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ScienceDirect
Chemosphere 71 (2008) 447–455
CHEMOSPHERE
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# Relationship between blood concentrations of heavy metals and cytogenetic and endocrine parameters among subjects involved in cleaning coastal areas affected by the 'Prestige' tanker oil spill

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Received 8 May 2007; received in revised form 18 October 2007; accepted 28 October 2007
Available online 25 January 2008

**Abstract**

The sinking of the 'Prestige' oil tanker in front of the Galician coast (NW of Spain) in November 2002 offered a unique opportunity to analyze intermediate cytogenetic and endocrine effects among people exposed to the complex mixture of substances that oil constitutes, including several toxic heavy metals. In this work we evaluated the relationship between exposure to heavy metals (blood concentrations of aluminium, cadmium, nickel, lead and zinc) and genotoxic parameters (sister chromatid exchanges, micronucleus test and comet assay) or endocrine parameters (plasmatic concentrations of prolactin and cortisol) in subjects exposed to 'Prestige' oil during cleaning tasks developed after the spillage. Concentrations of lead were significantly related to the comet assay even after adjusting by age, sex and smoking. Cortisol concentrations were significantly influenced by aluminium, nickel (both, inversely) and cadmium (positively). Women had clearly higher concentrations of prolactin and cortisol, even when adjusting by age, smoking, cadmium, aluminium or nickel. Plasmatic cortisol was jointly influenced by gender, smoking and aluminium or nickel (all $p < 0.05$). In women there was a strong relationship between concentrations of cadmium and prolactin ($\beta = 0.37$, $p = 0.031$). When the effects of cadmium, aluminium and nickel on cortisol were simultaneously assessed, only the latter two metals remained statistically significant. Among parameters analysed, cortisol appeared to be the most sensitive to the effects of metal exposure. Plasma levels of cortisol deserve further evaluation as a potentially relevant biomarker to assess the effects of exposure to heavy metals.


*Keywords:* Sister chromatid exchanges; Micronucleus test; Comet assay; Prolactin; Cortisol

## 1. Introduction

Heavy metals constitute a wide and special group of chemical agents: while many of them are essential to the development of a variety of physiological functions, others, as cadmium and lead, do not have well recognised roles or are toxic, even at low doses (Thompson et al., 1992; Merzenich et al., 2001). Furthermore, many heavy metals bioaccumulate in several compartments of the human body and have slow excretion rates (Elinder et al., 1994; Hayes, 1997). Hence, in some circumstances exposure to these agents may be a public health concern.

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doi:10.1016/j.chemosphere.2007.10.053

Among the large number of heavy metals present in the human environment several stand out because of their well known toxicity and noxious effects on living organisms: they include aluminium, cadmium and nickel, which are classified as human carcinogens (group 1) by the International Agency for Research on Cancer (IARC), and lead, classified as possible human carcinogen (group 2B). Besides interference at distinct steps of several DNA repair mechanisms (Hartwig and Schwerdtle, 2002), some of their carcinogenic effects are related to DNA–protein crosslinking (Kasprzak, 2002); the effects of metals on chromatin have been observed *in vitro* and *in vivo*. Some metals may also contribute to cause diseases of complex aetiology through indirect genotoxic and epigenetic mechanisms (Luch, 2005). In addition, some metals have been characterized as endocrine disruptors, and one of their targets is the hypothalamus-pituitary-gonadal (Lafuente et al., 2003, 2004a, 2005) and/or hypothalamus-pituitary-adrenal axis (Stejskal et al., 2006), which is crucial for vertebrates to cope with stressors (Lacroix and Hontela, 2004). Plasmatic prolactin and cortisol are especially sensitive to physical and psychical stress (Sobrinho, 2003; Dahlgren et al., 2005).

Aluminium and its compounds are common food and water contaminants. While no biological function of this element has been identified, some aspects of aluminium toxicity are well described (Berthon, 1996; Corain et al., 1996; Reinke et al., 2003). Its relation to some disorders such as microcytic anaemia, and to neurodegenerative disorders as Alzheimer's and Parkinson's diseases have been assessed (Berthon, 1996; Corain et al., 1996; Yokel, 2002). It has been also reported that physiologically relevant amounts of aluminium are capable of inducing alterations in gene expression programs that may support downstream pathogenic responses and brain cell dysfunction (Alexandrov et al., 2005).

Cadmium is one of the most toxic heavy metals, capable of generating mutagenic and genotoxic events (Waalkes, 2003). The carcinogenicity of cadmium and nickel have long been recognized; mechanisms include the induction of oxidative DNA damage (Kasprzak, 1995; Bal et al., 2000) and epigenetic alterations like gene silencing by changes in DNA methylation patterns (Klein and Costa, 1997; Luch, 2005).

Because of its persistence in the environment and effects on humans, exposure to lead has long been of public health concern. In eukaryotic cells this metal is usually genotoxic, though mechanisms are only partly known; they possibly involve indirect damage to DNA affecting the stabilization of chromatin, or interaction with repair processes (Vaglenov et al., 2001).

Zinc stands out for having some opposite effects to the other metals: it is co-released with neurotransmitters by neurons, and zinc supplements may delay the progression of Alzheimer's disease (Potocnik et al., 1997). Protective effects of zinc against cadmium and lead toxicity have also been described (Leblond and Hontela, 1999; Nampoothiri et al., 2007).

The sinking of the 'Prestige' oil tanker in front of the Galician coast (NW of Spain) in November 2002 offered a unique opportunity to analyze intermediate cytogenetic and endocrine effects among people who were exposed to a variety of environmental agents while cleaning the coast (Porta et al., 2004; Gestal et al., 2004). The composition of 'Prestige' oil was determined by the Spanish National Research Council as 50% aromatic hydrocarbons, 22% saturated hydrocarbons and 28% resins and asphalthene (CSIC, 2003a). In addition, presence of different quantities of heavy metals in emulsified samples (with 54–59% water) of 'Prestige' oil was also confirmed (CSIC, 2003b; Albaigès et al., 2006).

In order to test the hypothesis that exposure to some heavy metals might be associated with genotoxicity events or several endocrine alterations, we evaluated the relationship between blood concentrations of aluminium, cadmium, nickel, lead and zinc and genotoxic or endocrine effects in a human population exposed to 'Prestige' oil during cleaning tasks developed after the spillage. The assays sister chromatid exchanges (SCE), micronucleus (MN) test, and single cell gel electrophoresis (comet) assay were selected as genotoxicity biomarkers, and plasmatic prolactin and cortisol as indicators of endocrine alterations.

## 2. Materials and methods

### 2.1. Study population

The study population comprised 179 healthy individuals who participated in the cleaning of coastal areas affected by the 'Prestige' oil spill, which occurred on November 2002. Their mean age was 32 years and 49% were women (Table 1). Sixty individuals were college student volunteers (V) from Spanish universities engaged in the cleaning of the oil in beaches for five consecutive days (from Monday to Friday) during 4 h $day^{-1}$. The other subjects were workers hired by the Galician government for the manual collection of the oil in beaches (MW, $N = 59$), and workers hired to clean the rocks using high-pressure machines (HPW, $N = 60$) during 6.5 h $day^{-1}$. At the time of the study MW had been exposed to oil for 4 months and HPW for 3 months. Information on smoking was elicited through a self-administered questionnaire. Smoking was more common among males (Table 1); it was thus treated as a potential confounder in statistical analyses. None of the study participants had undergone diagnostic tests (e.g., X-rays) or received significant medical treatment in the 3 months prior to the study.

Peripheral blood samples were collected between 24 March and 12 May 2003. Blood was drawn before the beginning of the working shift in the morning (08:00–09:00 am), codified to ensure a blind study, and immediately transported refrigerated to the laboratory. Two samples were obtained from each donor, one in a lithium heparin container for determination of heavy metals, hormones, sister chromatid exchanges (SCE) and the micronu-