

## OCCURRENCE OF POLYCHLORINATED DIBENZO-PARA-DIOXINS AND DIBENZOFURANS IN BIVALVES FROM THE SPANISH COAST

E. Abad, F. Pérez, J.J. Llerena, J. Caixach, J. Rivera

Mass Spectrometry Laboratory, Dept. of Ecotechnologies, IIQAB-CSIC. C/ Jordi Girona 18-26, 08034 Barcelona (Spain).

### Introduction

Mussels and other bivalve species are widely distributed in coastal areas, they have a sedentary behaviour and they are able to bioaccumulate environmental pollutants. For these reasons, bivalves are currently used as bio-indicators to evaluate the impact of chemical pollution in the marine environment. Data related to the concentrations of several contaminants (i.e. PAHs, PCBs, pesticides and trace metals) in these organisms have been extensively documented in the literature<sup>1,2</sup>. In addition, PCDD/PCDF levels have also been reported. However, information about the comparison between PCDD/PCDF profiles of different species is scarce<sup>3,4</sup>.

The aim of this study was to evaluate the presence and the isomer distribution of PCDDs/PCDFs in four different bivalve species (mussels, oysters, carpetshells and cupped oysters) collected along The Spanish Coast.

### Methods and Materials

#### *Sampling, extraction and cleanup*

Different species of bivalves were collected at several locations from the Spanish coast and they were kept frozen until analysis. Briefly, 45-50 g of freeze-dried tissue was extracted in a Soxhlet for 24h with toluene:cyclohexane (1:1). After that, the extracts were spiked with known amounts of a <sup>13</sup>C<sub>12</sub>-PCDD/PCDF mixture. Then, they were treated with sulphuric acid to remove the fat content and organic matter, whereas PCDDs/PCDFs remained in the n-hexane fraction. Finally, the extracts were rotary concentrated and filtered prior to the cleanup process.

Purification was accomplished by an automated cleanup system (FMS<sup>TM</sup>, USA) based on the use of multilayer silica, basic alumina and PX-21 carbon absorbents<sup>5</sup>.

#### *HRGC-HRMS analysis*

Purified extracts were analysed by HRGC-HRMS/EI(+)-SIM on a GC 8000 Series gas chromatograph (Carlo Erba Instruments, Milan, Italy) coupled to an Autospec Ultima mass spectrometer (Micromass, Manchester, UK) equipped with a CTC A200S autosampler, at 10000 resolving power (10 % valley definition). Chromatographic separation was achieved with a DB-5 (J&W Scientific, CA, USA) fused-silica capillary column (60 m x 0.25 mm ID, 0.25 µm film thickness) with helium as the carrier gas at a linear velocity of 35 cm/s (T=100 °C) in the splitless injection mode (1-2 µl). A DB-DIOXIN (J&W Scientific, CA, USA) was used as a confirmation column. Quantification was performed by the isotopic dilution method.

The criteria for ensuring the quality control and quality assurance (QC/QA) measures have been previously reported elsewhere<sup>5</sup>.

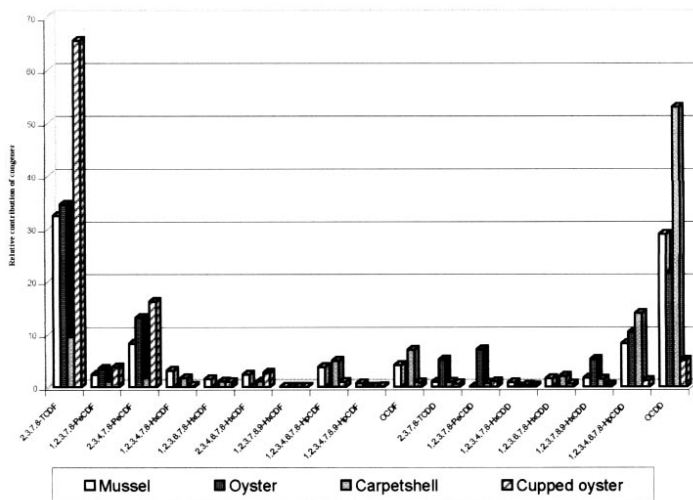
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## Results and discussion

A total of ~100 samples were processed during a period of eight years (1994-2001) following the method above described. Four types of bivalve species collected along the Spanish coast were studied: oyster (*Ostrea edulis*), mussel (*Mytilus spp.*), carpetshell (*Tapes spp.*) and cupped oyster (*Crassostrea spp.*).

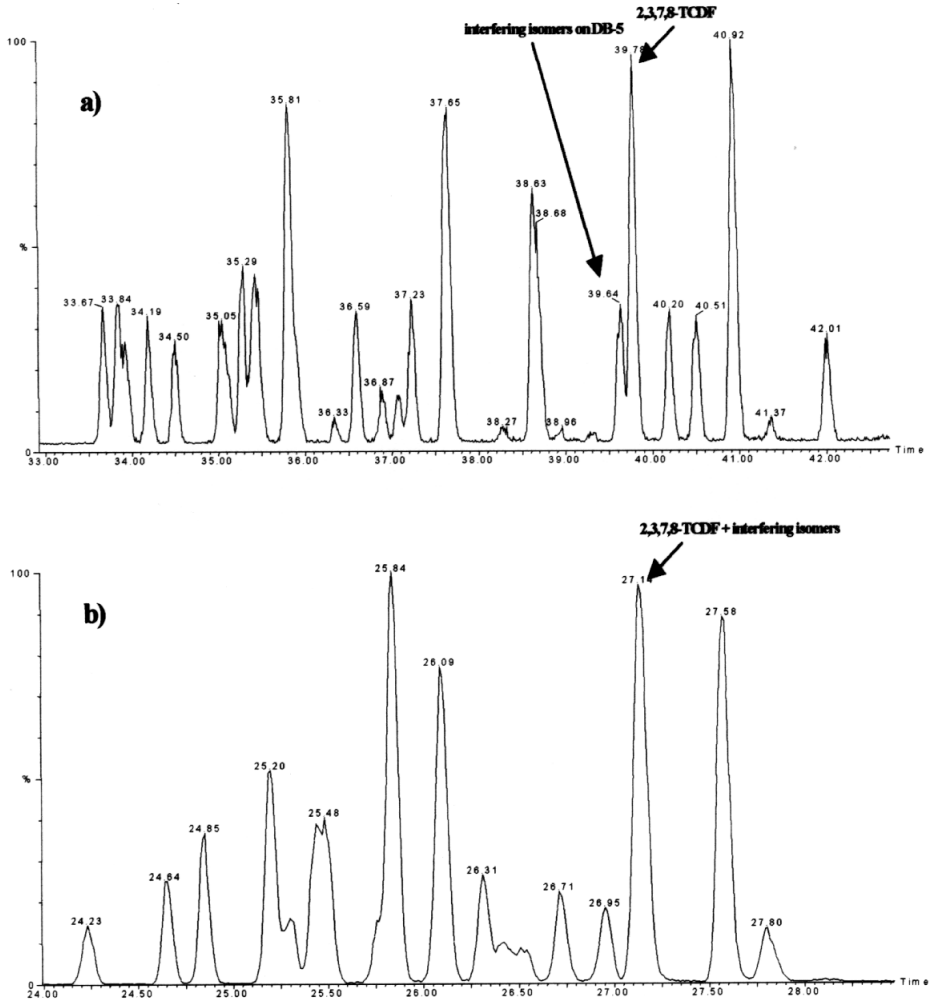
At present the European Community has set a limit of 4 WHO-TEQ pg/g (whole weight) for PCDDs/PCDFs in fish and fish products. However, bivalves are not included in this directive. Taking this into account, medium to high levels of PCDDs/PCDFs (i.e 3.14-7.61 WHO-TEQ pg/g) were found in most of analysed samples considering the limit value established for fish.

A comparison of the percentage ratio distribution of toxic PCDDs/PCDFs present in the four bivalves species (expressed in concentration) is given in Figure 1. In general, the predominant congeners in all species were TCDF and OCDD isomers. Mussels and oysters showed similar profiles with an analogous contribution of both isomers. By the other hand, carpetshell preferably contained OCDD (53 %), while the relative percentage of TCDF raised down to 10 %, being lower than HpCDD (14 %). On the contrary, TCDF is the main isomer that contribute to the total profile in cupped oyster (65.5 %) followed by a lower percentage of 2,3,4,7,8-PeCDF and OCDD. When the data were evaluated in TEQ basis the 2,3,7,8-TCDF and the 2,3,4,7,8-PeCDF presented the major contribution to the total TEQ values, followed by 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, remaining the rest of the isomers in a minor proportion.



**Figure 1.** Isomer distribution of different species of bivalves from Spanish coast

In this sense, it is well known that non-toxic isomers coelute with the 2,3,7,8-TCDF in 5 % phenyl-methylpolysiloxane phases. Therefore, representative samples of each specie were also analysed using a polar column, such as DB-DIOXIN, in order to separate the congeners. Thus, it was assured that the signal obtained in DB-5 was mostly due to the toxic isomer (Figure 2).



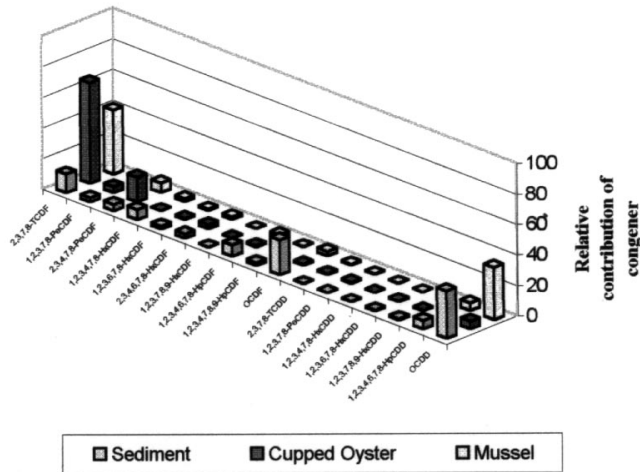
**Figure 2.** Chromatograms of TCDF isomers from a mussel sample. a) DB-DIOXIN column, b) DB-5 column

In addition, PCDD/PCDF profiles of different bivalve species and sediments were studied in some coastal areas. An example of this is given in Figure 3. In general, the results confirmed the behaviour found along the whole study. Thus, in mussels the principal contribution to the total levels of PCDDs/PCDFs came from TCDF, OCDF and OCDD isomers; whereas, cupped oysters contained mainly TCDF and PeCDF, with independence of the type of contamination source found in sediments.

Therefore, cupped oyster seems to have a preferential accumulation for the lower chlorinated isomer. In contrast, mussel trends towards a profile characterized by the major contribution of both, lowest and highest chlorinated isomers. Moreover, the results do not allow to establish a clear

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correlation between the profiles of bivalves and sediment nearshore, likely due to the observed differences in bioaccumulation behaviour.



**Figure 3.** Profiles of a sediment and two bivalve samples from a coastal area

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