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Endocrine and immunological parameters in individuals involved in *Prestige* spill cleanup tasks seven years after the exposure

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ABSTRACT

In November 2002 the oil tanker *Prestige* spilled 63,000 tonnes of heavy oil off the northwest coast of Spain, impacting more than 1000 km of coastline. A general concern led to a huge mobilization of human and technical resources, and more than 300,000 people participated in cleanup activities, which lasted up to 10 months. Some endocrine and immunological alterations were reported in *Prestige* oil exposed subjects for several months. Therefore, the objective of this study was to evaluate if these alterations are still present seven years after the exposure. Fifty-four individuals exposed for at least 2 months were compared to 50 matched referents. Prolactin and cortisol plasma concentrations, percentages of lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+16^+$), plasma levels of circulating cytokines (interleukin (IL) 2, IL4, IL6, IL10, tumour necrosis factor α, and interferon γ), and serum concentrations of neopterin, tryptophan and kynurenine were determined in peripheral blood samples. Results showed significant differences in exposed individuals *vs.* referents only in cortisol (increase), kynurenine and $\%CD16^+56^+$ lymphocytes (both decrease). Time of exposure to the oil or using protective clothes did not influence the results, but effect of using protective mask was observed on neopterin, $\%CD8^+$, $CD4^+/CD8^+$ ratio and IL4. Surveillance of the exposed individuals for early detection of possible health problems related to the endocrine or immunological systems is recommended.

1. Introduction

In November 19, 2002, the oil tanker *Prestige* broke into two and sank in the Atlantic Ocean 130 nautical miles off the Galician coast (NW Spain) at 3500 m depth, releasing about 63,000 tonnes of heavy oil (ITOPF, 2013). The impact of this spill was more serious than that in other similar catastrophes due to the extensive coastal area affected: more than 1000 km of Spanish, Portuguese and French coastlines. Due to its proximity to one of the busiest maritime routes in the world, Galicia had already been polluted in the last decades by previous oil spills, namely Polycommander in 1970, Urquiola in 1976, Andros Patria in 1978 and Aegean Sea in 1992.

The *Prestige* oil was fuel oil no. 6 (bunker C), determined as a complex mixture made of saturated hydrocarbons (22%), aromatic hydrocarbons (50%), and asphaltenes and resins (28%) (CSIC, 2003a). The aromatic fraction was mainly composed of naphthalene, phenanthrene and alkyl derivatives, and the saturated fraction was composed of lineal and cyclic hydrocarbons of variable length (Alzaga et al., 2004). In addition, the presence of different quantities of heavy metals in emulsified samples (with 54–59% water) of *Prestige* oil was also confirmed (Albaigès et al., 2006; CSIC, 2003b).

As a consequence of the high impact that *Prestige* spill had on such rich and valuable natural environment, a general concern led to a huge mobilization of human and technical resources, and more than 300,000 people participated in cleanup activities, which lasted up to 10 months. Some studies reported general acute health problems (Suárez et al., 2005), respiratory symptoms (Zock et al., 2007) and higher frequency of suboptimal scores in mental health (Carrasco et al., 2007) in residents and subjects involved in cleanup operation after *Prestige* spill, as it had already been extensively observed in previous oil spills (reviewed in Aguilera et al., 2010; Goldstein et al., 2011; Levy and Nassetta, 2011). Increases in different genotoxicity parameters, especially in individuals exposed to the oil for several months, were also observed (Laffon et al., 2006; Pérez-Cadahía et al., 2006, 2008a).

Furthermore, until the *Prestige* accident the possible effect of oil exposure on endocrine or immunological parameters had never been investigated. Alterations in prolactin and cortisol plasma levels

Abbreviations: CI, confidence interval; EDTA, ethylenediamine tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GTP, guanosine triphosphate; HPLC, high-performance liquid chromatography; HPW, high-pressure water jets; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; Kyn/Trp, kynurenine to tryptophan ratio; NK, natural killer cells; TNF, tumour necrosis factor.

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were reported in *Prestige* oil exposed subjects (Pérez-Cadahía et al., 2007, 2008b). Moreover, the levels of these hormones were related to some exposure biomarkers, since cortisol was influenced by plasma concentrations of aluminium and nickel inversely and by cadmium positively, and in women there was a strong association between Cd and prolactin levels. Indeed, cortisol concentration appeared to be the most sensitive parameter, among all the genotoxicity and endocrine toxicity parameters evaluated, to the effects of metal exposure (Pérez-Cadahía et al., 2008c). As for immunological variables, significant modifications were also observed in some lymphocyte subpopulations and concentrations of plasma cytokines in individuals who were exposed to *Prestige* oil for several months (Gestal et al., 2004).

Thus, the objective of this study was to evaluate if the endocrine and immunological alterations are still present in subjects involved in *Prestige* oil cleanup activities seven years after the exposure. Prolactin and cortisol plasma concentrations were evaluated as endocrine parameters, and percentages of lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+16^+$), plasma levels of circulating cytokines (interleukin (IL) 2, IL4, IL6, IL10, tumour necrosis factor (TNF) α, and interferon (IFN) γ), and serum concentrations of neopterin, tryptophan and kynurenine were determined as immunological parameters in referents and individuals exposed for at least two months in the period November 2002–September 2003.

2. Materials and methods

2.1. Study population

The exposed group was composed of 54 fishermen and people who worked collecting shellfish living in Galician villages seriously affected by the spill: Fisterra ($N = 17$), Muxía ($N = 17$), Lira ($N = 8$) and O Pindo ($N = 12$) (Fig. 1). They were contacted through the fisherman associations from every village by organizing previous informative sessions. Participation in the cleanup tasks for a minimum of 2 months and 4 h/day within the period November 2002–October 2003 was the inclusion criterion, which was fulfilled by all individuals who showed interest in participating in the study. These subjects were newly recruited, not evaluated in the previous study. The unexposed group included 50 individuals working in University offices or schools, not exposed to any known mutagen in their occupational activities and who did not collaborate in the *Prestige* oil cleanup. They were matched to the exposed on a group basis regarding age, sex and smoking habits. All individuals participating in the study were asked to complete a questionnaire whereby relevant information on demographic characteristics, lifestyle factors, health conditions and occupational history was obtained. Some questions were also included to characterize the exposure conditions (tasks performed, use of protective devices, time of exposure, *etc.*) (Table 1). The exposure time in h was calculated by multiplying the total number of days that the individuals collected oil and the number of hours per day. Exposed subjects were also interviewed about specific symptoms related to oil exposure, experienced from the initial exposure to the oil, including respiratory (breathing problems, suffocation, asthma), dermatological (itch, erythema, and skin, mucous and eye irritation), and psycho-neurological (headache, anxiety, depression, insomnia) symptoms. Regarding the cleanup labours, 76% of the exposed were involved in manual cleaning of coastal areas, 2% used high-pressure water jets to detach oil from the rocks, 7% cleaned both manually and with those machines, 6% were involved in cleaning the sea from boats, and 9% cleaned the sea and also did manual cleaning. Ethical standards laid down in the 1964 Declaration of Helsinki were followed in this work. All individuals signed an informed consent.

2.2. Sample collection

Samples were collected between November 2009 and February 2010. Peripheral blood was drawn early in the morning before the

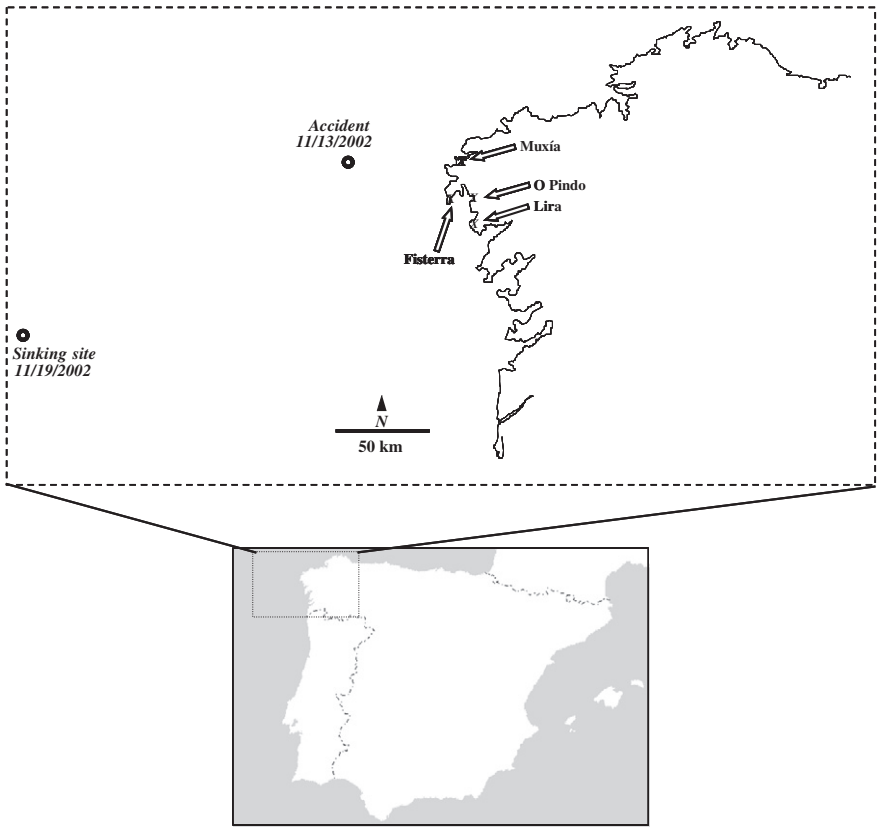

Fig. 1. Map of Galician shoreline (north-western Spain) indicating the study sampling places and the sites of *Prestige* initial accident and final sinking.