Environment International 37 (2011) 354–364

Contents lists available at ScienceDirect

Environment International

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

What can we learn from monitoring PCBs in the European eel? A Belgian experience

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ARTICLE INFO

Article history:
Received 16 July 2010
Accepted 12 October 2010

Keywords:
European eel
PCBs
Bioaccumulation
Human intake
Monitoring network
Flanders

ABSTRACT

Between 2000 and 2007 pooled muscle tissue samples of the European eel (*Anguilla anguilla*) from 48 sites in Flanders (Belgium) were analysed for 30 polychlorinated biphenyl (PCB) congeners. There was a large variation between individual sites (range 11–7752 ng/g wet weight (ww) for the sum of the ICES 7 PCBs), eels from the River Meuse basin (mean 1545 ng/g ww) being considerably more polluted than those from the River Scheldt (615) and IJzer (61) basins. Overall, PCB 153, PCB 138 and PCB 180 were the most prominent congeners, however PCB patterns varied between the monitored locations. Analysis of the weight percentage of congeners demonstrates obvious differences in PCB composition between sites, indicating differential sources of pollution. Due to the variation in patterns, atmospheric fallout does not seem to be the main source of the PCB spread, but instead both local and upstream sources linked to industrial activities seem to be the main cause for PCB presence in Flanders.

Considering the levels of the Sum 7 PCBs, eels are not compliant with the Belgian legal limits for consumption (75 ng/g ww) in 71% of the sites. Regular consumption of eels from polluted sites leads to a considerable excess of the WHO Acceptable Daily Intake value. Consumption of wild eels should by all means be prevented, as it presents risks for human health, especially for local anglers consuming their catch.

1. Introduction

The production of polychlorinated biphenyls (PCBs) was banned in North America in 1977 and in most European countries in the 1980s. However, these toxic compounds are omnipresent in our environment and continue to threaten human and animal life. Introduced around 1930, PCBs were commercially available (under various trade names such as *Aroclor*, *Clophen* and *Phenoclor*) as mixtures of multiple isomers with different degrees of chlorination. They were used for a variety of applications, such as dielectric fluids for capacitors, transformers, but also as lubricating oils and additives. PCBs have entered the environment through both use and disposal. They are persistent, bioaccumulating and highly lipophilic, which facilitates their uptake in aquatic and benthic biota.

The first biomonitoring studies date back from the 1970s as Jensen et al. (1969) detected PCBs in eagles, herring and other environmental samples from Sweden. Numerous reports followed, indicating their ubiquitous presence in humans, animals and a variety of other environmental matrices. Detrimental effects of PCBs on human health, such as acute toxic effects (e.g. chloracne; Erickson, 2001) were observed as early as 1936. Due to their ban, environmental levels of PCBs tended to decrease as reported for rural vegetation and air in the UK (Jones et al., 1992), human breast milk in Sweden (Norén and Meironyté, 2000), as well as in eel muscle (*Anguilla anguilla*) sampled between 1994 and 2005 in a monitoring network of 365 sites over Flanders (Belgium) (Maes et al., 2008).

Despite an overall decrease in PCB levels, current environmental levels have stabilized in the last years and remain a matter of serious concern due to their continuous accumulation in the human body (Schroijen et al., 2008), mainly through dietary intake (Baeyens et al., 2007). PCBs have been recognised as endocrine-disrupting chemicals (Soontornchat et al., 1994), and evidence is presented that PCBs continue to affect human and animal health. In humans, a variety of toxicological effects have been described, including several developmental effects which were documented from studies with cohorts of fish eaters (Jacobson and Jacobson, 2001). Dhooge et al. (2010) reported that body size (body mass index) in adolescents and adults is associated to their current internal exposure level of PCBs. Multiple reports are available documenting detrimental effects of PCBs on fish, amphibians, reptiles, birds and mammals (Bernanke and Köhler, 2009).

Recently, it has been suggested that PCBs and other dioxin-like chemicals play an important role in the actual decline of the European eel (van Ginneken et al., 2009; Belpaire et al., 2009; Geeraerts and Belpaire, 2010), although causative relationships between PCB

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0160-4120/$ – see front matter
doi:10.1016/j.envint.2010.10.006

exposure and effects on population level are difficult to demonstrate, considering the complex life cycle of this panmictic catadromous species.

Flanders, the northern part of Belgium, constitutes an area of 13 522 km^2, with a dense population (439 inhabitants/km^2), intensive agricultural and industrial activities, and hence considerable environmental pressure. Since the Belgian dioxin crisis in 1999 (Bernard et al., 1999; Covaci et al., 2008), public awareness for PCBs has grown considerably. The Flemish environmental report (Wevers et al., 2007) recognizes pollution by PCBs as a chemical of major concern. Previously, it has been demonstrated that PCB body burdens in Flemish eels attain high levels (Maes et al., 2008; Belpaire, 2008).

Here, we report on the status of PCBs throughout the aquatic environment in Flanders through monitoring in feral eel, in order to document the spatial variation in intensity and composition of these highly toxic compounds. Spatial analysis of the intensity and profiles of an extensive set of 30 PCB congeners will give insight in the mechanism regulating mobility and spreading of PCBs, hence helping policy makers, not only those involved in the management of hazardous compounds in our environment, but also those active in human health risk management and related epidemiological studies. PCBs have been included in many international directives aiming at their reduction or elimination from the environment. The results of this study also support (inter)national eel management and hopefully will help to understand the current threats the European eel is facing.

2. Materials and methods

2.1. Sampling yellow eel

This study was carried out in Flanders (Belgium), where eels were collected in 48 locations distributed over the catchments of the rivers IJzer (8 sites), Scheldt (21) and Meuse (13) and in canals around Bruges and Ghent (6) in the period 2000–2007 (Fig. 1). Sites differed in typology and included rivers (19 sites), canals (24) and closed waters (5). To avoid effects of possible variation in body burden of individual eels from a particular site due to variation in size, sex or age, we aimed to analyse pooled samples from 10 individuals per site. This objective could not be met at all sites as for practical reasons it was not possible to obtain 10 eels from each site. At each location, 3–10 individuals were collected through electrofishing and fykenetting, all of them being sedentary, yellow staged eels with their total length ranging from 31 to 88 cm. We refer to Belpaire et al. (2000) for a detailed description of fishing techniques. Table 1 gives extensive information on the location and the morphometrics of the sampled eels. Depending on the number of individuals obtained from each site, 1 to 4 g of muscle tissue from each eel was taken and pooled. Subsequently, each pool was analysed for 30 PCB congeners. The same samples also were analysed for brominated flame retardants and results were reported elsewhere (Roosens et al., 2010).

2.2. Analytical methods

2.2.1. Sample analysis

The following PCB congeners (IUPAC numbering) were targeted for analysis: 18, 28, 31, 44, 49, 52, 74, 87, 95, 99, 101, 105, 110, 118, 128, 138, 149, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195, 199, 206 and 209. CB 143 was used as the internal standard for the quantification of PCBs. All individual PCB standards were obtained from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). All solvents used for the analysis (acetone, dichloromethane, *iso*-octane, *n*-hexane, methanol) were of SupraSolv® grade (Merck, Darmstadt, Germany). Sodium sulphate (Merck) and silica gel (0.063–0.200 mm, Merck) were pre-washed with *n*-hexane and heated overnight at 150 °C before use. Extraction thimbles (25 × 100 mm, Whatman®, England) were pre-extracted for 1 h with hexane/acetone (3/1; *v*/*v*) and dried at 100 °C for 1 h. Empty polypropylene columns for clean-up (25 ml) were purchased from Alltech (Lokeren, Belgium).

The method used for sample extraction and clean-up has been previously described and validated (Voorspoels et al., 2004). Briefly, a homogenised sample of approximately 1 g pooled eel muscle was weighed, mixed with anhydrous Na_2SO_4 and spiked with 40 ng of internal standard (CB 143). Further, the samples were extracted for 2 h by hot Soxhlet (Büchi, Flawil, Switzerland) with 100 ml hexane/acetone (3:1, *v*/*v*). The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was cleaned on ~8 g acidified silica and successively eluted with 20 ml hexane and 15 ml dichloromethane. The cleaned extract was concentrated to approximately 2 ml using a rotary-evaporator and further to near dryness under a gentle nitrogen stream. The dried extract was reconstituted in 100 µl *iso*-octane and analysed for PCBs using gas chromatography–mass spectrometry (GC–MS) with electron impact ionization (EI).

An Agilent 6890 GC–5973 MS system operated in 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. One µl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) rising to 300 °C with 700 °C/min), pressure pulse 25 psi and pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as the carrier gas at constant flow (1.0 ml/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (kept for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, and kept for 20 min. The MS was used in the selected ion-monitoring (SIM) mode with 2 ions monitored for each PCB homologue group. Dwell times were set to 30 ms.

Samples with concentrations below LOQ were calculated as f*LOQ with "f" being the fraction of samples above LOQ (or the detection frequency). All results were expressed as ng/g wet weight (ww). Total PCB level is indicated as Sum 30 PCBs and totals the 30 PCB congeners previously mentioned. Seven congeners are considered as indicator PCBs (28, 52, 101, 118, 138, 153, 180) and their sum is commonly used in European countries to report PCB contamination. This is further abbreviated as Sum 7 PCBs.

2.2.2. Quality assurance

Multi-level calibration curves were created for the quantification, and good linearity ($r^2 > 0.999$) was achieved for tested intervals which included the whole concentration range found in samples. The area ratio between the analyte and internal standard was plotted against the corresponding absolute amount ratio. The analyte identification was based on their relative retention times to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions for GC–MS. A deviation of the ion intensity ratios within 20% of the mean values of the calibration standards was considered acceptable. The analytical procedures were validated through the regular analysis of procedural blanks, duplicate samples, recovery monitoring of spiked samples and analysis of certified material SRM 1945 (PCBs in whale blubber). Obtained values did not deviate more than 10% from the certified values and all samples were blank-corrected. The quality control scheme was also assessed through regular participation in interlaboratory comparison exercises organized by Arctic Monitoring Assessment Programme (AMAP) and the US National Institute for Standards and Technology (NIST), for which the obtained values did not vary more than 15% from the target values. Mean recovery of IS was 89% (SD 8%). For each analyte, the mean procedural blank value was used for subtraction. The method limits of quantification (LOQs) were calculated as 3 × SD of the procedural blanks, taking into account the amount of sample taken for