingerprint observed in these animals with non-targeted fingerprints in dolphins from other regions of the world will allow for regional contamination patterns to be documented as well as a better understanding of the sources and origins of naturally-produced HOCs.

Acknowledgements
This work was supported by Brazilian Research Council — CNPq (fellowship to M.B. Alonso Post Doctorate Abroad" - PDE), Rio de Janeiro State Government Research Agency — FAPERJ (Jovem Cientista do Nosso Estado #101.449/2010), Mount Sinai School of Medicine (NY/USA), and Fogarty International Center NIH/USA (grant 1D43TW0640) and the member agencies of SCCWRP. AFA and JL-B have research grant from CNPq (PQ-1D) and FAPERJ (JCNE). The authors also acknowledge also "The Brazilian Program" from San Diego State University (SDSU).

References

1. Hoh, E. *et al.* Nontargeted comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry method and software for inventorying persistent and bioaccumulative contaminants in marine environments. *Environ. Sci. Technol.* **46,** 8001–8008 (2012).
2. Shaul, N. J. *et al.* Nontargeted Biomonitoring of Halogenated Organic Compounds in Two Ecotypes of Bottlenose Dolphins (Tursiops truncatus) from the Southern California Bight. *Environ. Sci. Technol.* **49,** 1328–1338 (2015).
3. Alonso, M. B. *et al.* Anthropogenic (PBDE) and naturally-produced (MeO-PBDE) brominated compounds in cetaceans - A review. *Sci. Total Environ.* **481C,** 619–634 (2014).
4. Fruet, P. F. *et al.* Remarkably low genetic diversity and strong population structure in common bottlenose dolphins (Tursiops truncatus) from coastal waters of the Southwestern Atlantic Ocean. *Conserv. Genet.* 879–895 (2014). doi:10.1007/s10592-014-0586-z
5. Kucklick, J. *et al.* Bottlenose dolphins as indicators of persistent organic pollutants in the western North Atlantic Ocean and northern Gulf of Mexico. *Environ. Sci. Technol.* **45,** 4270–7 (2011).
6. Villanger, G. D. *et al.* Disruptive effects of persistent organohalogen contaminants on thyroid function in white whales (Delphinapterus leucas) from Svalbard. *Sci. Total Environ.* **409,** 2511–24 (2011).
7. Lailson-Brito, J. *et al.* High organochlorine accumulation in blubber of Guiana dolphin, Sotalia guianensis, from Brazilian coast and its use to establish geographical differences among populations. *Environ. Pollut.* **158,** 1800–8 (2010).
8. Alonso, M. B. *et al.* Pyrethroids: a new threat to marine mammals? *Environ. Int.* **47,** 99–106 (2012).
9. Gribble, G. W. *Naturally Occurring Organohalogen Compounds - A Comprehensive Update*. (Springer, 2010).