DIOXIN-LIKE COMPOUNDS IN PORPOISES AND SEALS FROM THE SOUTHERN NORTH SEA: RELATIONSHIP WITH BIOLOGICAL AND ECOLOGICAL FACTORS

Krishna Das[1], Edwin De Pauw[2], Gauthier Eppe[2], Anne-Cécile Massart[2], Virginie Debacker[2], Thierry Jauniaux[3] and Jean-Marie Bouquegneau[1]

[1]MARE Center, Laboratory for Oceanology, Liege University, B6C, 4000 Liege Belgium
[2]Mass Spectrometry Laboratory, Liege University, B6C, 4000 Liege, Belgium
[3]MUMM Gulledelle, 100, B-1200 Brussels, Belgium

Introduction

The North Sea represents a major ecosystem for the harbour porpoise (*Phocoena phocoena*) and the harbour seal (*Phoca vitulina*). The grey seal (*Halichoerus grypus*) occurs more occasionally in the southern part of the North Sea. Their population over this last decade has experienced major fluctuations likely linked to prey availability and seal epizootics[1, 2]. Despite being banned more than 30 years ago, levels of polychlorinated biphenyls (PCBs) in marine mammals are still of concern due to historical contamination of the North Sea. PCBs in harbour porpoises were recently linked to poor body condition[3], thymus atrophy[4] and thyroid disruption[5]. Certain non-*ortho* and mono-*ortho* PCBs and other chlorinated chemicals with comparable structural and biochemical properties are considered "dioxin-like compounds" and can act similarly in terms of dioxin-type toxicity. Seven polychlorinated dibenzo-p-dioxins (PCDDs), 10 dibenzofurans (PCDFs), 12 dioxin-like PCBs including non-ortho PCBs (77, 81, 126, 169) and mono-ortho PCBs (105, 114, 118, 123, 156, 157, 167, 189) exert dioxin-like effects. These compounds may provoke immunodepression phenomena (observed in harbour seals *Phoca vitulina* and otters *Lutra lutra)*, perturb lipid metabolism and impact the reproductive system (observed in harbour seals and mink *Mustela vison*) [6, 7, 8]. This raises concerns about actual levels, trends and effects of dioxin-like compounds in marine mammals from the southern North Sea. Dioxin-like compounds including PCDD, PCDF, non-*ortho* and mono-*ortho* PCBs have been determined in the blubber and liver of porpoises and seals stranded along the southern North Sea coast between 1994 and 2004.

Materials and Methods

Sampling

Marine mammals found dead along the Belgian, French and Dutch coasts were collected between 1994 and 2004 and necropsied following a standard procedure[9]. Thirty six blubber samples and 24 liver samples of harbour porpoises were used for determination of dioxin-like compounds. Tissue samples from one grey seal and one harbour seal were integrated in this study.

Dioxin-like compound analyses

Seventeen 2,3,7,8-substituted ^{13}C-labeled PCDD/F congeners, four non-orthos PCBs and 8 mono-ortho PCBs were quantified in the samples. All glassware was thoroughly cleaned using respectively methanol, acetone, toluene and a solution of dichloromethane / hexane (50 :50, V :V). Samples were weighed prior to lyophilisation (24 hours). After being finely crushed, the dry tissues were inserted in a steel extraction cell which was placed in an Accelerated Solvent Extractor (ASE 200, Dionex, operating under high pressure and temperature conditions (hexane, 10 minutes at 125°C and 1500psi). After drying over anhydrous sodium sulphate, the extracts were transferred to a tared round-bottomed flask and the remaining solvents completely removed by evaporation. The flask was then reweighed so that the amount of extracted fat could be determined. A solution of hexane/dichloromethane was added to the extracted lipids and the sample stored in a vial prior to purification. The lipid extracts were then spiked with a mixture containing seventeen ^{13}C-labeled 2,3,7,8-substituted dioxins isomers, 4 c-PCBs isomers (EDF-4144, LGC Promochem) and 8 mono-*ortho* PCB isomers (Campro Scientific WP-LCS). The purification was managed in a semi-automatic system made of four different columns. The first column is a HCDS column (High Capacity Disposable Silica) which was an acidic silica column; the second one

was a silica column (4g acid, 2g basic and 1.5g neutral); the third one was a basic alumina column; the fourth one was a carbon column (Power-Prep, Fluid Management System, U.S.A).
In the first two columns, the fat and the proteins were eliminated by the acid action; the basic alumina column then retained the PCBs, PCDDs and PCDFs. These were eluted by hexane/dichloromethane (50:50, V:V) and hexane/dichloromethane (98:2, V:V) and passed through a carbon column where PCDDs, PCDFs and non-ortho PCBs were retained while the other PCBs were eluted. Ethylacetate/toluene (50:50, V:V) and then toluene extracted PCDDs and PCDFs retained on the carbon column. As the PCDDs and PCDFs were adsorbed in the top of the column, they were eluted in backflush with toluene. The purified extracts in toluene were concentrated using a turbovap and were later transferred into 4 µl of nonane, used as keeper. The remainder of the toluene was evaporated under atmospheric pressure conditions. The different congeners retained on the sample were analysed using a gas chromatography equipped with a capillary column of 40 m coupled to a high resolution mass spectrometer (GC-HRMS, Autospec Ultima). Concentrations were calculated by comparing to the added internal ^{13}C standard. Congeners which produced a peak less than three times the signal to noise ratio in ^{12}C with the spectrometer were considered as 'non detected' (ND). Results were expressed as pg TEQ/g of lipid weight (Table 1). The TEQ values were calculated using the 'upper bound' limit which meant that for a non-detected congener or a value below the limit of quantification (LOQ), the minimum value considered in the TEQ calculation was LOQ. Toxic equivalents (TEQs) of dioxin-like PCBs were calculated by multiplying the molar concentrations of these PCB congeners by their respective toxic equivalent factors (TEFs)[10]. PCB TEQs were the sum of individual PCB TEQs.

Results and Discussion

Our results indicated that in addition to ICES PCBs[11], coplanar congeners are still very present in the tissues of harbour porpoises from the southern North Sea (Table 1). Indeed, blubber concentrations of all combined dioxin-like contaminants observed in this study were slightly higher than those described previously in harbour porpoises living in this area[12,13]. Of these, mono-*ortho* PCBs (in particular PCB 118) clearly dominated the mix (representing 81 – 99 % of the TEQs), followed by non-ortho PCBs (2 – 14 %), furans (0.5 – 2.8 %) and dioxins (0 – 1.26 %). Among the PCDD/Fs, the dominant congener was TCDF (concentrations) and PeCDD (TEQs). In the blubber, mono-*ortho* PCBs represented 91% of the TEQs while in the liver, non-*ortho* congeners dominated the mix (56%; Figures 1 and 2). PCB concentrations present in the blubber and liver samples were among the highest reported in North Sea biota. Toxicity assessment revealed the variable contribution of non-*ortho* and mono-*ortho* PCBs to the overall TEQ, and that the impact of PCDD/Fs was less than 10%. Surprisingly, despite their high PCB concentrations in blubber and liver tissues, PCDD/PCDFs concentrations remained low in harbour porpoises.

As a comparison, the level of PCDD/PCDF contamination in the oceanic seabird black guillemot (*Uria aalge*) collected along the southern North Sea coast was higher than that seen in our samples[14]. The reasons for such discrepancy are unclear but are possibly related to differences in the specific metabolic abilities and foraging areas for seabirds and mammals. Human adipose tissue contains higher amounts of PCDD/PCDFs with a quite different congener pattern (octaCDD being the predominant congener[15]), which can be associated with land-based waste incineration activities.

A mean PCDD/PCDF value of 3.4 pg TEQ/g lipid was measured in the blubber of the porpoises collected in 2003/2004, which is the same order of magnitude as that measured 10 years ago [12]. A slight decrease (but still the same order of magnitude) was observed for non-*ortho* and mono-*ortho* PCBs. It should also be noted that PCDD/F TEQ values for the two seals analysed were more than six times higher than those observed in harbour porpoises, highlighting the need for further investigations in seals. These results are likely to be related to the high position of harbour seal in the trophic web as inferred from $\delta^{15}N$ values and to specific food habits[16].

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Table 1. Dioxins expressed as a sum (Σ) of the 7 dioxin congeners (PCDDs), furans expressed as Σ of the ten furan congeners (PCDFs), non-*ortho* PCBs (Σ of 4 congeners) and mono-*ortho* PCBs (Σ of 8 congeners) detected in blubber and liver of marine mammals, expressed as Toxic Equivalent/ g lipid weight (pg TEQ/g lipids). Results expressed as mean and range

		Σ PCDDs	Σ PCDFs	Σ non-*ortho* PCBs	Σ mono-*ortho* PCBs
Harbour porpoise	Blubber	0.77 (0.2-1.55) n =36	1.84 (0.2-7.54) n=36	7.0 (0.7-29) n=36	103 (17-238) n=29
	Liver	1.90 (0.17-6.81) n=23	10.7 (0.36-35) n=23	107 (3.08-530) n=23	96 (2.95-431) n=24
Grey seal	Blubber	6.4	2.1	31.0	nd
	Liver	16	9	151	nd
Harbour seal	Liver	14.8	9.3	53.5	nd

Figure 1. TEQ distribution for dioxins, furans, non-ortho PCBs (c-PCBs), mono-*ortho* PCBs (mo-PCBs) in the blubber of harbour porpoises (%)

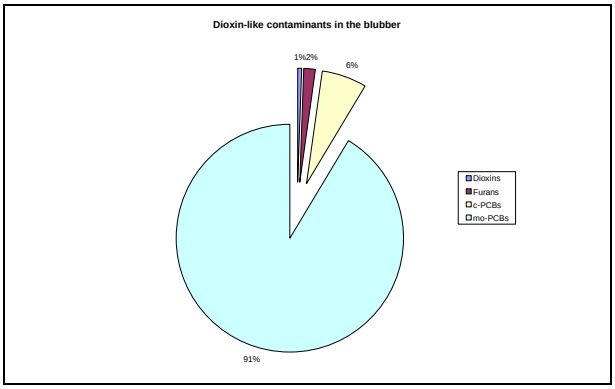

Figure 2. TEQ distribution for dioxins, furans, non-ortho PCBs (c-PCBs) and mono-*ortho* PCBs (mo-PCBs) in the liver of harbour porpoises (%)