Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in livers of an Atlantic seabird, the common guillemot *Uria aalge* : influence of the general body condition.

V. Debacker[1,2], G. Eppe[2], A-C. Massart[2], C. Xhrouet[2], T. Jauniaux[3], P. Huart[1], P. Hauteclair[1], J-M. Bouquegneau[1] and E. De Pauw[2].

1. Laboratory of Oceanology, University of Liège, B6c Sart Tilman, B-4000, Belgium.
2. Laboratory of Mass Spectrometry, Centre for the Analysis of Traces Residues (CART), University of Liège, B6c Sart Tilman, B-4000, Belgium.
3. Pathology Department of the Veterinary College, University of Liège, B43 Sart Tilman, B-4000, Belgium.

Introduction

Exposure to anthropogenic contaminants is clearly suspected as a potential cause for uncommon diseases and/or mortality observed in seabird populations and more specifically for piscivorous species[1-8]. A previous study conducted on a severely debilitated common guillemot (*Uria aalge*) population found stranded at the Belgian coast already gave a first insight of the complex interactions existing between contaminants – in this case heavy metals – their potential detrimental effects and the individuals' general fitness[9-12]. Recently, analyses have been extended to the 17 most toxic dioxin and furan congeners, a series of molecules recognized as highly detrimental.

Levels of dioxin and furan congeners detected in the livers of the common guillemots are more specifically studied when considering the general body condition or robustness of the individuals. In addition, samples of guillemots collected after the Erika's oil spill off the Brittany coasts (France) were available for toxicological analyses. Such a sample of robust individuals representative of the living population at sea provided a highly interesting toxicological comparison not only on the geographical level but also regarding the influence of robustness on polluants'tissue distribution.

Materials and methods

A total of 39 male guillemots were selected for the toxicological analysis. All were collected as dead stranded animals during the wintering seasons from 1993 to 2002. Twenty-one individuals washed ashore along the Belgian coast while the remaining 18 were sampled after the Erika's oil spill in December 1999 off the Brittany coasts. The latter were completely covered with oil. All fresh carcasses were necropsied following a standardised protocol[13]. Emaciation (cachexia) was evaluated using visible signs - presence or not of subcutaneous fat, light to severe atrophy of the pectoral muscles- and given a range from 1 to 3 depending on its severity. Non cachectic and severely cachectic individuals of both geographic origins are considered. Livers were collected, weighed and kept frozen (-18° C) prior to toxicological analyses.

All analyses were carried out in the Laboratory of Mass Spectrometry, Centre for the Analysis of Trace Residues (CART), University of Liège. Five to six grams of liver samples were weighed and lyophilised. Dry tissues were inserted in a steel extraction cell and placed in the **A**ccelerated **S**olvent **E**xtractor (ASE 200, Dionex). This machine using organic solvents operates under high pressure and temperature conditions (10 minutes at 125°C and 1500psi) and allows the extraction of the different organic compounds present in the biological matrice. After filtration on Na_2SO_4

anhydre the fat extracts are then spiked with a mixture containing seventeen ^{13}C-labeled 2,3,7,8-substituted dioxins. The purification is managed in a semi-automatic system made of four different columns. The first column is a HCDS column (**H**igh **C**apacity **D**isposable **S**ilica) which is a silica-modified column; the second one is a Na_2SO_4 column made of neutral and acid silica; the third one is a basic aluminium column; and the fourth one is an active coal column (Powder-Prep, Fluid Management System, U.S.A). The purified extracts in the toluene are concentrated and are transferred into 4 ml of nonane.

The different congeners present in the sample are then analysed using a **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). They can be quantified and their concentration calculated when compared to the added internal ^{13}C standard[14]. Results are expressed either as pg/g of lipids weight or in terms of toxicity, using WHO TEF for birds[15] as pg TEQ/g of lipids weight

All statistical tests were performed using Statistica software (5.1, 1996) with a significant level of $p<0.05$. Data were checked for adherence to the standard assumptions of parametric tests using the Kolmogorov-Smirnov test for normality and the Levene's test for homogeneity of variances.

Results and discussion

Global comparison

The observed mean PCDD/F concentrations in this study are much higher than those described in the tissues of granivorous, omnivorous or other piscivorous birds[7] .They, nevertheless, are quite comparable to levels detected in the fish-eating common cormorant in Japan[16], described as high. Significantly higher levels ($p<0.01$) of both dioxin and furan congeners (multiplied by a factor 3 and 5 respectively) were detected in the livers of the Belgian guillemots compared to their Brittany counterparts (Table 1).

However, when considering the congeners' profile a similar situation is observed at both locations with a predominance of the furan congeners when expressed as concentrations and TEQ (⩾60%). More specifically, the Pe_2CDF congener with a bird TEF equal to 1 particularly contributes to the total TEQ.

Table 1 : Comparison of levels of PCDD/Fs expressed as concentrations and TEQ.

	Concentration pg/g lipid weight		WHO TEF (birds). pg TEQ/g lipid weight	
	Brittany coast n = 18	Belgian coast n = 21	Brittany coast n = 18	Belgian coast n = 21
Σ PCDDs	2607 ± 2089 49 – 6347	7778 ± 6879 824 – 24915	319 ± 336 21 - 1299	2109 ± 1941 309 - 7527
Σ PCDFs	2108 ± 1928 94 – 6615	11084 ± 9954 1649 – 40353	1430 ± 1371 62 - 4574	6899 ± 5966 882 - 23693

Influence of the bird's robustness

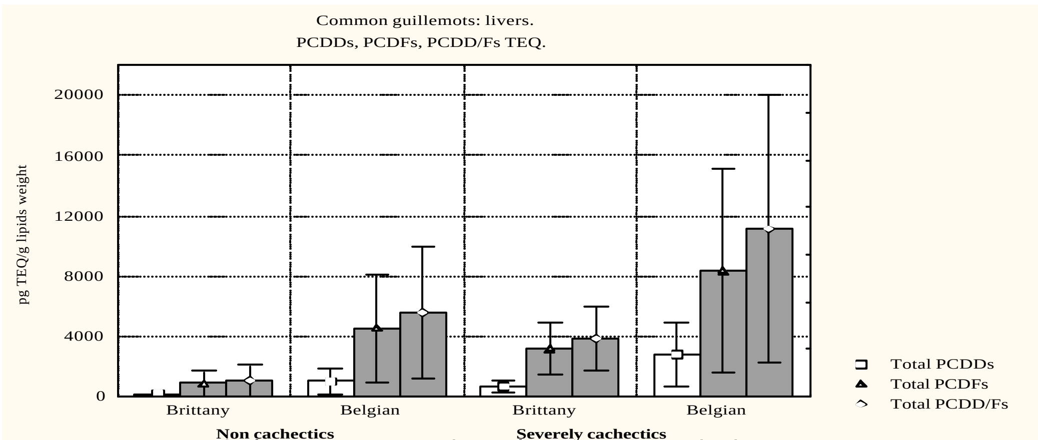
Among the factors susceptible to influence the pollutants' distribution within the tissues, it appears that the individuals'robustness is not negelectable (Table 2). It appears that increasing levels of both dioxin and furan congeners are found parallel to degrading body condition (increasing cachectic conditions). Levels of dioxin congeners are significantly higher in severely debilitated birds, whereas furans tend to increase, but not significantly. Total dioxin load in the liver of severely cachectic guillemots also increases significantly ($p<0.05$), probably indicating a remobilisation of dioxin compounds accompanying fat and proteins catabolism. However, the dioxin profile remains similar for both nutritional status and is dominated by the Hx$_2$CDD (35%) and PeCDD (20%) whereas the remaining 5 congeners each represent 14% and less of the total concentration.

Table 2 : Sum of the 7 dioxins, 10 furans and sum of both dioxin and furan congeners expressed as mean ± std dev. and min-max. ↗ : indicate an increasing trend, significantly different at $p<0.05$, n.s. : not significant.

Livers	**Belgium coast**		
pg/g lipid weight	Non cachectic n = 8		Severely cachectic n = 13
Σ PCDDs	3671 ± 2982	**↗**	10305 ± 4751
	824 – 8299	**p < 0.05**	2448 - 24915
Σ PCDFs	6960 ± 5475	↗	13622 ± 11373
	1649 – 13401	n.s.	3362 - 40353
Σ PCDD/Fs	10632 ± 8340	↗	23927 ± 18682
	2977 - 21700	n.s.	6124 - 65268
pg TEQ/g lipid weight			
Σ PCDDs	*1034 ± 867*	**↗**	*2770 ± 2144*
	309 - 2762	***p < 0.05***	*531 - 7527*
Σ PCDFs	*3769 ± 3612*	↗	*8352 ± 6760*
	882 - 8848	*n.s.*	*2179 - 23693*
Σ PCDD/Fs	*5572 ± 4405*	↗	*11122 ± 8865*
	1221 - 11610	*n.s.*	*2710 - 31221*

A similar situation is observed when considering the contaminants' distribution in the livers of guillemots sampled in Brittany. However it is worth noting that for a similar cachectic status all levels are higher in the livers of Belgian guillemots (Fig. 1).

Figure 1 : Comparison of levels observed depending of the cachectic status of the individuals.



Conclusions

The high levels of dioxins and furans observed in the livers of wintering guillemots are clearly shown to increase in parallel to a progressive debilitation process ultimately leading the individuals to death. Questions arise as to what extent these toxic compounds may enhance/contribute to the general debilitation observed in this seabird population.

In addition, results demonstrated that wintering seabirds in the Southern North Sea have to face higher pollutant levels compared to their Brittany counterparts.

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