Distribution and profile of polybrominated diphenyl ethers in European eel (Anguilla anguilla) from a Mediterranean coastal lagoon (Orbetello, Italy)

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Introduction

Brominated flame retardants (BFRs) include a large number of different types of brominated substances. Many BFRs are persistent and have been detected in environmental samples, raising concern about the biological/toxicological risk associated with their use. Polybrominated diphenyl ethers (PBDEs) are a class of BFR, produced commercially in mixtures and characterized as persistent and highly lipophilic. They share some of the same mechanisms of toxicity, in particular on endocrine systems with the well-known polychlorinated biphenyls (PCBs)[1]. They accumulate in the environment and concentrate in biological systems. As no current information is available on occurrence, distribution and profile of PBDEs in Italian brackish waters PBDEs were determined in European eels *(Anguilla anguilla)* from Orbetello lagoon (Tuscany, Italy). This lagoon is of enormous scientific interest because of the variety of its environments and the richness of its flora and fauna, despite the fact that it is threatened by increasing pollution and degradation due agriculture, aquaculture and urban effluent[2]. The decline in eel wild stock and fishery throughout the world has been acknowledged by the scientific community since the early 1980's and although the causes are still unknown, pollution by domestic and industrial effluent is though to affect the reproductive biology and physiology of eels[3].

Materials and Methods

Sampling was conducted in June 2002 in Orbetello lagoon that covers an area of a 2700 ha on the southern coast of Tuscany (42°30'N, 11°10'E) (Fig. 1). At each of the seven sampling sites, 15 yellow eels were captured and shipped to the laboratory in oxygenated coolers with aerated collection-site water. Immediately after sacrifice, the specimens were individually weighed and measured. A portion of dorsal fillets was excised, flash frozen and stored at –20° for chemical analysis.

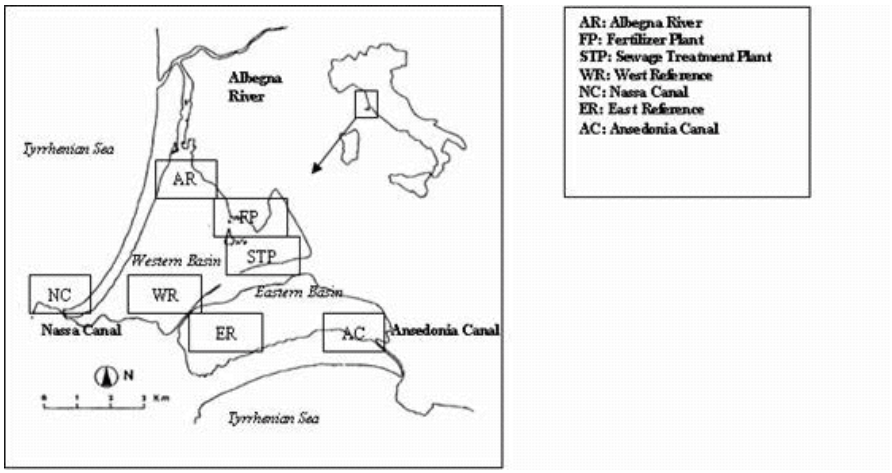

Figure 1: Map of the Orbetello lagoon showing eel sampling site.

PBDEs were extracted according to Kannan *et al.*[4]. A GC/MS (ion trap mass detector) from ThermoFinnigan (Trace^{TM} GC 2000/GCQ Plus) operating in MS/MS mode, was used. Samples were separated in a Resteck RTX-5MS capillary column (30 m x 0.25 mmi.d., 0.25 µm).A Wellington Laboratories Inc. solution containing 17 PBDEs in

nonane was used as calibration standard. PCB-141 ($^{13}C_{12,}$ 99%) in isooctane, supplied by Cambridge Isotope Laboratory, was used as internal standard. A blank sample, prepared using the same procedure as for the samples, was included every five samples. PBDE-99, -153 -154 and -156 were detected in the blanks. For the other compounds, the analytical detection limit was based on a signal-to-noise ratio of 3. Limits of detection (LOD) of PBDEs, calculated as mean blank +3SD, was 0.7 pg/µl = 0.9 ng/g lipid [5].

Results and discussion

The results reported in Table 1 showed heterogeneous PBDE contamination in eels collected from the different sites in Orbetello lagoon. Eels collected near the former fertilizer plant (site FP) and sewage treatment plant (site STP) had the highest PBDE levels, higher than those observed in eels from the River Elbe (Germany) (median values: 6.3 ng/g l.w.)[6]and than values reported by Akutsu *et al.*[7] in eels from the inland Sea of Seto (Japan). However the average total concentration of PBDEs in eels from the lagoon was rather low compared to the literature: Eljarrat *et al.*[8] found total PBDE concentrations ranging from 0.1 to 436 ng/g w.w. in barbel (*Barbus graellsi)* muscle tissue from the Spanish Cinca River (a heavily industrialized area) while Andersson and Blomkvist[9] reported levels of 17,000 ng/g l.w. in muscle of eels from south-west Sweden, suggesting that there was a local source of pollution along the River Haggan.

Table 1: Mean concentration and standard deviation (S.D.) of PBDEs (ng/g l.w.) in muscle tissue of European eels captured in Orbetello lagoon.

Sampling sites	No. of eels	mean weight (SD) (g)	mean length (SD) (cm)	% lipid (SD)	mean conc. (SD) (ng/g l.w.)
NC	15	35 (14.1)	30.4 (3.4)	3.5 (0.7)	1.7 (1.3)
FP	15	27.5 (3.5)	26.8 (1.8)	3.5 (0.7)	20.9 (9.6)
AR	15	63.4 (27)	34.2 (4.5)	10 (7.1)	1.2 (0.2)
STP	15	36.3 (8.8)	29 (2.8)	3.5 (2.1)	14.1 (10.3)
WR	15	121.3 (19.4)	42.1 (2)	14 (5.7)	4.4 (1.4)
ER	15	82.2 (21.6)	30.4 (12.2)	6 (5.7)	5.6 (4.1)
AC	15	57.9 (14.7)	33.1 (2.4)	9 (5.7)	< LOD (0.9 ng/g l.w.)

Seventeen congeners were identified in the PBDE residue. The most abundant congeners in samples included 2,2',4,4'-tetrabromodiphenylether (BDE-47), 2,2,4,5'-tetraBDE (BDE-49), 2,2',4,4',5-pentaBDE (BDE-99), 2,2',4,4',5,5'-hexaBDE (BDE-153) and 2,3,3',4,4',5-hexaBDE (BDE-156) (Fig. 2). In our study, not all congeners could be detected in every sample, as also found by Lepom *et al.* [6] but BDE-47, BDE-49, BDE-99 and BDE-156 were found in 80% of eels analyzed. As reported in other countries, the dominant congener was BDE-47 in eels from FP, STP, WR and ER sites. In fact, it is a major component of Bromkal 70-5DE which was produced and used in many European countries [10]. De Boer [11] reported concentrations of BDE-47 in eels captured in Dutch freshwater environments. It was found to be the main PBDE component and its concentration ranged from < 20 to 1700 ng/g l.w.

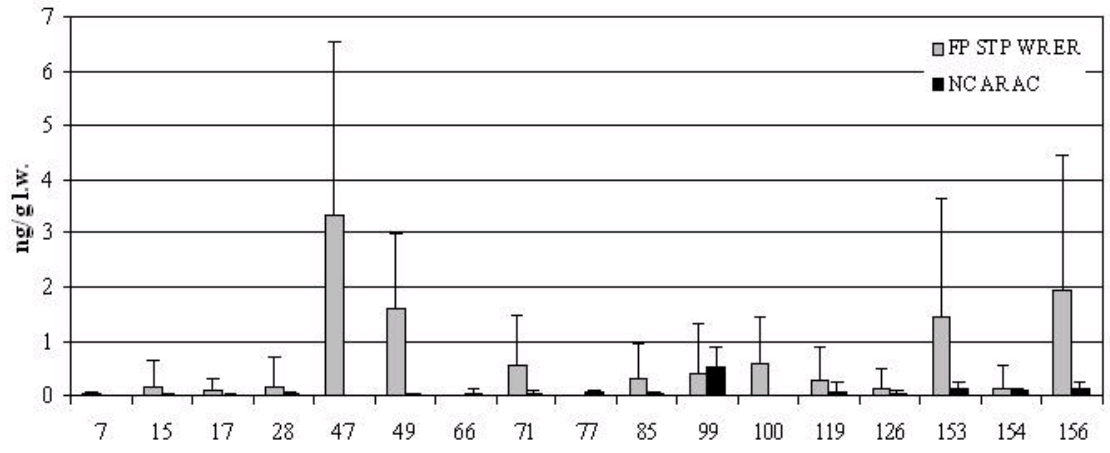

Fig. 2: Patterns of bioaccumulation of individual PBDEs in European eels from Orbetello lagoon.

BDE-47 has the highest biomagnification factors (BMFs) as verified by Andersson *et al.*[12] in zebrafish *(Danio rerio*). Moreover, BDE-99 is biotransformated to BDE-47 in the intestinal tract of the common carp *(Cyprinus carpio),* as found by Stapleton *et al.* [13], leading to appreciable accumulation of the less brominated congener. Although BDE-99 is the major component (35%) of Bromkal 70-5DE mixture together with BDE-47 (37%) [14], it was as low as BDE-100 in samples from FP, STP, WR and ER sites. Its effective molecular cross-section (EMCS) is bigger than that of BDE-100 and it is therefore less bioavailable to organisms since it has greater difficulty crossing cell membranes [15]. On the other hand, BDE-99 predominated in eels from NC, AR and AC sites. This different PBDE profile, may be related to low metabolization of BDE-99 to BDE-47 in eels from the above sites, as well as to recent exposure of these eels to PBDEs. In all eels, BDE-28 was detected in very low levels as in eels from the River Elbe Lepom *et al.* [6]. It is only 0.11% of the total BDEs in Bromkal 70-5DE [14]. The isomer profiles observed for eels were different from that of the commercial pentaBDE mixture. TetraBDEswere the most abundant isomers in samples from FP, STP, WR and ER sites followed by hexaBDEs and pentaBDEs (Fig. 3). On the other hand pentaBDEs, followed by hexaBDEs and tetraBDEs, predominated in eels from NC, AR and AC sites. These isomer profiles were slightly different from those reported in other fish. In salmon from lake Michigan the relative amounts of PBDE isomers were 56% for tetraBDEs, 21% for pentaBDEs and 10.2% for hexaBDEs[16] while in carp *(Cyprinus carpio)* and large mouth bass (*Micropterus salmoides)* from Detroit River (USA) isomer percentages were: 53-56% for tetraBDEs, 18% for pentaBDEs and 17% for hexaBDEs[17]. Differences in BMFs among PBDE congeners [12] and biotransformation processes [13] may explain these trends.

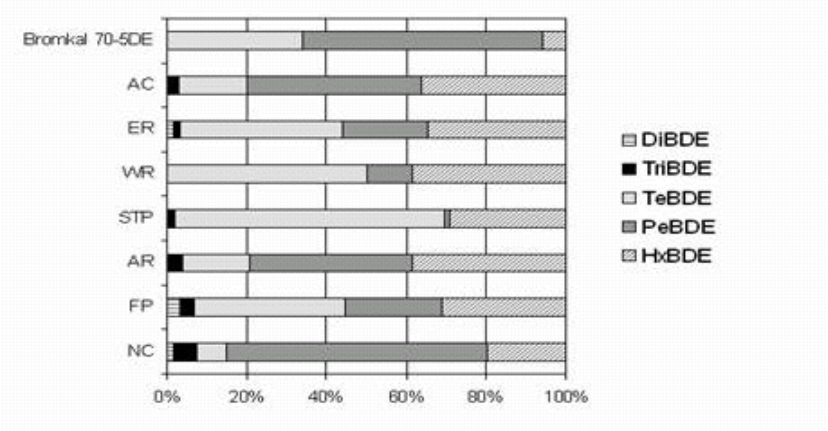

Fig. 3: Percentages of isomer classes in Bromkal 70-5DE and in eels from the sampling sites.

The present study provides new information on levels of PBDE pollution in European eels from Italian waters. Two hot spots were identified in Orbetello lagoon, one near the former fertilizer plant and the other at the sewage treatment plant outlet. Levels throughout the lagoon were nevertheless quite low, suggesting a complete absence of local sources of PBDEs. However measurable concentrations of PBDEs (from di- to hexaBDEs) in muscle of European eels and lack of information on the effects of PBDEs in the aquatic environment suggest the need for further research to clarify the impact of PBDEs on the European eel.

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References

1. Legler, J. and Brouwer, A. (2003) *Environ. Int.* 29: 879-885.
2. Corsi, I., Mariottini, M., Sensini, C., Lancini, L., Focardi, S. (2003) *Oceanol. Acta* 26: 129-138.
3. Robinet, T. and Feunteun, E. (2002) *Ecotoxicology* 11: 265-277.
4. Kannan, K., Hilscherova, K., Yamashita, N., Williams, L.L. and Giesy, J.P. (2001) *Environ. Sci. Technol.* 35: 441-447.
5. Keith, L.H. (1991) Environmental Sampling and Analysis: a Practical Guide, Lewis Publishers, 143.
6. Lepom, P., Karasyova, T. and Sawal, G. (2002) *Organohalogen Compounds* 58: 209-212.
7. Akutsu, K., Obana, H., Okihashi, M., Kitagawa, M., Nakazawa, H., Matsuki, Y., Makino, T., Oda, H., Hori, S (2001) *Chemosphere* 44: 1325-1333.
8. Eljarrat, E., de la Cal, A., Barcelò, D. (2003) *J. Chromatog. A* 1008: 181-192.
9. Andersson, O. andBlomkvist, G. (1981) *Chemosphere* 10: 1051-1060.
10. WHO (1994), Environmental Health Criteria 162. Brominated diphenyl ethers, International Program on Chemical Safety, World Health Organization.
11. de Boer, J. (1990) *Organohalogen Compounds* 2: 315-318.
12. Andersson, P. L., Blom, A., Johannisson, A., Pesonen, M., Tysklind, M., Berg, A.H., Olsson, P.-E. , Norrgren, L. (1999) *Arch. Environ. Contam. Toxicol.* 37: 145-150.
13. Stapleton, H.M., Letcher, R.J. and Baker, J.E. (2004) *Environ. Sci. Technol.* 38: 1054-1061.
14. Sjodin, A., Jakobsson, E., Kierkegaard, A., Marsh, G., Sellstrom, U. (1998) *J. Chromatogr. A* 822: 83-89.
15. Boon, J.P., Booij, K., Lewis, W.E., Zegers, B.N. (2000) in: De Boer J., Leonards P.E.G., Boon J.P., Law R.J. (Eds.), BSEF workshop on polybrominated diphenyl ethers. The Netherland Institute for Fisheries Research (RIVO), Ijmuiden, The Netherlands.
16. Manchester-Neesvig, J.B., Valters, K. and Sonzogni, W.C. (2001) *Environ. Sci. Technol.* 35: 1072-1077.
17. Rice, C.P.,Chernyak, S. M., Begnoche, L., Quintal, R. and Hickey, J. (2002) *Chemosphere* 49: 731-737.