Environmental Research 109 (2009) 851–856

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Polychlorinated biphenyl and organochlorine pesticide contamination signatures in deep-sea fish from the Mediterranean Sea

M.M. Storelli [a,*], S. Losada [b,c], G.O. Marcotrigiano [a], L. Roosens [b], G. Barone [a], H. Neels [b], A. Covaci [b,d]

[a] Pharmacological–Biological Department—Chemistry and Biochemistry Section, Veterinary Medicine Faculty, University of Bari-Strada Prov. le per Casamassima Km 3, 70010 Valenzano (Ba), Italy
[b] Toxicological Center, University of Antwerp, Univeriteitsplein 1, 2610 Wilrijk, Belgium
[c] Analytical Chemistry Department, University of Barcelona, Martí i Franqués 1-11, E-08028 Barcelona, Spain
[d] Laboratory of Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

ARTICLE INFO

Article history:
Received 16 March 2009
Received in revised form
13 July 2009
Accepted 20 July 2009
Available online 13 August 2009

Keywords:
Deep-sea fish
Polychlorinated biphenyls
Organochlorine pesticides
Hexaclorobenzene
PCB-TEQs

ABSTRACT

Polychlorinated biphenyl (PCB) and OCP concentrations were determined in the livers of two deep-sea fish species, roughsnout grenadier and hollowsnout grenadier, from the Adriatic Sea. In both species, contaminant concentrations were in the following order: PCBs > DDTs > > HCB. Contaminant load was higher in roughsnout grenadier (PCB: 12,327 ng g^{-1}; DDTs: 5357 ng g^{-1}; HCB: 13.1 ng g^{-1}) than in hollowsnout grenadier (PCB: 1234 ng g^{-1}; DDTs: 763 ng g^{-1}; HCB 6.3 ng g^{-1}). PCB patterns were dominated by higher chlorinated congeners (hexa-CBs: 50.3–52.1%, hepta-CBs: 29.6–35.5%, penta-CBs: 8.0–11.1% and octa-CBs :5.2–5.4%). PCBs 138, 153 180 and 187 were the most abundant. Regarding the DDT pattern, *p,p′*-DDE was prevalent in both species (roughsnout grenadier: 99.7%, hollowsnout grenadier: 90%), suggesting no recent DDT input. In both species, the total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalent (TEQ) concentrations (roughsnout grenadier: mean 43.77 pg/g, hollowsnout grenadier: mean 20.49 pg/g), calculated from non- and mono-*ortho* PCBs, reached those encountered in marine organisms at higher levels in the trophic chain.

1. Introduction

Liphophilic persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (DDTs) and hexachlorobenzene (HCB), are chemicals of the greatest concern owing to their persistence in environment and the fact that they are readily bioaccumulated and highly toxic for living organisms. Up to 70% of all organochlorinated compounds entering the environment are concentrated in the world's oceans (Tanabe, 1985). Transported by living biota or dead biota or absorbed to the detritus, these hydrophobic pollutants can reach the sea floor. From this final resting ground, they can be resuspended by physical mixing or by the activity of bottom-dwelling organisms and bioaccumulate as one moves up the food chain. Consequently, the deep environment not only will not be free from these pollutants but may even act as a sink for them, to the point that restrictive regulations for the use and disposal of these chemicals, which have resulted in a significant decrease of source inputs in recent decades, may still not be recognizable in these remote areas (Ramu et al., 2006). Different researches have continued to reveal that contamination in deep-sea biota is more elevated than surface living species, highlighting transport processes of POPs to the deep-sea environments. For example, Froescheis et al. (2000) and Looser et al. (2000) showed that the PCB levels in bottom dwellers at depths greater than 800 m were between 10 and 17 times higher than in the related surface species. Similarly, Kramer et al. (1984) find an enrichment of up to 600 times for DDT in Atlantic deepwater fish relative to surface living species. Nevertheless, there is a paucity of information regarding exclusively the feature of organochlorine contamination in deep-sea biota (Berg et al., 1997, 1998; Froescheis et al., 2000; de Brito et al., 2002a–c; Mormede and Davies, 2003), probably as a combination of the difficulty in obtaining samples and the low commercial interest of these fish species. The scarcity of data extends also to the Mediterranean basin (Solé et al. 2001; Storelli et al., 2004a, 2007), although it has been often reported that this marine area is heavily contaminated (Meadows, 1992; Kuetting, 1994; Borrell et al., 1997), as a consequence of its particular hydrographical characteristics and high anthropogenic pressure. However, many efforts have attempted to protect this delicate and complex ecosystem. The Barcelona Convention for the Protection of the Mediterranean Sea, including the Mediterranean Action Plan (MAP) and the Mediterranean Marine Pollution Monitoring and Research Program (MED POL), has encouraged the implementation of monitoring programs for evaluating the health status of this water body. Recently, United Nations Environment

---

* Corresponding author. Fax: +39 805443863.
E-mail address: m.m.storelli@veterinaria.uniba.it (M.M. Storelli).

0013-9351/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2009.07.008

Program (UNEP) produced a regionally based assessment of sources, environmental levels, transport pathways and effects of persistent toxic substances in the environment, considering the Mediterranean Sea as one of the regions for the study (UNEP, 2002, 2003). In this renewed political context, the monitoring of PCBs, DDTs and HCB in the deep-sea environment of the Mediterranean basin remains of crucial importance not only to fill knowledge gaps on the state of its chemical contamination, but also to produce baseline data for taking decisions regarding future issues of environmental conservation.

Taking the above into account, this manuscript, which represents a component of a broader investigation including other environmental pollutants such as polybrominated diphenyl ethers (PBDEs; Covaci et al., 2008) and heavy metals (Storelli et al., in preparation), determines the occurrence and potential impact of PCBs, DDTs and HCB in two deep-sea fish species, *Trachyrincus trachyrincus* and *Coelorhinchus coelorhynchus*, collected in the Mediterranean Sea. Particular attention has been paid to the seven "target" PCBs (congeners 28, 52, 101, 118, 138, 153 and 180) recommended for PCB monitoring by international organizations (e.g. European Union, International Council for the Exploration of the Sea (ICES), World Health Organization) and to the "dioxin-like" PCBs (non- and mono-*ortho* PCBs) useful in the assessment of ecotoxicological impact.

2. Materials and methods

2.1. Sample collection

Specimens of *Trachyrinchus trachyrinchus* (roughsnout grenadier; specimen number: 307, length: 35.0–49.3, average: 40.60 ± 4.70) and *C. coelorhynchus* (hollowsnout grenadier; specimen number: 203, length: 5.10–8.70 cm, average: 6.70 ± 1.30) were caught along the Apulian coast from Trani to Castro (about 200 km) in the Southern Adriatic Sea (Mediterranean Sea) between June and September 2006. The sampling sites (Fig. 1) were selected based on the geographical features of the Adriatic Sea, which generally presents shallow waters except for a deep depression along the Apulian coast (300–1200 m). Species choice was based on their availability at the sampling location. The analyses were conducted in composite samples. From the total number of specimens, pools were formed (hollowsnout grenadier: no. 6; roughsnout grenadier: no. 9) within which individual fish were gathered as a function of their similar size. From fish of each pool, liver was taken, homogenized and kept in deep freeze at −20 °C until chemical analysis.

2.2. Organochlorine compound

The following PCB congeners (no. 18, 28, 31, 44, 47, 49, 52, 74, 77, 87, 95, 99, 101, 105, 110, 118, 126, 128, 132, 138, 146, 149, 151, 153, 156, 158, 169, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 196/203, 199, 205, 206 and 209), together with the DDT compounds (*p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE and *o,p′*-DDD) and HCB were investigated using previous methods (Covaci et al., 2002; Voorspoels et al., 2004).

Briefly, approximately 0.6 g of pooled fish liver was grinded with Na_2SO_4 and spiked with 20 ng of PCB 143 used as internal standard. Samples were extracted for 2 h by hot Soxhlet with a mixture of acetone/hexane (1/3, v/v). The extract was evaporated and cleaned by passing through 8 g of acid silica (H_2SO_4, 44% w.w.), using 50 mL of a mixture of hexane/dichloromethane (1/1, v/v) for elution of the analytes. The eluate was evaporated to dryness and redissolved in 100 μL of iso-octane. For the separation of non-*ortho* PCB congeners (no. 77, 126 and 169) from other PCBs, the method reported by Tanabe et al., (1987), involving fractionation on 125 mg of activated carbon (434455 C. Erba, Milano, Italy), was used.

2.3. High-resolution gas chromatography–mass spectrometry (GC–MS)

For the analysis of PCBs, an Agilent 6890 GC connected with an Agilent 5973 MS operated in electron impact ionization (EI) mode was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The MS was used in the SIM mode with two ions monitored for each PCB homolog group or pesticide in specific windows. One microliter of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 700 °C/min) for splitless time 1.50 min, pulse pressure time 1.50 min and pressure pulse 25 psi. Helium was used as the carrier gas at constant flow (1.0 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min, further increased to 280 °C at a rate of 5 °C/min, further increased to 300 °C at a rate of 40 °C/min and held for 7 min.

2.4. Quality assurance and quality control

QA/QC was performed through the analysis of procedural blanks, a duplicate sample and a standard reference material (SRM 1945, PCBs and OCPs in whale blubber) for each set of 10 samples. For the replicate and SRM 1945, the relative standard deviations (RSDs) were $<10\%$ for all the detected compounds. Additionally, the method performance was assessed through successful participation to interlaboratory studies organized by NIST (NIST/NOAA Interlaboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissues). Obtained values were deviating with less than 20% from the consensus values. Multi-level calibration curves ($r^2 > 0.999$) in the linear response interval of the detector were created for the quantification. The calibration curves were prepared to result in a range of 2–10,000 ng g^{-1} lipid weight for *p,p*-DDE, PCB 153, PCB 138 and PCB 180 and in a range of 2–2000 ng g^{-1} lipid weight for the other PCB congeners. The identification of target analytes was based on the relative retention times (RRTs) to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. A deviation of the ion intensity ratios within 20% of the mean values obtained for calibration standards was considered acceptable. For analytes detected in the procedural blanks (PCBs 101, 138, 153 and 180), the mean value of each analyte in the procedural blanks was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3*SD of the value obtained in the procedural blanks. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. LOQs for individual PCBs and OCPs ranged between 1 and 2 ng g^{-1} lipid weight. Concentrations of individual compounds below LOQ were substituted with a value equal to zero. The RSD value below 3.6% for all pesticides and PCB congeners shows the good repeatability of the method used. Concentrations of PCBs, DDTs and HCB means of 2 replicate measurements are presented as ng g^{-1} on a lipid weight basis.

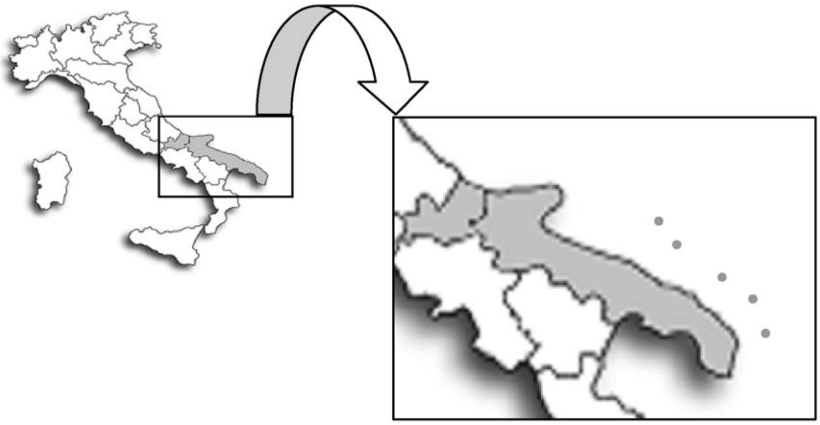

Fig. 1. Location of sampling stations along the southeast coast of Italy.