

**LEVELS OF 2,3,7,8-SUBSTITUTED PCDD AND PCDF IN CETACEANS
FROM THE MEDITERRANEAN SEA**

B. Jimènez¹ O. Jiménez², S. Reich³, E. Eljarrat⁴, J. Rivera⁴ and M.J. González¹

¹Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, C.S.I.C., Juan de la Cierva 3, 28006 Madrid, SPAIN.

²University of Alcalá, 28871 Alcalá de Henares, Madrid, SPAIN. ³ University of Tübingen, Germany. ⁴Laboratory of Mass Spectrometry, Research and Development Centre, CSIC, Jordi Girona 18-26, 08034 Barcelona, SPAIN.

Introduction

Cetacean populations living in the Mediterranean Sea are at considerable risk from levels of persistent chemicals in the marine environment which have risen rapidly in the second half of this century (1). Marine mammals are at the top of the food chain, making them among the most exposed of all marine animals. Cetaceans do not have sweat or sebaceous glands, fur or active blood-water exchange via gills. Thus they can be regarded as closed systems in which contaminants can act practically unopposed. Moreover, they have been found to be very much less able to metabolise organochlorine than birds and land mammals (2). Although there is as yet no evidence that pollutants are causing the death of marine mammals, organochlorines such as PCBs, PCDDs and PCDFs are known to cause immune and reproductive dysfunction (3-4). In a number of regions contamination in cetaceans has now reached concentrations at which there are known sub-lethal effects sufficient to harm populations of other species. There is also evidence to suspect that organochlorines may be altering the reproductive potential and health of cetaceans (1).

Material and Methods

Sampling

Liver samples used for ecotoxicological studies were obtained from five species of cetaceans: *Stenella coeruleoalba*, *Tursiops truncatus*, *Balaenoptera physalus*, *Grampus griseus* and *Globicephala melaena*. All the animals studied were found dead along the Italian shore of the Tirrenian, Adriatic and Ligurian Seas in the period 1987-1992. Collection and transport of the carcasses was authorised and supervised by the Centro Studi Cetacei (Milan). Liver samples were frozen and stored at -20° C until use. Samples for residue analysis were lyophilised and quantities of approximately 0.5 grams of liophilised liver tissue were used for analysis.

Analytical determination

Extraction and clean up were performed as previously described in detail elsewhere (5). Basically this consisted of low pressure chromatography on neutral and base-modified silica gel and activated carbon dispersed on glass fibres. Three fractions were eluted from the carbon column for each sample. These contained ortho-substituted PCBs, non-ortho-substituted PCBs and PCDD/Fs,

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respectively. Further cleanup was done using silica gel impregnated with sulphuric acid, and Florisil®.

Resolution and quantification of PCDDs and PCDFs were performed by HRGC-HRMS using a VG AutoSpec Ultima (VG Analytical, Manchester, UK) coupled to a Fisons Series 8000 (8060) gas chromatograph. A fused silica capillary DB-5 column (60m, 0.25 mm id., 0.25µm film thickness, J&W Scientific, USA) and a DB-DIOXIN column were used. The carrier gas was Helium at a column head pressure of 175 Kpa. A minimum resolution of 10,000 was used when operating with the HRMS instrument.

Results and discussion

Total PCDD and PCDF levels and calculated I-TEQs [6] in liver samples from all cetaceans studied are shown in Table 1. Most of all 2,3,7,8-substituted PCDD/Fs were detected in all the samples studied. Total values were calculated assuming that all values less than the limit of detection (LOD) are equal to half LOD. Total PCDD/F levels ranged from 13.03 ppt to 91.32 ppt on a wet weight basis.

Total PCDD/F levels

The highest levels were found in Risso's dolphins (*Grampus griseus*) with a maximum value of 91.32 ppt and Striped dolphins (*Stenella coeruleoalba*) with a maximum value of 79.78 ppt. The lowest levels were found in Bottlenose dolphins (*Tursiops truncatus*). Regarding the contribution of PCDDs and PCDFs to total levels, in general a higher percentage contribution was found from PCDFs, ranging from 44 to 77%.

Table 1. Ranges of total PCDD/F levels and calculated I-TEQs (pg/g wet basis) found in all cetaceans studied.

Species	<i>Grampus griseus</i> (n=3)	<i>Tursiops truncatus</i> (n=5)	<i>Stenella coeruleoalba</i> (n=5)	<i>Globicephala melaena</i> (n=1)	<i>Balaenoptera physalus</i> (n=1)
Common name	Risso's dolphin	Bottlenose dolphin	Striped dolphin	Long-finned pilot whale	Fin whale
Total PCDD/Fs	19.46 – 91.32	13.03 – 15.75	39.86 – 79.78	22.74	23.86
% PCDDs	23 - 56%	44 - 52%	40 – 67%	48%	39%
% PCDFs	44 - 77%	48 - 56%	33 – 60%	52%	61%
Total I-TEQs	1.45 – 5.25	0.88 – 1.48	1.35 – 6.39	1.82	2.44
% I-TEQ PCDDs	33-45%	46 - 70%	16-45%	44%	39%
% I-TEQ PCDFs	55-67%	30 - 54%	55-84%	56%	61%

In general the most abundant congener was OCDD which accounted for between 23 and 54% of the total. The contribution of OCDF, which lay in the range 8 to 35%, was also noteworthy, and in one case it had the highest contribution with a value of 44%. In one case 2,3,7,8-TCDF had a contribution similar to that of OCDD.

I-TEQs

The species exhibiting the highest I-TEQ levels was the Striped dolphin (*S. coeruleoalba*) with a maximum level of 6.39 ppt, followed by the Risso's dolphin (*Grampus griseus*) with a maximum level of 5.25 ppt.

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Total I-TEQ levels were lower in the rest of the species studied, the lowest value observed being found in Bottlenose dolphins (*T. truncatus*).

Some differences were found in the contribution of dioxins or furans to total I-TEQs. In general it was found that the contribution from PCDFs was almost always higher than 50%, in some cases being as high as 84%, in the case of Striped dolphins (*S. coeruleoalba*). This situation was different in the case of one Bottlenose dolphin specimen which had a PCDD contribution of 70%. The significant contribution made by the congener 1,2,3,7,8-PnCDD to total I-TEQs in all cases, with percentage contributions ranging from 17 to 38%, was particularly noteworthy. In some cases, such as that of Bottlenose dolphins (*T. truncatus*), this isomer was responsible for the largest contribution to total I-TEQs. The situation was similar in the case of the long-finned pilot whale (*G. melaena*) and two of the Risso's dolphins (*Grampus griseus*).

In the case of PCDFs, some clear species-related trends were apparent:

- In the Bottlenose dolphin (*T. truncatus*) specimens an important contribution from 2,3,4,7,8-PnCDF was found in all cases, making a total contribution to I-TEQs of between 10 to 20%. Also, significant levels of congener 1,2,3,7,8,9-HxCDF making a contribution to total I-TEQs of 10 to 13%. The contribution made by the remaining 2,3,7,8- hexa-furans was also important but generally the half of the former.
- In the case of Risso's dolphins two types (*G. griseus*) of pattern were found. Those with a major 2,3,7,8-TCDF contribution (approximately 20%) and patterns where 2,3,4,7,8-PnCDF contributed 18%. This situation was fairly similar in the Fin Whale studied (*B. physalus*).
- In the case of the Long-finned pilot whale (*G. melaena*) congener 2,3,7,8-TCDF did not make an important contribution to total I-TEQs but the contribution of 2,3,4,7,8-PnCDF (22%) was noteworthy.
- In the case of striped dolphins (*S. coeruleoalba*) there were at least 3 different cases regarding the percentage contribution of each isomer. The important contribution of congeners such as 2,3,7,8-TCDF, 2,3,4,7,8-PnCDF and 1,2,3,7,8-PnCDD deserves note.

Dioxin toxic equivalent of coplanar PCBs.

It has been reported (1) that in aquatic animals approximately 40% of toxicity is due to furans, 22% to dioxins, and 38% to the more highly chlorinated PCBs. In a previous study concerning PCBs (7) carried out with *Stenella coeruleoalba*, a high contribution to total I-TEQs from PCBs has been reported. When the contribution from PCDD/Fs is compared with that of PCBs it can be seen that it is lower than that coming from PCBs. The 2,3,7,8-TCDD toxic equivalent factors (TEFs) recommended by WHO (8) were used for PCB hazard risk assessment in a previous study (7). They are usually 5-10 times lower than those obtained using the TEFs developed by Safe (9), which was used in hazard risk assessment studies conducted up until 1994. Average TEQs were found to be 0.24 ng/g (wet weight basis) in liver samples from *Stenella coeruleoalba* specimens. The contribution of the most toxic PCB congeners (#77, #126 and #169) was not very high, together accounting for less than 1% of total TEQs. The mono-ortho PCB congeners #105, #118, #156, #157 and the di-ortho #170 and #180 made the largest contribution to total PCB toxicity in the dolphins studied. Together they account for about 80% of the total calculated TEQs. These results agreed with those obtained by other authors (10,11) who found that the contribution to total TEQs by non- and mono-ortho PCB congeners in marine mammals is consistently higher than that

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of non-ortho PCB congeners, while in the case of terrestrial mammals, the toxic equivalents of non-ortho PCB congeners are comparable to those of mono-ortho PCB congeners.

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