DIOXINS, FURANS AND DIOXIN-LIKE PCBS IN JUVENILE HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) FROM THE NORTH SEA.

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Introduction

As apex predators, marine mammals are very important for the regulation of ecosystems. Their survival and diversity in the Northeast Atlantic Ocean is at risk due to past whaling activities, current overfishing of their prey as well as their own accidental capture in fishing nets. To this must be added an intoxication by anthropogenic pollutants which end up accumulating in marine mammal tissues due to their long life span and the long half-life of these contaminants (i.e. organohalogens, heavy metals). These animals are rather unique in their ability to accumulate fatty reserves (particularly in sub-cutaneous fat, or blubber). They are characterized by a growing exposure to pollutants with age, and their young are subject to lipophilic pollutants (via lactation or the placenta)[1]. High concentrations of contaminants (trace metals, organochlorines) have been observed in stranded animals, including harbour porpoises [23]. These observations have caught the scientific community's attention on the possible relation between these pollutants and the decline of several populations such as that of the harbour porpoise *Phocoena phocoena* in the North and Baltic Seas[1,4].

The species chosen for this study was the harbour porpoise *Phocoena phocoena* as the most common marine mammal in the North Sea[4]. Juveniles were selected in order to minimise age differences between individuals as well as differences due to reproductive output in females (transmission of pollutants to the young)[1,3].

Our interest in this study lies in the group of organochlorines related to 2,3,7,8 substituted dioxins which are submitted to an intense bioaccumulation in living organisms and are highly toxic. These are the 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 dibenzofurans (PCDFs) and the dioxin-like polychlorinated biphenyls: coplanar PCBs (77, 81, 126, 169) and mono-ortho PCBs (105, 114, 118, 123, 156, 157, 167, 189). They provoke immunodepression phenomena (observed in harbour seals *Phoca vitulina* and otters *Lutra lutra*)[5], perturb lipid metabolism and can have an impact on the reproductive system (observed in harbour seals and mink *Mustela vison*)[6,7]. They are susceptible of contaminating the food web (ending with man) where they are subject to bioaccumulation. The impact of dioxins on marine food chains is not well known and deserves further attention. Species occupying the summit of food chains (such as marine mammals) concentrate important quantities of different pollutants. These pollutants have a worldwide distribution due to atmospheric transfer and as the result of agricultural and industrial activities[8].

A common toxic pathway characterizes these four groups of molecules. Being lipophilic they can easily cross the cell membrane and enter the cytoplasm where they form a complex with the **Aryl**

hydrocarbon **R**eceptor (AhR) which will enter the nucleus and provoke the transcription of diverse genes (such as cytochrome P450)[9]. In order to determine the actual toxicity of the contaminants detected in the samples the WHO 2,3,7,8-TCDD **T**oxic **E**quivalent **F**actors will be used (TEF)[10].

Material and methods

Collection and sampling
Blubber samples were obtained from 23 juvenile (immature, <3 years of age) harbour porpoises stranded along the coasts of Belgium and northern France. Necropsies were performed following a standardised protocol and samples were stored frozen (-18°C) prior to toxicological analysis. Emaciation was evaluated using visible signs such as the thickness of the subcutaneous fat and the aspect of the dorsal muscles[11]. The emaciated animals had an average blubber thickness of 8mm, while that of the non-emaciated animals was 21.6 mm.

Extraction and clean-up
All analyses were carried out in the Laboratory of Mass Spectrometry, Centre for the Analysis of Residues in Traces (CART), University of Liège. Three to four grams of blubber were sliced and weighed prior to pre-filtering on Na_2SO_4 anhydrous. Lipids and solvent were collected in a weighted balloon and the rest of the sample (+ Na_2SO_4) was inserted in a steel extraction cell which was placed in the **A**ccelerated **S**olvent **E**xtractor (ASE 200, Dionex) in order to extract the organic compounds present in the matrix, under high pressure and temperature conditions. The extracts were then filtered on Na_2SO_4 anhydrous to eliminate the water and the solvents with lipids were collected in the balloon previously mentioned. After evaporating the remaining solvents, the extracted fat was spiked with a mixture of ^{13}C-labeled 2,3,7,8-substituted dioxin, furan, coplanar PCB and mono-ortho PCB isomers. The purification was done in a semi-automatic system of four different columns: a HCDS column (**H**igh **C**apacity **D**isposable **S**ilica) which is a silica-modified column; a Na_2SO_4 column made of neutral and acid silica; a basic aluminium column; and an active coal column (Powder-Prep, Fluid Management System, USA). The purified extracts in the toluene were concentrated using a turbovap and were transferred into 4 ml of nonane.

Instrumental analysis
The different PCDD/F and coplanar PCB congeners present in the sample were analysed using **G**as **C**hromatography equipped with a capillary column of 40 m coupled to a **H**igh **R**esolution **M**ass **S**pectrometer (GC-HRMS). **G**as **C**hromatography coupled with tandem ion trap **M**ass **S**pectrometry (GC-MS/MS) was used to analyse the mono-ortho PCBs. Congeners were quantified and their concentrations calculated when compared to the added internal ^{13}C standard. Results are expressed either as pg/g of lipid weight (lw) or in terms of toxicity, using WHO TEFs for mammals as pg TEQ/g of lipid weight[10].

Data analysis
Statistical analysis of the results was done with the computer programme Statistica (6.0, 2002). The non-parametric U-Mann Whitney Test was used to determine if the differences between the groups (male *vs* female; emaciated *vs* non-emaciated) were significant (at $p < 0.05$).

Results and discussion
The average concentration of all combined dioxin-like contaminants observed in this study are relatively low 95.4 ± 65. 8 pg TEQs /g lw, and yet they are slightly higher (but still the same order of magnitude) than those from a previous study on harbour porpoises in the Wadden Sea[12].

Of these mono-ortho PCBs (in particular PCB 118) clearly dominated the mix (representing 81 - 99 % of the TEQs), followed by coplanar PCBs (2 – 14 %), PCDFs (0.5 – 2.8 %) and PCDDs (0 – 1.26 %). Among the PCDD/Fs, the dominant congener was TCDF (concentrations) and PeCDD (TEQs).

A first comparison showed that although females tend to concentrate higher levels of dioxin-like pollutants than males, their toxicity is lower whereupon they show a selection of less toxic congeners. However, these results were not statistically significant ($p>0.1$). Apart from sex it appeared that individuals' general body condition significantly influenced the pollutants' distribution in the tissues. Pollutant levels in emaciated and non emaciated porpoises were thus compared.

Whether considering concentrations (pg/g lw) (Table 1) or toxicity (pgTEQs/g lw) (Table 2) emaciated animals showed much higher contaminant levels than did the non-emaciated ones. In fact, global toxicity values in emaciated animals were twice those in non-emaciated animals ($p<0.05$) (Figure 1). The differences were significant for PCDFs ($p<0.01$) and mono-ortho PCBs ($p<0.01$) when considering concentrations, and for PCDDs ($p<0.05$), PCDFs ($p<0.005$) and mono-ortho PCBs ($p<0.05$) when considering toxicity. The difference in c-PCBs between the two groups was never significant ($p>0.2$). Emaciated animals seem to have a higher proportion of more toxic dioxin congeners: the difference with the non-emaciated animals for PCDD concentrations is not enough to be significant, whereas it is for results expressed in TEQs.

This study has shown there to be a relationship between harbour porpoises' body condition and the concentration of dioxin-like pollutants. Nevertheless, as of the moment we cannot establish whether this is a causal relationship; more data is needed. Currently analysis of livers from the same individuals studied here is under way. This should shed some light on the dynamics of these pollutants during the emaciation process.

Table 1 : PCDD/Fs and coplanar and mono-ortho PCB concentrations in pg/g lw (average, standard deviation, minimum-maximum, sample size).

Group	PCDDs	PCDFs	Coplanar PCBs	mono-ortho PCBs
Non-emaciated	0.8 ± 1 (0 – 2.6) n=7	3.2 ± 2.5 (1.4 – 8.2) n=7	193.4 ± 182.4 (42 – 464) n=6	$525\ 015 \pm 248\ 847$ (140 260 – 853 330) n=6
Emaciated	13 ± 31 (0 – 109) n=16	10 ± 4.4 (3.4 – 16) n=16	331 ± 273.5 (0 – 867) n=16	$1\ 080\ 903 \pm 489\ 744$ (467 580 – 2 038 970) n=14

Table 2 : PCDD/Fs and coplanar PCB and mono-ortho PCB concentrations in pg TEQs/g lw (average, standard deviation, minimum-maximum, sample size).

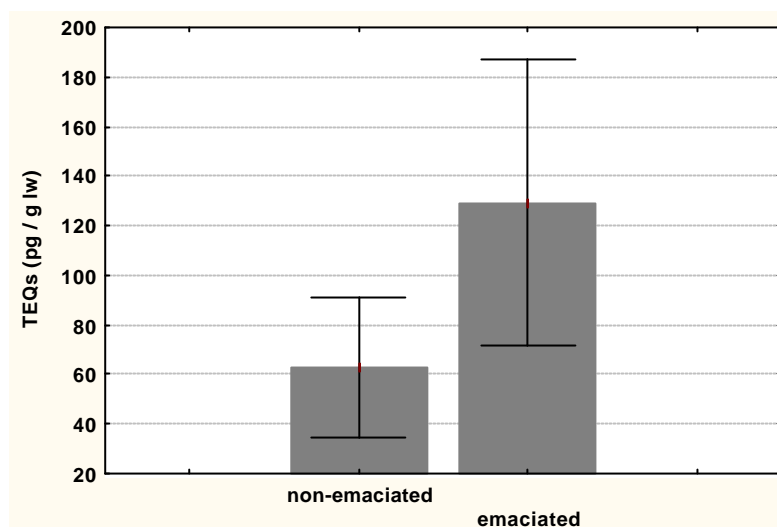| Group	PCDDs	PCDFs	Coplanar PCBs	mono-ortho PCBs	Total
Non-emaciated	0.15 ± 0.3 (0 – 0.84) n=7	0.82 ± 0.6 (0.39 – 1.97) n=7	3.67 ± 3.22 (0.11 – 9.59) n=6	58.4 ± 26.8 (15.29 – 93.29) n=6	**62.44 ± 28.38** (15.79 – 97.16) n=6
Emaciated	0.55 ± 0.54 (0 – 1.46) n=16	1.87 ± 0.6 (0.93 – 3.15) n=16	6.37 ± 5.63 (0.04 – 17.25) n=16	119.7 ± 54.3 (52.44 – 215.94) n=14	**129.33 ± 57.4** (62.68 – 236.67) n=16

Figure 1: Global toxicity expressed as the sum of PCCD, PCDF, coplanar and mono-ortho PCBs in emaciated and non-emaciated animals (pg TEQs/g lw).

Acknowledgements

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