PCDD/F CONCENTRATIONS AND EROD INDUCTION IN FISH FROM THE BIOBIO RIVER IN CHILE

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Introduction

Dioxins and furans could enter into the aquatic environment from different sources some of them include the use of Pentachlorophenol and pulp and paper mill effluents as well as incineration process. All of the processes mentioned above are present in the Biobio river basin in Central Chile. The Biobio river basin is the third largest basin in Chile and is an aquatic ecosystem of utmost social-economic importance to the Biobio region, serving as a resource for drinking water supply, water supply for industry, hydroelectric power, and irrigation for agriculture and forestry. Because of its multiple uses and importance to a population of approximately 1,200,000 people who reside in the area of the Biobio basin, there is an increasing concern relative to the effect of antropogenic stressors (*i.e.* contaminants), which degrade water quality and affect the biological and ecological integrity of this aquatic ecosystem. The overall quality of the river has been moderately to severely impacted by physical disturbances caused by operation of hydroelectric dams, chemical pollution by organic and inorganic contaminants from heavy industry, inputs of untreated sewage, and non-point pollution from pesticides and fertilisers used in agricultural and forestry activities. Previous studies have demonstrated the existence of a pollution gradient of organochlorine compounds in the river, which extends from the Andes Mountains (relatively unpolluted), to the river mouth (severely polluted) at the Pacific Ocean[1]. The majority of the people who depend on the resources of the Biobio river live along the lower reaches of the basin, where pollution is the most severe and the biological integrity and resources of the river have been the most impacted.

Discharges from four pulp and paper mills, making use of the same area of the river, are the main sources of stress on the resources of the river. Pulp and paper mill effluents are very complex mixtures, whose characteristics are dependent on numerous factors including the type of raw material, the technology of the process, and the treatment technology of the effluent. The toxicological properties of these effluents can therefore vary depending on the characteristics of the discharge. Furthermore, the behaviour of the chemical substances, originating from processing of wood products (cellulose) is controlled by the chemical properties of the effluent itself and by the physicochemical characteristics of the river in which these effluents are discharged. Partitioning of chemicals between distinct environmental compartments such as particulate material, sediments and water basically accounts for the concentrations, availability, and distribution of contaminants in the different tissues of the organisms that reside in the river. From the previous antecedents it was considered useful the analysis

of PCDD/F in resident fish in the area, in order to evaluate if PCDD/F were present and if there is any risk due to these pollutants to fish fauna inhabiting the river.

Material and Methods

Fish were sampled in three pre-defined areas of the river (Figure 1), a pre impact area where there are neither industrial discharges nor activities that could discharge chlorinated pollutants into the environment, an impact area where industrial effluents are discharged. Sampling devices involved nets and electro fishing. Sampling was performed in April 2001 (Early Autumn).

Residue analysis

Extraction and Clean-up

Freeze-dried liver samples were analysed for dioxin and furans contents. Basically the extraction of PCDD/Fs and PCBs involved a solid phase matrix dispersion (SPMD) procedure. Fractionation among the studied compounds and other possible interferences was achieved by using SupelcleanTM Supelco ENVITM-Carb tubes as described elsewhere[2]. Three fractions were eluted. The first fraction contained the bulk of PCBs and DDTs. The second and third fraction contained non-ortho substituted PCBs and PCDD/Fs, respectively analysed for this study.

Identification and Quantification.

Resolution and quantification of PCDDs, PCDFs and co-planar PCBs were performed by HRGC-HRMS using a VG AutoSpec Ultima (VG Analytical, Manchester, UK) coupled to a Fisons Series 8000 (8060) gas chromatograph. A minimum resolution of 10,000 was used when operating with the HRMS instrument. Methods blanks were routinely analysed, and no contributions were detected. Resolution and quantification of mono-ortho PCBs was carried out by HRGC-ECD using a Varian GC Instrument CX Series, STAR 3400. A fused silica capillary DB-5 column (60m, 0.25 mm id., 0.25mm 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.

Biomarker Analysis.

EROD activity was measured in fish livers collected in the three sampling areas. Only Post mitochondrial supernatant was used for testing the enzyme activity. Methods are the same as described by Gavilán et al.[3].

Results and Discussion

Only four different fish species were analysed in this study, *O. mykiss* was found in the pre-impact zone and *C. carpio* in the impact zone. Finally at the mouth of the river two species were sampled *E. maclovinus* and *M. cephalus*. About 10 specimens per species were analysed for liver EROD activity at each sampling site, however only three fish were catch up in the impact zone besides the high capture effort.

EROD activity resulted to be higher at the lower stretch of the Biobio river when compared to impact and pre-impact areas of the river (see figure 2). Besides a direct comparison it is difficult because different species were analysed, results showed that EROD activity is higher at the mouth of the river, indicating probably two reasons for that, the first one is that pollution levels are higher in that area, resulting in a high EROD induction, the second could be that both species (*E. maclovinus and M. cephalus*) have naturally higher activities, however the last option have been discarded by previous research conducted in the same area[3].

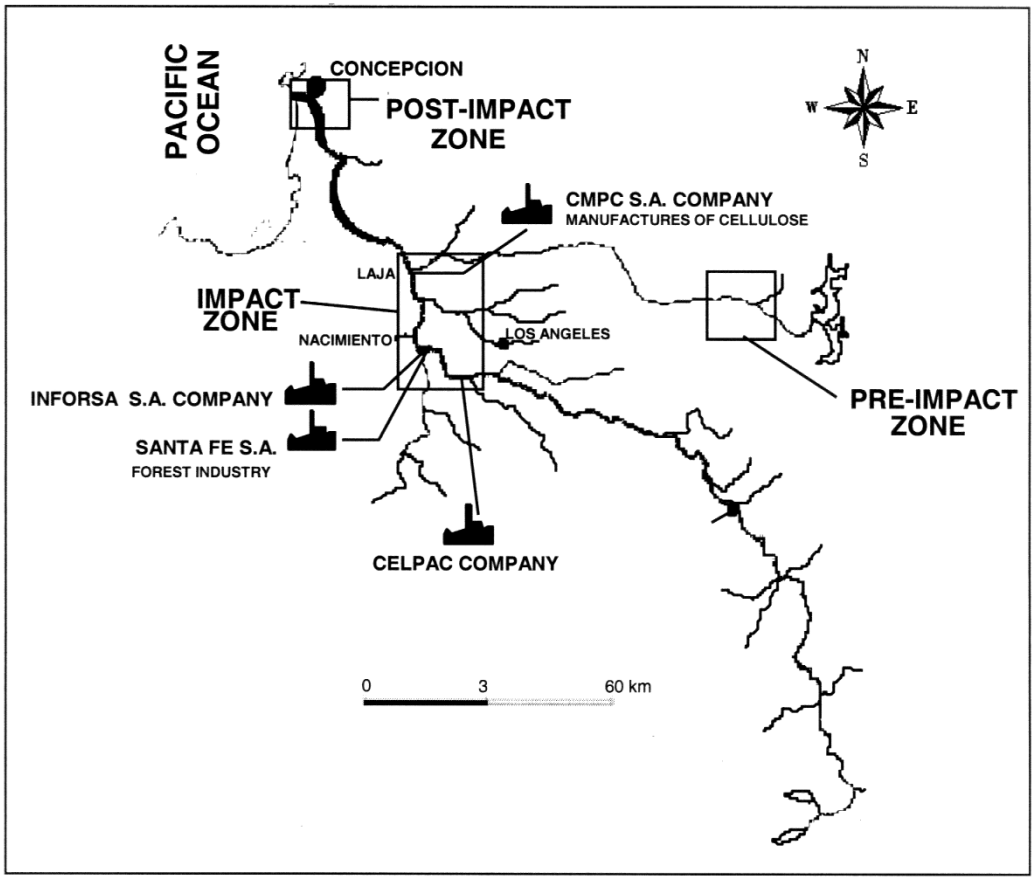

Figure 1. Sampling sites and location of the study area (Biobio River) in Chile.

On the other hand, observed EROD induction at the impact zone seems to be not very high, also these effect have been previously observed since it is well known that severe pollution may cause liver damage resulting in low EROD induction. We did not observe, however, any liver damage in fish captured downstream the pulp mill discharges.

Previous results from our group indicates that EROD induction could be due to PAHs exposure[4], more than organochlorine levels, even though this is the first time that co-planar PCB, PCDD and PCDF levels are measured in the are of study using the fish species mentioned.

Total PCDD/F levels found in this study ranged from 18.48 to 21.7 pg/g on a wet weight basis (WW), being very low and quite similar in all the sampling points studied. Total levels found for the co-planar PCBs analysed (#77, 126 and 169) were slightly higher than those found for PCDD/Fs and ranged from 43,05 to 181 ppt (WW). Calculated TEQs for PCDD/Fs and coplanar PCBs were low ranged from 5.05 to 12.46 ppt (WW). The low TEQ levels found in this study do not explain such a high induction detected at the mouth of the river, where a high industrial setting is established, discharging different pollutants into the river.

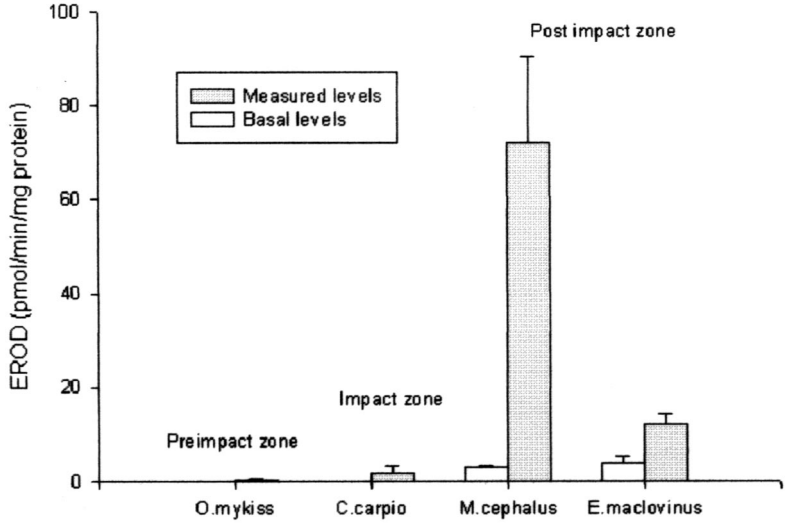

Figure 2. Mean (± SD) cytochrome P450 1A1 (based on 7-ethoxiresorufin O-deethylase dealkylation, EROD) activity in different fish species collected in the Biobio river basin in 2001. EROD basal levels for each species are shown in white bars.

Acknowledgements

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