Environment International 59 (2013) 103–111

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

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

Blanca Laffon [a,*], Francisco Aguilera [a,b], Julia Ríos-Vázquez [a], Julia García-Lestón [a], Dietmar Fuchs [c], Vanessa Valdiglesias [a], Eduardo Pásaro [a]

[a] Toxicology Unit, Department of Psychobiology, University of A Coruña, Edificio de Servicios Centrales de Investigación, Campus Elviña s/n, 15071-A Coruña, Spain
[b] Medical Technology, Faculty of Medicine, University of Valparaiso, Chile
[c] Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Center for Chemistry and Biomedicine, Innrain 80, 4th Floor, Room M04-313, A-6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 6 March 2013
Accepted 27 May 2013
Available online 20 June 2013

Keywords:
Circulating cytokines
Endocrine toxicity
Lymphocyte subsets
Neopterin
Prestige oil spill
Tryptophan degradation

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.

* Corresponding author at: Toxicology Unit, University of A Coruña, Edificio de Servicios Centrales de Investigación, Campus Elviña s/n, 15071-A Coruña, Spain. Tel.: +34 981 167000; fax: +34 981 167172.
E-mail address: blaffon@udc.es (B. Laffon).

0160-4120/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envint.2013.05.014

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

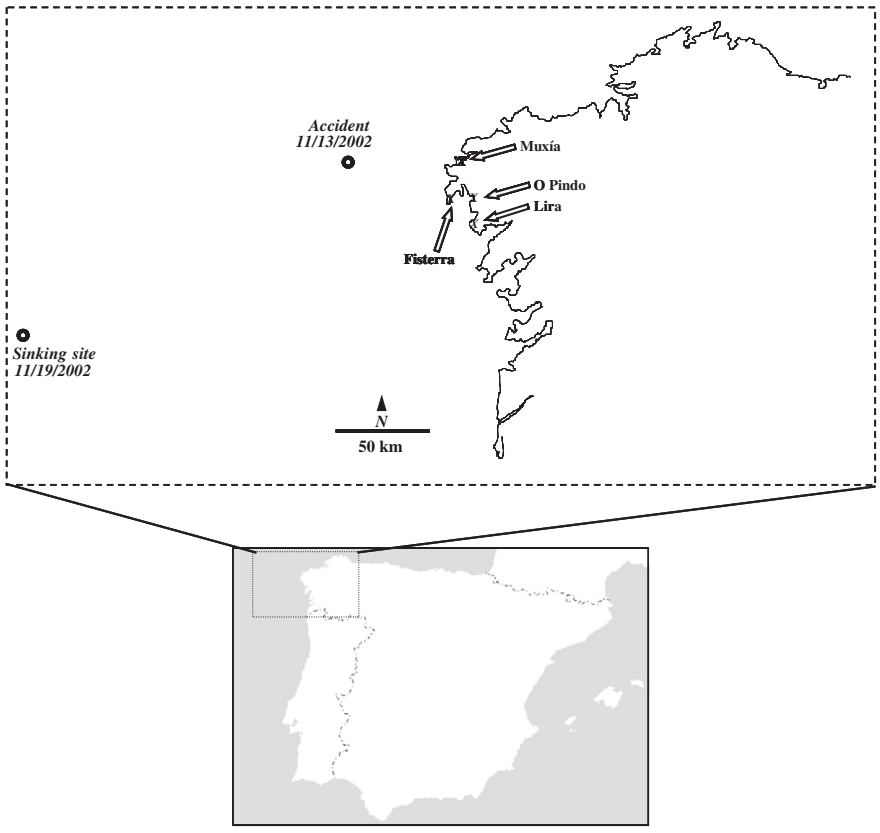

Fig. 1. Map of Galician shoreline (north-western Spain) indicating the study sampling places and the sites of *Prestige* initial accident and final sinking.

Table 1
Description of the study population.

	Controls	Exposed	*P*-value
Total	50 (48.1%)	54 (51.9%)	
Gender			
Males	30 (60.0%)	33 (61.1%)	0.908[b]
Females	20 (40.0%)	21 (38.9%)	
Age[a]	47.0 ± 10.2 (28–65)	47.9 ± 11.2 (29–68)	0.672[c]
Smoking habits			
Non-smokers	40 (80.0%)	37 (68.5%)	0.182[b]
Smokers	10 (20.0%)	17 (31.5%)	
Cigarettes/day[a]	17.0 ± 11.7 (2–35)	15.8 ± 9.4 (2–30)	0.779[c]
Years smoking[a]	27.5 ± 10.4 (5–40)	21.9 ± 13.1 (6–49)	0.233[c]
Pack-years[a]	23.2 ± 14.2 (0.5-45.0)	16.7 ± 15.9 (1.6-67.5)	0.280[c]
Exposure time[a] (months)		9.0 ± 1.9 (2–10)	
Exposure time[a] (h)		1887 ± 616 (450–3000)	
Protective devices			
Gloves		49 (90.7%)	
No gloves		5 (9.3%)	
Clothes		44 (81.5%)	
No clothes		10 (18.5%)	
Mask		12 (22.2%)	
Mask occasional		28 (51.9%)	
No mask		14 (25.9%)	

[a] Mean ± standard deviation (range).
[b] Chi-square test.
[c] Student's *t* test.

working shift (between 8 and 10 am) by venipuncture in vacuum blood tubes containing ethylenediamine tetraacetic acid (K_2-EDTA) or no anticoagulant. In order to ensure a blind study, all samples were coded just after collection. Fresh whole EDTA-blood was used to determine the percentages of lymphocyte subpopulations. The remaining EDTA-blood was centrifuged, and supernatant plasma was aliquoted and stored at −80 °C to be used for analysing the concentrations of cytokines. Serum obtained from the whole blood without anticoagulant was aliquoted and stored at −80 °C for determination of cortisol, prolactin, neopterin, tryptophan, and kynurenine. All analyses were performed in duplicate.

2.3. Prolactin and cortisol

Commercially available enzyme-linked immunosorbent assays (ELISAs) were used for the analysis of prolactin and cortisol concentrations in serum samples, following instructions provided (DRG International Inc.). Sensitivities of the assays were 0.35 ng/ml for prolactin and 2.5 ng/ml for cortisol.

2.4. Lymphocyte subpopulations

Fresh whole blood (100 μl) was used to analyse the percentage of the different lymphocyte subpopulations by means of flow cytometry (García-Lestón et al., 2011). The lymphocyte subsets determined were $CD3^+$ T-lymphocytes, $CD4^+$ T-helper lymphocytes, $CD8^+$ T-cytotoxic lymphocytes, $CD19^+$ B-lymphocytes, and $CD56^+16^+$ natural killer (NK) cells.

2.5. Circulating cytokines

The cytokines IL2, IL4, IL6, IL10, IFNγ, and tumour TNFα were quantified in plasma using the commercial kit BD Cytometric Bead Array (CBA) Human Th1/Th2 Cytokine Kit II (Becton Dickinson), according to the instructions provided by the manufacturer. In brief, phycoerythrin-conjugated detection antibodies were mixed with bead populations coated with capture antibodies specific for the mentioned cytokines and then incubated with test samples to form sandwich complexes. At least 2000 events were captured for each sample in a FACSCalibur flow cytometer using the BD CBA Software (Becton Dickinson) for quantification. The limits of detection were 2.4 pg/ml for IL2, 1.3 pg/ml for IL4, 3.2 pg/ml for IL6, 1.8 pg/ml for IL10, 2.6 pg/ml for TNFα and 5.8 pg/ml for IFNγ.

2.6. Neopterin

A commercially available ELISA kit (BRAHMS, Hennigsdorf, Germany), was used to determine serum neopterin concentration according to the manufacturer's recommendations. The limit of detection was 2 nmol/l neopterin.

2.7. Tryptophan and kynurenine

Tryptophan and kynurenine concentrations in serum samples were measured by a high-performance liquid chromatography (HPLC) methodology with 3-nitro-L-tyrosine as internal standard following Widner et al. (1997). The kynurenine to tryptophan ratio (Kyn/Trp), expressed in micromoles kynurenine per millimole tryptophan, was calculated to estimate the extent of tryptophan breakdown.

2.8. Statistical analysis

Univariate analysis was used to carry out a population description, comparing exposed and unexposed groups by socio-demographic and lifestyle factors. The Student's *t*-test and the Pearson's Chi-square test were applied for continuous and categorical variables, respectively.

Student's *t*-test was carried out to preliminarily assess the effect of exposure. A logarithmic transformation of the data was applied to prolactin, cortisol, kynurenine, Kyn/Trp to achieve a better approximation to the normal distribution. No transformation was needed for tryptophan, lymphocyte subsets and cytokines. As no improvement was achieved with transformation, the Mann–Whitney *U*-test was applied to neopterin. A multiple linear regression analysis was performed to estimate the effect of exposure and possible confounders on the log-transformed data. All models included age, gender and smoking habits (pack-years).

An ancillary regression analysis was carried out to assess the effect of exposure time and using protective devices during the cleanup tasks, only in the exposed population. Adjustment for age, gender and smoking habits was applied. Furthermore, a logistic regression model including adjustment for age, gender and smoking was also performed in the exposed group to identify the relationship between selected symptoms and exposure time, use of protective equipment, neopterin or tryptophan breakdown parameters.

Associations between variables were analysed by Spearman's rank correlation. The level of statistical significance was set at 0.05. All analyses were performed using the SPSS software package V. 20 (SPSS, Inc.) and STATA software package V. 9.1 (StataCorp LP).

3. Results

Table 1 shows the general characteristics of the study population. No significant differences were observed regarding gender, age or smoking habit distribution between the referent and exposed groups. Ex-smokers were grouped with non-smokers, since they gave up smoking at least 4 years ago (mean 12.7 ± 7.0 years) and they were few (only 11 unexposed and 6 exposed). The mean exposure period was around 9 months, and it was quite long for most individuals (8–10 months for 82% of them).

According to the reference ranges previously proposed for prolactin and cortisol (Melmed et al., 2011), neopterin (Murr et al., 2002), and all the lymphocyte subpopulations (García-Dabrio et al., 2012), 3.7% control males exceeded the range for prolactin, 14.0% controls and 9.3% exposed individuals exceeded the range for neopterin, 36.0% controls and 11.1% exposed were out of the range for $\%CD3^+$ cells, 28.0% controls and 20.4% exposed were out of the range for $\%CD4^+$ cells, 18.0% controls and 13.0% exposed were out of the range for $\%CD8^+$ lymphocytes, 26.0% controls and 16.7% exposed were out of the range for $\%CD19^+$ cells,

Table 2
Results of effect biomarkers in the study groups.

	Controls		Exposed		*P*-value
	N	Mean ± SD	*N*	Mean ± SD	
Prolactin (ng/ml)	50	7.42 ± 0.38	54	9.30 ± 1.00	0.081
Cortisol (ng/ml)	50	149.56 ± 2.27	54	160.91 ± 3.11	**0.004**
Neopterin (nmol/l)	50	5.83 ± 0.29	54	5.53 ± 0.20	0.469
Tryptophan (μmol/l)	50	73.70 ± 2.17	53	69.96 ± 1.78	0.186
Kynurenine (μmol/l)	49	3.08 ± 0.23	53	2.53 ± 0.18	0.039
Kyn/Trp (μmol/mmol)	49	38.19 ± 2.49	53	34.86 ± 2.19	0.327
%$CD3^+$	45	65.96 ± 1.74	44	69.87 ± 1.35	0.080
%$CD4^+$	45	35.61 ± 1.64	44	38.76 ± 1.72	0.189
%CD8	45	25.63 ± 1.32	44	22.90 ± 1.29	0.142
$CD4^+/CD8^+$	45	1.62 ± 0.15	44	2.05 ± 0.18	0.070
%$CD19^+$	45	9.23 ± 0.67	44	10.47 ± 0.76	0.223
%$CD16^+56^+$	45	23.46 ± 1.64	44	16.75 ± 1.33	**0.002**
IL2 (pg/ml)	35	5.65 ± 0.32	40	5.64 ± 0.09	0.572
IL4 (pg/ml)	30	1.72 ± 0.95	37	0.83 ± 0.09	0.555
IL6 (pg/ml)	35	8.73 ± 0.73	40	8.77 ± 0.29	0.439
IL10 (pg/ml)	35	2.43 ± 0.50	40	2.34 ± 0.08	0.110
TNFα (pg/ml)	35	10.80 ± 0.13	40	10.87 ± 0.04	0.481
IFNγ (pg/ml)	35	6.32 ± 0.23	40	6.65 ± 0.19	0.221

and 33.0% controls and 18.5% exposed exceeded the range for %$CD16^+56^+$ lymphocytes (Table 2). Results for tryptophan, kynurenine and Kyn/Trp ratio were similar to the ones previously described (Capuron et al., 2011; Frick et al., 2004). There are no reference levels for cytokine concentrations, and the referent values reported are very diverse among studies (COPE, 2011). Still, concentrations obtained were similar to those reported by Gestal et al. (2004) in individuals exposed to *Prestige* oil.

Univariate comparison by study group showed a significant increase in cortisol concentration and significant decreases in kynurenine levels and %$CD16^+56^+$ lymphocytes in the exposed individuals when compared to the referents (Table 2). Multivariate analysis was then applied to assess the effect of exposure, gender, age and smoking habits, mutually adjusted; data are shown in Tables 3–5. They confirmed the results of univariate analysis regarding differences between exposed and unexposed. In females, a significant decrease in the mean ratio with regard to males was obtained for tryptophan and %$CD16^+56^+$ lymphocytes, and significant increases for %$CD4^+$ and %$CD19^+$ cells, for $CD4^+/CD8^+$ ratio, and for IFNγ. The effect of age was observed in prolactin (decrease), and in neopterin (increase). Smoking (pack-years) influenced significantly only %$CD16^+56^+$ cells, with a slight decrease in the mean ratio.

An additional multivariate analysis was performed just in the exposed group to evaluate the effect of the time of exposure and wearing protective devices while working in contact with the oil (clothes and mask). Since 91% of the exposed individuals used gloves the effect of using them was not assessed. Fig. 2 shows only the significant results obtained. A tendency of decrease in neopterin levels was observed in the individuals who did not wear always the mask, significant for those who never used it. Increase in %$CD8^+$ lymphocytes, and subsequent decrease in $CD4^+/CD8^+$ ratio, were observed related to the absence of using mask, significant in the group with occasional use. Besides, all individuals who did not use mask showed significant increase in IL4 concentrations. No effect of either wearing protective clothes or exposure time was obtained.

Associations between parameters were analysed and significant correlations were observed for neopterin with the three tryptophan metabolism parameters ($r = 0.312$, $P = 0.001$ for kynurenine, $r = -0.217$, $P = 0.028$ for tryptophan, and $r = 0.395$, $P < 0.001$ for Kyn/Trp).

The exposed individuals also provided information on certain symptoms related to exposure to the oil compounds, namely respiratory, dermatological, and psycho-neurological, experienced from the exposure. A quarter of them (26%) reported respiratory symptoms, 22% dermatological symptoms and 39% psycho-neurological symptoms. Results of logistic regression models showed that none of the symptoms was influenced by the time of exposure or the use of protective equipment (data not shown). Nevertheless, significant increases in the mean ratio of psycho-neurological symptoms were observed with neopterin levels (3.27, 95% confidence interval 1.11–9.57, $P = 0.031$) and in females as compared to males (28.45, 95% confidence interval 2.75–294.24, $P = 0.005$).

4. Discussion

Numerous compounds contained in heavy oils, such as heavy metals and polycyclic aromatic hydrocarbons, present recognised endocrine disrupting properties (reviewed in De Coster and van Larebeke, 2012). Besides, the immunotoxic potential of several of these chemicals is also well known. For instance, Veraldi et al. (2006) reviewed the immunotoxic effects of different substances widely used in work environments, taking into account the type of immunotoxicity and strength of evidence and power. They found evidence of immunosuppressive activity for some oil compounds and, as an index of immunotoxicity, classified benzene and polycyclic aromatic hydrocarbons as “strong”, and toluene as “weak”. Nevertheless, until *Prestige* accident endocrine and immunological biomarkers had never being evaluated in subjects exposed to spilled oils.

Alterations in the levels of the hormones prolactin and cortisol reflect some kind of stress processes (Dahlgren et al., 2005; Sobrinho, 2003), and prolactin has also been proposed as a biomarker of neuroendocrine effect in people exposed to pollutants targeting the central dopaminergic function (Catalani et al., 2012). These two endocrine parameters were evaluated in plasma samples from people exposed to *Prestige* oil, finding decreases in cortisol – in all exposed groups

Table 3
Effect of exposure, age, gender and smoking habits on hormones, neopterin and tryptophan metabolism products.

	Prolactin		Cortisol		Neopterin		Tryptophan		Kynurenine		Kyn/Trp	
	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI
Exposure												
Controls	1.00		1.00		1.00		1.00		1.00		1.00	
Exposed	1.16	(0.99–1.36)	1.07**	(1.02–1.12)	0.96	(0.87–1.06)	0.95	(0.88–1.02)	0.82*	(0.70–0.98)	0.92	(0.78–1.08)
Gender												
Males	1.00		1.00		1.00		1.00		1.00		1.00	
Females	1.03	(0.87–1.21)	1.02	(0.98–1.07)	0.94	(0.86–1.05)	0.91*	(0.84–0.98)	0.99	(0.84–1.19)	1.08	(0.91–1.27)
Age (years)	0.99*	(0.98–1.00)	0.99	(0.99–1.00)	1.01*	(1.00–1.01)	1.00	(0.99–1.00)	1.01	(0.99–1.02)	1.01	(0.99–1.01)
Smoking habits (pack-years)	0.99	(0.98–1.01)	1.00	(0.99–1.00)	1.00	(0.99–1.00)	0.99	(0.99–1.00)	1.00	(0.99–1.01)	1.00	(0.99–1.01)

CI: confidence interval.
* $P < 0.05$.
** $P < 0.01$.

Table 4
Effect of exposure, age, gender and smoking habits on lymphocyte subsets.

	%CD3$^+$		%CD4$^+$		%CD8$^+$		CD4$^+$/CD8$^+$		%CD19$^+$		%CD16$^+$56$^+$	
	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI
Exposure												
Controls	1.00		1.00		1.00		1.00		1.00		1.00	
Exposed	1.07	(0.99–1.15)	1.10	(1.96–1.25)	0.90	(0.77–1.04)	1.22	(0.97–1.54)	1.13	(0.95–1.35)	0.69**	(0.56–0.86)
Gender												
Males	1.00		1.00		1.00		1.00		1.00		1.00	
Females	1.04	(0.97–1.12)	1.26**	(1.10–1.44)	0.91	(0.78–1.07)	1.38**	(1.09–1.76)	1.39**	(1.16–1.67)	0.70**	(0.56–0.88)
Age (years)	0.99	(0.99–1.00)	1.00	(0.99–1.01)	0.99	(0.98–1.00)	1.01	(0.99–1.02)	1.00	(0.99–1.01)	1.01	(0.99–1.01)
Smoking habits (pack-years)	1.02	(0.94–1.10)	1.01	(0.99–1.01)	1.00	(0.99–1.01)	1.01	(0.99–1.01)	1.01	(0.99–1.01)	0.99*	(0.98–0.99)

CI: confidence interval.
* $P < 0.05$.
** $P < 0.01$.

regarding to the referents, including short-term exposed volunteers and long-term exposed workers – and in prolactin – only in long-term exposed subjects using high-pressure water jets (HPW) – (Pérez-Cadahía et al., 2007). In the same study, increasing sample size from 60 to 180 exposed individuals the results showed a significant decrease in cortisol only in HPW individuals but no alteration in prolactin related to the exposure (Pérez-Cadahía et al., 2008b). Long-term follow up studies on endocrine disruption and genotoxic impacts on humans and wildlife were recommended by Ji et al. (2011), since they observed these capabilities in sediments collected from *Hebei Spirit* oil spill (South Korea, 2007) two years after the spill.

Changes in cells of the immune system are important indicators of systemic response of the body to xenobiotics introduced *via* different routes (inhalation, dermal, ingestion), such as those contained in the air pollution (Dutta et al., 2012). There are evidences linking these changes with exposure to immunotoxic compounds and in turn with alterations of the immune response (Oh et al., 2005; Tulinska et al., 2004). Gestal et al. (2004) reported significant decreases in %CD3$^+$ and %CD4$^+$ lymphocytes and increases in %CD8$^+$ cells together with IL2, IL4, IL10 and IFNγ plasma concentrations in workers involved in manual cleanup of *Prestige* oil for 3 months. They also observed some of these effects in workers using high-pressure water jets on the rocks and exposed for 4 months, namely increases in IL2, IL4 and IL10. But no alteration was observed in a group of volunteers exposed for only 5 days, neither in %CD19$^+$ subset and IL6 and TNFα levels in any of the groups analysed. Nevertheless, all levels reported in the exposed and referent individuals were within the reference ranges. After Deepwater Horizon oil spill (Gulf of Mexico, 2010) an *in vivo* study using murine models showed the sensitizer potential of COREXIT 9500A, the primary dispersant used in cleanup efforts (Anderson et al., 2011), but dispersants were not used in most previous great spills.

In this new evaluation of endocrine and immunological biomarkers (18 endpoints analysed) carried out seven years after the exposure to *Prestige* oil, significant differences in exposed individuals *vs.* unexposed were only observed in cortisol (increase), kynurenine and %CD16$^+$56$^+$ lymphocytes (both decrease). The increase in cortisol in the exposed subjects, contrasting with the decrease initially detected, suggests an alteration in the endocrine system. Significantly higher levels of plasma cortisol have been reported in outdoor workers chronically exposed to urban pollution, which shares several compounds with oil (Rosati et al., 2011; Tomei et al., 2003), and it has been established that a chronic increase in hypothalamic pituitary–adrenal axis activity, and the subsequent increase in cortisol, is associated with negative health outcome (Rosati et al., 2011). Still, it must be taken into account that, apart from the exposure, cortisol levels can be influenced by a great variety of factors, including primarily not only circadian rhythms but also genetic and environmental factors (Franz et al., 2010). Furthermore, the fact that the difference between the exposed and the referents can include some occupational exposure inherent to the fishing work, apart from their participation in the cleanup, must also be considered. Moreover, values obtained fell within the reference range for healthy subjects in both study groups.

Tryptophan is an essential proteinogenic amino acid and neurotransmitter precursor which is degraded by the enzymes tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase (IDO) to form kynurenine. The latter enzyme is stimulated by IFNγ (Taylor and Feng,

Table 5
Effect of exposure, age, gender and smoking habits on circulating cytokines.

	IL2		IL4		IL6		IL10		TNFα		IFNγ	
	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI	Mean ratio	95% CI
Exposure												
Controls	1.00		1.00		1.00		1.00		1.00		1.00	
Exposed	1.02	(0.95–1.11)	1.16	(0.63–2.12)	1.04	(0.93–1.15)	1.17	(0.98–1.40)	1.01	(0.99–1.03)	1.06	(0.97–1.16)
Gender												
Males	1.00		1.00		1.00		1.00		1.00		1.00	
Females	0.99	(0.92–1.07)	1.60	(0.88–2.91)	1.03	(0.93–1.15)	1.16	(0.97–1.39)	1.02	(0.99–1.04)	1.13**	(1.03–1.24)
Age (years)	0.99	(0.99–1.00)	0.99	(0.97–1.03)	0.99	(0.99–1.01)	1.00	(0.99–1.01)	1.00	(1.00–1.00)	1.00	(0.99–1.00)
Smoking habits (pack-years)	1.00	(0.99–1.00)	1.01	(0.98–1.03)	1.00	(0.99–1.01)	0.99	(0.98–1.00)	1.00	(0.99–1.00)	1.00	(0.99–1.01)

CI: confidence interval.
** $P < 0.01$.

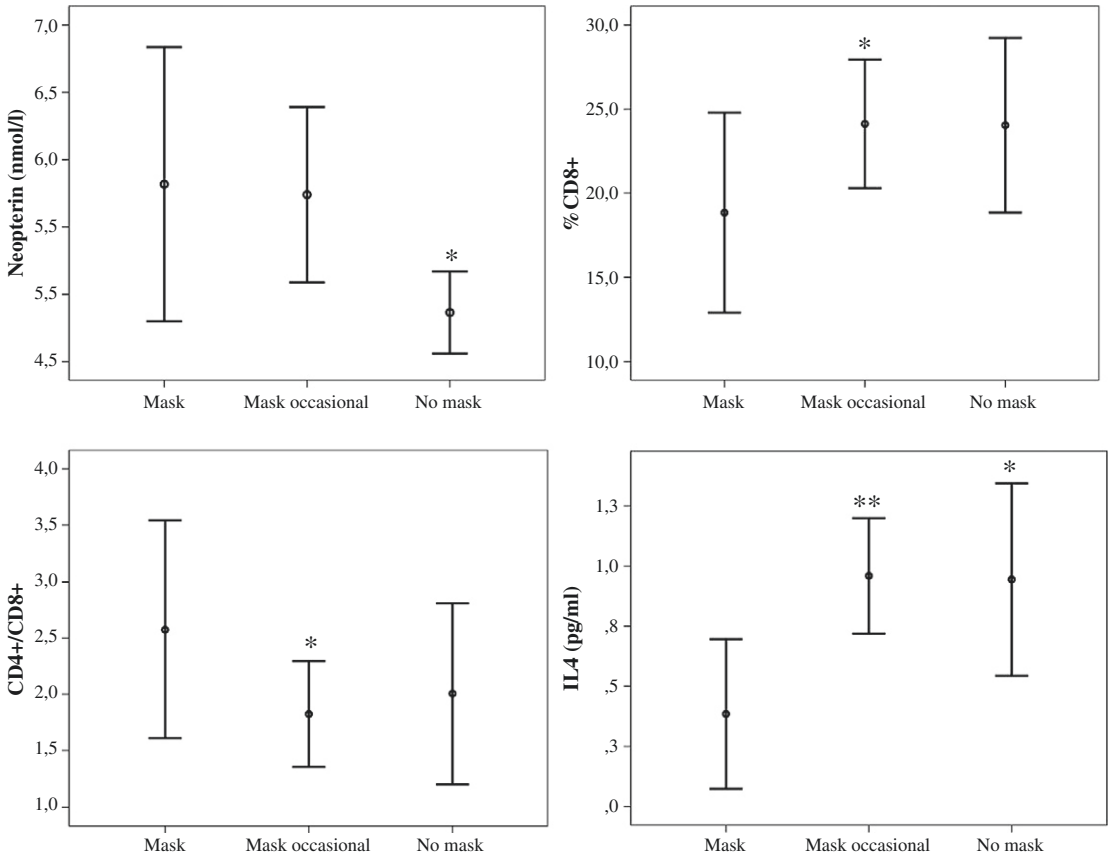

Fig. 2. Effect of using protective mask on neopterin, %$CD8^+$ lymphocytes, $CD4^+/CD8^+$ ratio and IL4 concentration. Results corrected by age, gender and smoking habits. Bars represent the mean 95% confidence interval. *$P < 0.05$; **$P < 0.01$, significant difference with regard to using mask.

1991). IFNγ stimulation is also involved in the production of neopterin by the enzyme guanosine triphosphate (GTP)-cyclohydrolase I from GTP by monocytes and macrophages (Schroecksnadel et al., 2010). Thus, neopterin concentration in body fluids is a sensitive marker of activation of the immune system, specifically Th1 or cell-mediated response. Neopterin concentrations are increased in infectious, autoimmune, cardiovascular and neurodegenerative diseases, malignant tumours, and during rejection episodes in allograft recipients (reviewed in Murr et al., 2002). Besides, the spectrum of diseases in which increased degradation of tryptophan has been described is very similar to the one with increased neopterin production (reviewed in Schröcksnadel et al., 2006; Widner et al., 2002). Serum neopterin concentrations and the extent of tryptophan breakdown have been used as immunotoxicity biomarkers in occupational exposures to different chemicals, such as silica (Altindag et al., 2003), aluminium (Baydar et al., 2005), ionizing radiation (Engin et al., 2005), lead (Engin et al., 2006; García-Lestón et al., 2012) and zinc (Sarac et al., 2013). Furthermore, a strong association has been described between neopterin concentration and the rate of tryptophan degradation (Kyn/Trp ratio) (Schröcksnadel et al., 2006). Although this association was confirmed in the present study, since neopterin significantly correlated to tryptophan (inversely), kynurenine and Kyn/Trp (both positively), the slight decrease in kynurenine concentration in the exposed group ($P = 0.039$) was not accompanied by an increase in tryptophan level, or by similar decreases in the Kyn/Trp ratio, and neopterin and IFNγ concentrations, so the relevance of this finding is uncertain. Furthermore, since numerous biomarkers were analysed in this work, finding statistical significances purely by chance may be expected. Applying Bonferroni's correction for multiple comparisons, the result for kynurenine loses its statistical significance ($P = 0.027$, value obtained in the multiple linear regression analysis), but cortisol and %$CD16^+56^+$ cells remain significant ($P \leq 0.001$ for both).

In the current study we did not observe significant differences in any of the immunological parameters reported to be initially altered in the *Prestige* exposed individuals (lymphocyte subsets and circulating cytokines) (Gestal et al., 2004). The only variable significantly modified was %$CD16^+56^+$ cells, not evaluated in the previous work. NK ($CD16^+56^+$) cells are effector lymphocytes of the innate immune system that control several types of tumours and microbial infections by limiting their spread and subsequent tissue damage, and are also engaged in reciprocal interactions with dendritic cells, macrophages, T cells and endothelial cells (reviewed in Vivier et al., 2008). Since cortisol suppresses the immune response, maybe the overall decrease of %$CD16^+56^+$ cells observed in the exposed group is an indirect consequence of the increase in cortisol in those individuals. The decrease obtained in NK cells, although falling within the reference range, might have serious consequences, since these cells are an important defence against viral infections and are the major cell type involved in immune surveillance against neoplastic cells. Previous studies on human populations also reported a decrease in NK cell count or function related to relatively similar exposures. Moszczyński et al. (1996) showed that the counts of NK cells were reduced in men exposed to organic solvents, including benzene, toluene and xylene; decrease of NK-mediated cytotoxicity against human NK-sensitive target was reported in people from an industrial area exposed to environmental pollution (Bulog et al., 2011), and woman workers at a chemical reagent factory exposed to volatile organic compounds – benzene, ethylbenzene, toluene and xylene among them – showed significantly decreased expression of NK cell activation receptors (De Celis et al., 2008).

According to the questionnaires, there was no incidence of cancer among the exposed and unexposed subjects in the present study, and only 1 case of recent infection (less than 2 months) corresponding to an exposed individual. Unfortunately, no information was collected on former infection processes. Besides, evaluation of NK cell function is also a key point to be addressed in future studies.

Some physiological and lifestyle factors (gender, age and smoking) influenced the endocrine and immunological biomarkers evaluated. Gender modified tryptophan, %$CD4^+$, $CD4^+/CD8^+$, %$CD19^+$ and %$CD16^+56^+$ cells, and IFNγ; all parameters but tryptophan and %$CD16^+56^+$ cells were higher in females than in males. Our results on lymphocyte subpopulations support other previous studies in Spanish and Italian subjects consistently reporting higher %$CD4^+$ and lower %$CD16^+56^+$ lymphocytes in females (Andreu-Ballester et al., 2012; García-Dabrio et al., 2012; Santagostino et al., 1999).

Decrease in immunocompetence is one of the most important changes associated with ageing. It may lead to infections or autoimmune diseases and be involved in the pathogenesis of several age-related disorders, such as cardiovascular or neurodegenerative disorders (Wick and Grubeck-Loebenstein, 1997). Although changes in some lymphocyte subsets with age (García-Dabrio et al., 2012) and alterations in the cytokine production in the elderly (reviewed in Rink et al., 1998) have been reported, none of these changes was observed in our study. One possible explanation for this lack of influence of age on such parameters is the fact that most of the subjects were quite young, with very few aged over 60 years. Similarly, Santagostino et al. (1999) also failed to observe any effect of age on lymphocyte subpopulations analysing a group of 968 individuals aged 18–70 years.

Furthermore, the observed increase in neopterin production with increasing age has been profusely documented (Frick et al., 2004; García-Lestón et al., 2012; Murr et al., 2002; Spencer et al., 2010), suggesting that ageing in healthy people is related to a process of immune activation that may be clinically non-detectable but may lead to associated diseases such as atherosclerosis or dementia.

Smoking has been demonstrated to alter the levels of prolactin (Xue et al., 2010), cortisol (Rohleder and Kirschbaum, 2006; Steptoe and Ussher, 2006), different lymphocyte subsets (García-Dabrio et al., 2012; Jubri et al., 2013; Santagostino et al., 1999) and neopterin (Diamondstone et al., 1994; Schennach et al., 2002; Walter et al., 2003). Influence of smoking on these parameters was not detected in this study, probably due to the low number of smoker individuals included (10 referents and 17 exposed).

Time of exposure to the oil or using protective clothes did not influence the results of the endocrine and immunological parameters. Nevertheless, clear influences were observed for the use of protective mask on neopterin, %$CD8^+$, $CD4^+/CD8^+$ and IL4. These associations are strengthened by the fact that all these biomarkers fit into one frame, namely Th2-type immune activation. *In vitro* it was observed that not only Th1-type cytokine IFN-γ stimulates neopterin production in peripheral blood mononuclear cells but also that Th2-type cytokine IL-4 is able to counteract this process (Weiss et al., 1999). Thus, higher IL-4 together with lower neopterin concentrations points to an influence to drive Th2-type (=humoral) immunity and slow-down Th1-type (=cellular) immunity in the individuals who did not wear a mask and were therefore exposed more intensely to oil contaminants. Interestingly such finding is also in line with the *in vitro* observation that antioxidant compounds are likely to suppress Th1-type immune response (Jenny et al., 2011); electron-rich hydrocarbons of mineral oil can be considered to belong to the group of antioxidants rather than pro-oxidants. Moreover, increased exposure to antioxidant compounds not only slows down immunosurveillance mechanisms but also appears to be associated with an increased risk of allergy development also in human individuals (Zaknun et al., 2012). But certainly the small number of individuals included in each group of analysis limits the validity of these results, and there may be also an information bias, due to the long time elapsed since the participation in the cleanup and the request of the data.

Regarding the symptoms related to the exposure, the most common human health effect associated with chronic/repeated exposure to fuel oils is dermatitis (Laffon, 2013). Also, Spanish fishermen who participated in the cleanup of *Prestige* oil spill had a higher prevalence of respiratory symptoms (Rodríguez-Trigo et al., 2010), and Zock et al. (2012) showed the persistence of these symptoms up to five years after exposure. Besides, Sabucedo et al. (2010) reported lower levels of mental health in the *Prestige* affected population one year after the exposure. Our results, although only based on a simple questionnaire and a small population size, seem to suggest that these symptoms are still present seven years after the exposure, but consistent studies with that aim are necessary. Interestingly, higher neopterin levels were observed related to the presence of psycho-neurological symptoms, as increased concentrations of neopterin have been previously demonstrated in patients with depression and to correlate with depressive symptoms (Maes et al., 1993; Widner et al., 2002). An association between altered neopterin concentrations and psychoneurological performance was demonstrated earlier in several clinical conditions including infections and cancer. However, this is usually regarded as a consequence of biochemical alterations due to the chronic immune pathological status of such patients which may disturb neurotransmitter metabolism by interfering strongly with tryptophan and tyrosine biochemistry (Capuron et al., 2011; Widner et al., 2002). Still there is no proof for a direct role of neopterin with this respect. Moreover, the higher prevalence of psycho-neurological symptoms in females has been extensively described (Fernández-Guasti et al., 2012; Kessler, 2003; Valentino et al., 2012).

In summary, our results showed that most endocrine and immunological parameters analysed in the exposed population were not different form the referent values. Nevertheless, alterations observed in cortisol, kynurenine and NK cells recommend the surveillance of these individuals for early detection of possible health problems related to the endocrine or immunological systems.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

Research was funded by the Spanish Ministry of Science and Innovation (project PSI2010-15115) and by the Austrian Research Funds (project 25150-B13). F. Aguilera was supported by a fellowship from the Fundación Carolina (AECI, Spain).

In memoriam

This article is dedicated to the memory of Francisco Aguilera. He carried out a great portion of the work compiled here as a part of his Doctoral Thesis, but unfortunately passed away in August 5, 2012, before having the opportunity to finish it. He was a very motivated researcher, with a great initiative, interest and creative spirit. All of his colleagues will keep him in our memory by his enormous camaraderie and as an excellent friend in thick and thin. Rest in peace.

References

Aguilera F, Méndez J, Pásaro E, Laffon B. Review on the effects of exposure to spilled oils on human health. J Appl Toxicol 2010;30:291–301.

Albaigès J, Morales-Nin B, Vilas F. The Prestige oil spill: a scientific response. Mar Pollut Bull 2006;53:205–7.

Altindag ZZ, Baydar T, Isimer A, Sahin G. Neopterin as a new biomarker for the evaluation of occupational exposure to silica. Int Arch Occup Environ Health 2003;76:318–22.

Alzaga R, Montuori P, Ortiz L, Bayona JM, Albaigés J. Fast solid-phase extraction-gas chromatography–mass spectrometry procedure for oil fingerprinting. Application to the Prestige oil spill. J Chromatogr A 2004;1025:133–8.

Anderson SE, Franko J, Lukomska E, Meade BJ. Potential immunotoxicological health effects following exposure to COREXIT 9500A during cleanup of the deepwater horizon oil spill. J Toxicol Environ Health A 2011;74:1419–30.

Andreu-Ballester JC, García-Ballesteros C, Benet-Campos C, Amigó V, Almela-Quilis A, Mayans J, et al. Values for αβ and γδ t-lymphocytes and CD4+, CD8+, and CD56+ subsets in healthy adult subjects: assessment by age and gender. Cytometry B Clin Cytom 2012;82B:238–44.

Baydar T, Engin A, Aydin A, Sahin G. Effect of aluminum exposure on pteridine metabolism. Biol Trace Elem Res 2005;106:153–64.

Bulog A, Karaconji IB, Sutić I, Mićović V. Immunomodulation of cell-mediated cytotoxicity after chronic exposure to vapors. Coll Antropol 2011;35:61–4.

Capuron L, Schroecksnadel S, Féart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. Biol Psychiatry 2011;70:175–82.

Carrasco J, Perez-Gomez B, Garcia-Mendizabal M, Lope V, Aragones N, Forjaz M, et al. Health-related quality of life and mental health in the medium-term aftermath of the Prestige oil spill in Galicia (Spain): a cross-sectional study. BMC Public Health 2007;7:245.

Catalani S, De Palma G, Tomasi C, Alinovi R, Mutti A, Apostoli P. Lack of correlation between blood lead and serum prolactin levels among lead exposed workers. Biomarkers 2012;17:513–9.

COPE (Cytokines & Cells Online Pathfinder Encyclopedia). Cytokine concentrations in biological fluids. Last consulted 5 March 2013. http://www.copewithcytokines.de/cope.cgi?key=Cytokine%20Concentrations%20in%20Biological%20Fluids, 2011.

CSIC (Centro Superior de Investigaciones Científicas). Caracterización del vertido y evolución preliminar en el medio. CSIC Technical Report No. 01; 2003a. (Last consulted 5 March 2013. http://csicprestige.iim.csic.es/desarro/informcsic/1/index.htm).

CSIC (Centro Superior de Investigaciones Científicas). Presencia de metales pesados en la zona del hundimiento del petrolero Prestige y composición de metales y complejantes del fuel emulsionado de la costa. CSIC Technical Report No. 02; 2003b. (Last consulted 5 March 2013. http://csicprestige.iim.csic.es/desarro/informcsic/2/index.htm).

Dahlgren A, Kecklund G, Akerstedt T. Different levels of work-related stress and the effects on sleep, fatigue and cortisol. Scand J Work Environ Health 2005;31:277–85.

De Celis R, Feria-Velasco A, Bravo-Cuellar A, Hicks-Gómez JJ, García-Iglesias T, Preciado-Martínez V, et al. Expression of NK cells activation receptors after occupational exposure to toxics: a preliminary study. Immunol Lett 2008;118:125–31.

De Coster S, van Larebeke N. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. J Environ Public Health 2012;2012:52.

Diamondstone LS, Tollerud DJ, Fuchs D, Wachter H, Brown LM, Maloney E, et al. Factors influencing serum neopterin and β2-microglobulin levels in a healthy diverse population. J Clin Immunol 1994;14:368–74.

Dutta A, Bhattacharya P, Lahiri T, Ray MR. Immune cells and cardiovascular health in premenopausal women of rural India chronically exposed to biomass smoke during daily household cooking. Sci Total Environ 2012;438:293–8.

Engin AB, Ergun MA, Yurtcu E, Kan D, Sahin G. Effect of ionizing radiation on the pteridine metabolic pathway and evaluation of its cytotoxicity in exposed hospital staff. Mutat Res 2005;585:184–92.

Engin AB, Tuzun D, Sahin G. Evaluation of pteridine metabolism in battery workers chronically exposed to lead. Hum Exp Toxicol 2006;25:353–9.

Fernández-Guasti A, Fiedler JL, Herrera L, Handa RJ. Sex, stress, and mood disorders: at the intersection of adrenal and gonadal hormones. Horm Metab Res 2012;44:607–18.

Franz C, York T, Eaves L, Mendoza S, Hauger R, Hellhammer D, et al. Genetic and environmental influences on cortisol regulation across days and contexts in middle-aged men. Behav Genet 2010;40:467–79.

Frick B, Schroecksnadel K, Neurauter G, Leblhuber F, Fuchs D. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. Clin Biochem 2004;37:684–7.

García-Dabrio MC, Pujol-Moix N, Martínez-Pérez A, Fontcuberta J, Souto JC, Soria JM, et al. Influence of age, gender and lifestyle in lymphocyte subsets: report from the Spanish gait-2 study. Acta Haematol 2012;127:244–9.

García-Lestón J, Roma-Torres J, Vilares M, Pinto R, Cunha LM, Prista J, et al. Biomonitoring of a population of Portuguese workers exposed to lead. Mutat Res 2011;721:81–8.

García-Lestón J, Roma-Torres J, Mayan O, Schroecksnadel S, Fuchs D, Moreira A, et al. Assessment of immunotoxicity parameters in individuals occupationally exposed to lead. J Toxicol Environ Health A 2012;75:807–18.

Gestal JJ, Smyth E, Figueiras A, Montes A, Méndez J, Laffon B, et al. Recollida e limpeza do fuel do Prestige. Avaliación da exposición e danos á saúde en voluntarios e traballadores. Santiago de Compostela: Área de Medicina Preventiva e Saúde Pública84-688-6048-4; 2004.

Goldstein BD, Osofsky HJ, Lichtveld MY. The Gulf oil spill. N Engl J Med 2011;364:1334–48.

ITOPF (The International Tankers Owners Pollution Federation Limited). Last consulted 5 March 2013. http://www.itopf.com/information-services/data-and-statistics/statistics/index.html, 2013.

Jenny M, Klieber M, Zaknun D, Schroecksnadel S, Kurz K, Ledochowski M, et al. In vitro testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors. Inflamm Res 2011;60:127–35.

Ji K, Seo J, Liu X, Lee J, Lee S, Lee W, et al. Genotoxicity and endocrine-disruption potentials of sediment near an oil spill site: two years after the Hebei Spirit oil spill. Environ Sci Technol 2011;45:7481–8.

Jubri Z, Latif A, Top AG, Ngah WZ. Perturbation of cellular immune functions in cigarette smokers and protection by palm oil vitamin e supplementation. Nutr J 2013;12:2.

Kessler RC. Epidemiology of women and depression. J Affect Disord 2003;74:5e13.

Laffon B. Fuel oils. In: Wexler P, editor. Encyclopedia of Toxicology. 3rd Edition. New York: Elsevier; 2013. http://dx.doi.org/10.1016/B978-0-12-386454-3.00024-5. (in press).

Laffon B, Fraga-Iriso R, Pérez-Cadahía B, Méndez J. Genotoxicity associated to exposure to Prestige oil during autopsies and cleaning of oil-contaminated birds. Food Chem Toxicol 2006;44:1714–23.

Levy BS, Nassetta WJ. The adverse health effects of oil spills: a review of the literature and a framework for medically evaluating exposed individuals. Int J Occup Environ Health 2011;17:61–167.

Maes M, Meltzer HY, Scharpe S, Bosmans E, Suy E, DeMeester I, et al. Relationships between lower plasma L-tryptophan level and immune-inflammatory variables in depression. Psychiatry Res 1993;49:151–65.

Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, editors. Williams Textbook of Endocrinology. 12th ed. Philadelphia, PA: Saunders Elsevier; 2011.

Moszczyński P, Rutowski J, Słowiński S. The effect of cigarettes smoking on the blood counts of T and NK cells in subjects with occupational exposure to organic solvents. Cent Eur J Public Health 1996;4:164–8.

Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker of the immune system activation. Curr Drug Metab 2002;3:175–87.

Oh E, Lee E, Im H, Kang H-S, Jung W-W, Won NH, et al. Evaluation of immuno- and reproductive toxicities and association between immunotoxicological and genotoxicological parameters in waste incineration workers. Toxicology 2005;210:65–80.

Pérez-Cadahía B, Laffon B, Pásaro E, Méndez J. Genetic damage induced by accidental environmental pollutants. ScientificWorldJournal 2006;6:1221–37.

Pérez-Cadahía B, Lafuente A, Cabaleiro T, Pásaro E, Méndez J, Laffon B. Initial study on the effects of Prestige oil on human health. Environ Int 2007;33:176–85.

Pérez-Cadahía B, Laffon B, Porta M, Lafuente A, Cabaleiro T, López T, et al. 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. Chemosphere 2008a;71:447–55.

Pérez-Cadahía B, Laffon B, Valdiglesias V, Pásaro E, Méndez J. Cytogenetic effects induced by Prestige oil on human populations: the role of polymorphisms in genes involved in metabolism and DNA repair. Mutat Res 2008b;653:117–23.

Pérez-Cadahía B, Méndez J, Pásaro E, Lafuente A, Cabaleiro T, Laffon B. Biomonitoring of human exposure to Prestige oil: effects on DNA and endocrine parameters. Environ Health Insights 2008c;2:83–92.

Rink L, Cakman I, Kirchner H. Altered cytokine production in the elderly. Mech Ageing Dev 1998;102:199–209.

Rodríguez-Trigo G, Zock J-P, Pozo-Rodríguez F, Gómez FP, Monyarch G, Bouso L, et al. Health changes in fishermen 2 years after clean-up of the Prestige oil spill. Ann Intern Med 2010;153:489–98.

Rohleder N, Kirschbaum C. The hypothalamic-pituitary adrenal (HPA) axis in habitual smokers. Int J Psychophysiol 2006;59:236–43.

Rosati MV, Sancini A, Tomei F, Andreozzi G, Scimitto L, Schifano MP, et al. Plasma cortisol concentrations and lifestyle in a population of outdoor workers. Int J Environ Health Res 2011;21:62–71.

Sabucedo JM, Arce C, Senra C, Seoane G, Vázquez I. Symptomatic profile and health-related quality of life of persons affected by the Prestige catastrophe. Disasters 2010;34:809–20.

Santagostino A, Garbaccio G, Pistorio A, Bolis V, Camisasca G, Pagliaro P, et al. An Italian national multicenter study for the definition of reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. Haematologica 1999;84:499–504.

Sarac ES, Girgin G, Palabiyik SS, Charehsaz M, Aydin A, Sahin G, et al. A pilot study on neopterin levels and tryptophan degradation in zinc-exposed galvanization workers. Biol Trace Elem Res 2013;151:330–4.

Schennach H, Murr C, Gächter E, Mayersbach P, Schönitzer D, Fuchs D. Factors influencing serum neopterin concentrations in a population of blood donors. Clin Chem 2002;48:643–5.

Schröcksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta 2006;364:82–90.

Schroecksnadel S, Jenny M, Kurz K, Klein A, Ledochowski M, Überall F, et al. Lps-induced nf-[kappa]b expression in thp-1blue cells correlates with neopterin production and activity of indoleamine 2,3-dioxygenase. Biochem Biophys Res Commun 2010;399:642–6.

Sobrinho LG. Prolactin, psychological stress and environment in humans: adaptation and maladaptation. Pituitary 2003;6:35–9.

Spencer ME, Jain A, Matteini A, Beamer BA, Wang N-Y, Leng SX, et al. Serum levels of the immune activation marker neopterin change with age and gender and are modified by race, BMI, and percentage of body fat. J Gerontol A Biol Sci Med Sci 2010;65A:858–65.

Steptoe A, Ussher M. Smoking, cortisol and nicotine. Int J Psychophysiol 2006;59:228–35.

Suárez B, Lope V, Pérez-Gómez B, Aragonés N, Rodríguez-Artalejo F, Marqués F, et al. Acute health problems among subjects involved in the cleanup operation following the Prestige oil spill in Asturias and Cantabria (Spain). Environ Res 2005;99:413–24.

Taylor MW, Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 1991;5:2516–22.

Tomei F, Rosati MV, Ciarrocca M, Baccolo TP, Gaballo M, Caciari T, et al. Plasma cortisol levels and workers exposed to urban pollutants. Ind Health 2003;41:320–6.

Tulinska J, Jahnova E, Dusinska M, Kuricova M, Liskova A, Ilavska S, et al. Immunomodulatory effects of mineral fibres in occupationally exposed workers. Mutat Res 2004;553:111–24.

Valentino RJ, Reyes B, Van Bockstaele E, Bangasser D. Molecular and cellular sex differences at the intersection of stress and arousal. Neuropharmacology 2012;62:13–20.
Veraldi A, Costantini AS, Bolejack V, Miligi L, Vineis P, van Loveren H. Immunotoxic effects of chemicals: a matrix for occupational and environmental epidemiological studies. Am J Ind Med 2006;49:1046–55.
Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol 2008;9:503–10.
Walter RB, Fuchs D, Weiss Gn, Walter TR, Reinhart WH. HMG–CoA reductase inhibitors are associated with decreased serum neopterin levels in stable coronary artery disease. Clin Chem Lab Med 2003;41:1314–9.
Weiss G, Murr C, Zoller H, Haun M, Widner B, Ludescher C, et al. Modulation of neopterin formation and tryptophan degradation by Th1- and Th2-derived cytokines in human monocytic cells. Clin Exp Immunol 1999;116:435–40.
Wick G, Grubeck-Loebenstein B. Primary and secondary alterations of immune reactivity in the elderly: impact of dietary factors and disease. Immunol Rev 1997;160:171–84.
Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. Clin Chem 1997;43:2424–6.
Widner B, Laich A, Sperner-Unterweger B, Ledochowski M, Fuchs D. Neopterin production, tryptophan degradation, and mental depression—what is the link? Brain Behav Immun 2002;16:590–5.
Xue Y, Morris M, Ni L, Guthrie SK, Zubieta JK, Gonzalez K, et al. Venous plasma nicotine correlates of hormonal effects of tobacco smoking. Pharmacol Biochem Behav 2010;95:209–15.
Zaknun D, Schroecksnadel S, Kurz K, Fuchs D. Potential role of antioxidant food supplements, preservatives and colorants in the pathogenesis of allergy and asthma. Int Arch Allergy Immunol 2012;157:113–24.
Zock J-P, Rodríguez-Trigo G, Pozo-Rodríguez F, Barberá JA, Bouso L, Torralba Y, et al. Prolonged respiratory symptoms in clean-up workers of the Prestige oil spill. Am J Respir Crit Care Med 2007;176:610–6.
Zock J-P, Rodríguez-Trigo G, Rodríguez-Rodríguez E, Espinosa A, Pozo-Rodríguez F, Gómez F, et al. Persistent respiratory symptoms in clean-up workers 5 years after the Prestige oil spill. Occup Environ Med 2012;69:508–13.