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Short communication

The Prestige oil spill: A laboratory study about the toxicity of the water-soluble fraction of the fuel oil

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Abstract

The Prestige oil spill caused severe effects on the coastal fauna and flora due to direct contact of organisms with the fuel oil. However, the water soluble fraction (WSF) of the fuel oil can also provoke deleterious effects in the long term and even in regions not directly affected by the spill. Our objective was to determine the toxicity of the WSF using a battery of laboratory toxicity tests. To obtain a WSF in the laboratory, a sample of the spilled fuel was mixed with adequate medium, sonicated, agitated and filtered. No cytotoxic effects were detected in RTG-2 cells exposed to the WSF. In an algae growth inhibition test (OECD test guideline 201) the WSF did not affect the growth of *Chlorella vulgaris*. Furthermore, acute and reproductive toxicity tests (OECD test guideline 202) carried out using *Daphnia magna* did not indicate any deleterious effect of the WSF. In a bioassay designed in our laboratory, *D. magna* were fed with algae previously exposed to the fuel, but no toxic effects were detected. However, the WSF was able to induce a dose-dependent increase of ethoxyresorufin-*O*-deethylase activity in RTG-2 cells, indicating the presence of chemicals that could cause sub-lethal effects to organisms. After chemical analyses it was established that the final total quantity of polyaromatic hydrocarbons dissolved in medium was approximately 70 ng/ml. These low concentrations explain the observed lack of toxicity.

Keywords: Prestige; Spill; Acute toxicity; Reproduction toxicity; EROD

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The oil tanker Prestige, carrying a cargo of 77,000 mt of fuel oil, sank off the coast of Galicia (NW Spain) on 19 November 2002. Most of the Galician coast was severely polluted with fuel oil. Nevertheless, the oil spill dispersed and reached the Spanish Cantabrian and the French Atlantic shorelines up to Brittany, as well as the northern coast of Portugal.

The spilled fuel-oil affected the organisms by direct contact or after ingestion. Reductions in the populations of some macrofaunal species (Junoy et al., 2005) and an important impact on the seabird populations (Camphuysen et al., 2002) have been reported. In addition, a fraction of the fuel-oil is soluble in water and could cause long-term effects. This water soluble fraction (WSF) has been observed to consist mainly of a variety of polyaromatic hydrocarbons (PAHs) and some quantities of aliphatic hydrocarbons (Díez et al., 2005; Informe Técnico CSIC "Prestige" nr 13). The main objective of this work was to characterize the toxicity of the WSF using a battery of different bioassays carried out under laboratory conditions.

To obtain the WSF different quantities of fuel (45, 10 or 5 g) were mixed with 100 ml of medium. Results from preliminary experiments were similar and no toxicity was detected with any of the three quantities of fuel used. Since the solubility of PAHs in water is very low (in the order of ng/ml), it was considered that in all cases the solution was saturated of PAHs. In order to facilitate the use of the fuel sample and to guarantee the total dispersion of the sample, finally 5 g of fuel in 100 ml of medium were used. The mixture was first sonicated for 10 min to disperse the fuel sample, and then it was gently agitated for 72 h in a closed bottle and in obscurity. Finally, the medium was filtrated in order to obtain only the WSF, avoiding any material in suspension.

Total PAHs of each medium were determined by high performance liquid chromatography (HPLC) with diode array detection, using solid phase extraction (SPE) as clean-up and concentration procedure. The only exception was the cell culture medium, in which, probably due to the high protein concentration, it was not possible to extract the PAHs adequately. Briefly, 100 ml of sample (water soluble fraction) was acidified at pH 2 with 6 N HCl and extracted by SPE (Strata 8B-S100-UBJ, Phenomenex) previously conditioned with 5 ml of dichloromethane, 5 ml of methanol and 5 ml of Milli-Q deionised water. After washing with 5 ml of Milli-Q water and drying the cartridge, PAHs were desorbed with 10 ml of dichloromethane. The eluate was dried in a vacuum evaporator (EZ-2, Genevac, UK) at 30 °C and dissolved with 1 ml of acetonitrile. Total PAHs were determined by HPLC (column: PAHs 00E-3029-EO, Phenomenex, Torrance, CA, USA) using a gradient from 60/40 water/acetonitrile to 100% acetonitrile in 60 min at 1.2 ml/min. External standard quantification was performed using EPA 610 mixture (Supelco, USA) as standard.

For the assessment of the cytotoxity of the WSF, RTG-2 cells were exposed to this WSF and β-galactosidase activity, neutral-red uptake and protein content of cells in 96-well culture plates were measured as previously described (Babín and Tarazona, 2005). The toxicity on *Chlorella vulgaris* was assessed by means of the alga growth inhibition test (OECD test guideline 201). Algae growth was estimated by measuring increases of absorbance ($\lambda = 450$ nm), fluorescence (indicating variations in the chlorophyll content, $\lambda_{emission} = 680$; $\lambda_{excitation} = 430$) and by direct counting using a Neubauer chamber. Toxicity to the cladoceran *Daphnia magna* was studied in acute and reproductive toxicity tests (OECD test guideline 202). The *D. magna* reproduction test was also performed feeding *D. magna* with algae grown in the WSF. All these bioassays were repeated four times. In