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Genotoxicity associated to exposure to *Prestige* oil during autopsies and cleaning of oil-contaminated birds

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Abstract

After the accident involving the oil tanker *Prestige* in November 2002 near 63,000 tons of heavy oil reached Galician coast (Northwest of Spain). This unleashed a large movement of volunteers to collaborate in several cleaning tasks. The aim of this study was to determine whether handling of *Prestige* oil-contaminated birds during autopsies and cleaning may have resulted in genotoxic damage. We have also evaluated the possible influence of DNA repair genetic polymorphisms (XRCC1 codons 194 and 399, XRCC3 codon 241 and APE1 codon 148) on susceptibility to the genotoxic effects evaluated. Exposure levels were analysed by determining volatile organic compounds in air samples. Peripheral blood samples were obtained from 34 exposed and 35 controls, and comet assay and micronucleus (MN) test were carried out. Genotyping was performed following PCR-RFLP procedures. Results obtained have shown significantly higher DNA damage, but not cytogenetic damage, in exposed individuals than in controls, related to time of exposure. Among exposed individuals, carriers of the variant alleles XRCC1 *399Gln* and APE1 *148Glu* have shown altered DNA damage with regard to wild-type homozygotes, suggesting exposure–genotype interactions. No effect of the DNA repair genetic polymorphisms analysed was observed in the MN test.

Keywords: *Prestige* oil; Genotoxicity; Micronucleus test; Comet assay; DNA repair genetic polymorphisms

1. Introduction

The wreck of the *Prestige* oil tanker off the Galician coast (Northwest of Spain), in November 2002, involved one of the greatest environmental catastrophes in European navigation in which the initial spill and subsequent leakage resulted in the release of nearly 63,000 tons of heavy oil during the period up to August 2003. This event mobilised a large number of volunteers, who collaborated in several tasks, such as cleaning beaches, rocks, sea and oil-contaminated birds. As many as 12,000 oil-contaminated birds had been collected in Galicia by September 2003; about 9500 of them were dead and 2500 alive, but contaminated.

Extraction of the rest of the oil from the tanker, that sank to rest at a depth of 4000 m, was performed between June and October 2004 by means of rigid shuttle bags. Bioremediation techniques were then applied to accelerate the natural degradation of the oil stuck on the tanker walls, and this process was concluded with the hermetic sealing of the tanks.

The oil carried by the *Prestige* was categorized as fuel oil no. 2 (maximum sulphur content 4%). The International Agency for Research on Cancer (IARC) has classified this kind of oil in Group 2B, as possibly carcinogenic to humans (IARC, 1989). The volatility of this oil was low (about 5–10% was considered volatile), due to its elevated content of high molecular weight hydrocarbons, such as polycyclic aromatic hydrocarbons (PAH). These have a lower acute toxicity than oils with a higher content of simple aromatic

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hydrocarbons such as benzene. According to the analysis made by the Spanish National Research Council, the composition of the *Prestige* oil could be described as 50% aromatic hydrocarbons, 22% saturated hydrocarbons and 28% resins and asphalthene (CSIC, 2003). The aromatic hydrocarbon fraction included light aromatic hydrocarbons (naphthalene and its alkylated derivatives), high molecular weight hydrocarbons (complex mixtures of asphalthenes and PAH) and volatile aromatic hydrocarbons (primarily benzene, toluene and xylenes), although the latter were in low proportion in the original oil. Moreover, it contained six PAHs categorized as probable or possible human carcinogens (naphthalene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and dibenz[*ah*]anthracene), and included in the 16 PAH designated by the United States Environmental Protection Agency (USEPA) as primary contaminants.

The DNA damage detected by the alkaline version of the comet assay can arise through various mechanisms, including DNA double- and single-strand breaks, DNA interstrand cross-linking, alkali labile sites, and incompletely repaired excision sites (Rojas et al., 1999). On the other hand, the micronucleus (MN) test represents a sensitive indicator of chromosome damage that provides a measure of both chromosome breakage and chromosome loss, and has the advantages of relative ease of scoring and high statistical power obtained from scoring larger number of cells than are typically used for metaphase analysis (Fenech et al., 1999). Moreover, the use of cytochalasin-B allows an accurate detection of MN in once divided cells, as opposed to those undivided cells (Fenech and Morley, 1985).

The objective of this study was to determine whether handling of *Prestige* oil-contaminated birds during autopsies and cleaning may result in DNA or cytogenetic damage, evaluated by the comet assay and MN tests, respectively. Exposure was measured by determining oil volatile organic compounds (VOC) in environmental air samples. Moreover, we have genotyped the individuals for common DNA repair polymorphisms (XRCC1 codons 194 and 399, XRCC3 codon 241 and APE1 codon 148), in order to analyse their possible influence on susceptibility to genotoxic effects evaluated.

2. Material and methods

2.1. Determination of exposure to volatile organic compounds

Concentrations of VOC through a whole day were determined in the environmental air of the room where volunteers performed the autopsies and cleaning of the oil-contaminated birds. The volume of the room was approximately 50 m^3; it had two windows on one of the walls and the door on the adjacent wall. The windows and door were maintained open during the work. There were 6–8 contaminated birds at any one time in the room. Twelve 1-h air samples were taken by means of a Perkin–Elmer STS-25 automated sampler (Norwalk, CT, USA), in 2 h intervals, sampling air at 50 ml/min for 60 min. The sampler was placed on the work bench, approximately 0.5 m away from the individuals. Analyses of VOC were carried out by thermal desorption coupled to gas chromatography–mass spectrometry, as described in Fernández-Martínez et al. (1999) and Fernández-Villarrenaga et al. (2004a). The VOC analysed were: aromatic compounds (benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, *p*-xylene, styrene, isopropylbenzene, *n*-propylbenzene, 1,3,5-trimethylbenzene, *tert*-butylbenzene, 1,2,4-trimethylbenzene, *sec*-butylbenzene, *n*-butylbenzene and 1,2,3-trimethylbenzene) aliphatic compounds (*n*-heptane, *n*-octane and *n*-decane), halogenated compounds (trichloromethane, 1,1,1-trichloroethane, tetrachloromethane, trichloroethene, 1,2-dichloropropane, bromodichloromethane, 2-chloroethoxyethene, *trans*-1,3-dichloropropene, *cis*-1,3-dichloropropene, tetrachloroethene, dibromochloromethane, chlorobenzene, tribromomethane, 1,2,2,2-tetrachloroethane, 1,2-dichlorobenzene, 1,3-dichlorobenzene and 1,4-dichlorobenzene) and natural compounds (α-pinene, β-pinene, Δ^3-carene and limonene). The sum of the total quantity of all these compounds was considered as the index total VOC (TVOC). The sum of the total quantity of benzene, toluene, ethylbenzene and *o*-, *m*- and *p*-xylene was considered as the BTEX index.

2.2. Study subjects

The exposed group consisted of 34 Caucasian volunteers from the University of A Coruña, engaged in the autopsies and cleaning of oil-contaminated birds. The cleaning of the birds was performed only with water and soap. The control group consisted of 35 Caucasian students from the University of A Coruña, not exposed to *Prestige* oil or any other known genotoxic substance. Data on protective measures adopted by exposed subjects, lifestyle, consumption habits such as smoking, alcohol intake, medication, recent viral infections, vaccinations, diagnostic tests, or previous occupational exposures to chemicals were obtained via questionnaire. The control and exposed groups were matched by gender and smoking; the other variables detailed in the questionnaires were collected in order to exclude non-suitable subjects for the study. The objective of the study was fully explained to the participant individuals, and informed consent was obtained. The exposed population was divided in three groups according to time of exposure; the time segments were established on the basis of trying to equate the number of subjects in each sub-group. Venous blood samples were drawn from each donor early in the morning, before the beginning of the work, in heparin and EDTA containers.

2.3. Comet assay

Isolation of mononuclear leukocytes was performed using BD Vacutainer™ CPT™ Cell Preparation Tubes with sodium heparin (Becton Dickinson), following manufacturer's instructions. Cells were suspended in freezing medium (50% foetal calf serum, 40% RPMI 1640, 10% DMSO) to obtain 10^7 cells/ml, and frozen at −80 °C in a Nalgene® Cryo 1 °C Freezing Container (Nalgene Nunc International) until the time of analysis (less than 2 weeks). Cells were quickly thawed at 37 °C, and viability was assessed by trypan blue exclusion technique, being higher than 80% in all cases.

The alkaline comet assay was performed basically as described by Singh et al. (1988), with minor modifications (Laffon et al., 2002). Two slides were prepared for each donor. One hundred cells were examined for each donor from replicated slides by a single "blind" scorer using a magnification of 400×. The selection of the cells to be scored was made as they were appearing in the microscopic examination field. The only criterion followed to exclude a cell from the analysis was the appearance of DNA clouds, as recommended by Hartmann and Speit (1997), since these are assumed to represent dead cells and thus confusion due to cytotoxicity is avoided.

Image capture and analysis were performed using the QWIN Comet software (Leica Imaging Systems, Cambridge, UK). Comet tail length (TL), measured from the estimated centre of the cell, was evaluated for each cell as DNA damage parameter.

2.4. Cytokinesis-block MN assay

Cultures were established in duplicate by adding 0.5 ml of whole blood to 4.5 ml of culture medium (RPMI 1640 containing 15% heat inactivated

foetal bovine serum, 1% phytohemagglutinin, 1% L-glutamine (200 mM) and 1% penicillin (5000 U/ml)/streptomycin (5000 μg/ml), all from Invitrogen, Barcelona, Spain). Cultures were incubated at 37 °C in the dark for 64 h, and cytochalasin-B (6 μg/ml) was added at 44 h to prevent cytokinesis. Cells were collected by centrifugation, treated with a mild hypotonic solution (0.075 M KCl at 4 °C), and then centrifuged immediately and fixed in Carnoy (3:1 methanol–acetic acid). Air dried slides were prepared and stained with 5 μg/ml 4,6-diamidino-2-phenylindole (DAPI) in antifade solution.

Microscope analyses were performed with a Leica DM-RXA fluorescence microscope, equipped with a 100 W mercury lamp and a 100× magnification objective. A total of 1000 binucleated cells with well-preserved cytoplasm were examined for each individual to determine the number of MN. For identifying binucleated cytokinesis-blocked cells and MN, the criteria described by Fenech et al. (2003) were followed.

2.5. Genotyping

Genomic DNA was isolated by means of Puregene™ DNA isolation kit (Gentra Systems, Minneapolis, USA). All genotype analyses were performed at least in duplicate to confirm the study results.

Polymorphisms in codons 194 and 399 of the XRCC1 gene were determined by a multiplex PCR assay followed by digestion with *Msp* I restriction endonuclease. Two fragments of 490 and 520 bp for codons 194 and 399, respectively, were amplified using 0.75 U *Taq* polymerase, 0.2 μM of each primer (5′-GCC CCG TCC CAG GTA A-3′ and 5′-GCC CCA AGA CCC TTT CAC-3′ for codon 194, and 5′-TTG TGC TTT CTC TGT GTC CA-3′ and 5′-CCC GCT CCT CTC AGT AGT CT-3′ for codon 399), 0.2 mM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, and 60 ng genomic DNA in a total volume of 30 μl. PCR conditions consisted of an initial denaturation of 90 s at 94 °C, 35 cycles of 30 s at 94 °C, 1 min at 62 °C and 45 s at 72 °C, and a final extension of 10 min at 72 °C. Ten microlitres of the PCR product were digested with 10 U *Msp* I, generating fragments of 292, 178 and 20 bp in the case of codon 194 wild-type allele, 312 and 178 bp in the case of codon 194 variant allele, 375 and 145 bp in the case of codon 399 variant allele, and remaining unchanged with 520 bp in the case of 399 wild-type allele. Fragments were evaluated on a 3% agarose electrophoresis gel and stained with 0.5 mg/l ethidium bromide.

Two PCR-RFLP assays were used to determine polymorphisms in codon 241 of the XRCC3 gene and codon 148 of the APE1 gene, following Hu et al. (2001).

2.6. Statistical analysis

The statistical evaluation was conducted using the SPSS for Windows statistical package, version 11.0 (Illinois, USA). Given data characteristics, the contribution of the effects of exposure, gender, age and smoking habits to the variability of TL and MN frequency in the total population was evaluated by means of the multifactorial analysis of variance (ANOVA). Bonferroni's correction was used for multiple comparisons among groups. Moreover, the effects of the selected polymorphisms in the control and exposed populations were determined by using the same statistical model. Homozygous and heterozygous carriers of the variant alleles were combined in the statistical analysis, due to the low number of variant homozygotes. The associations between two variables were analysed by Pearson's correlation.

3. Results

Results obtained in the chromatographic analyses of the room air samples are shown in Fig. 1. Maximum concentrations of TVOC obtained did not practically exceed 200 μg/m^3. These levels increased through time, especially during night hours. The same behaviour was observed for the BTEX index, the concentrations of which ranged between 15.92 and 67.82 μg/m^3 (Fig. 1a). Detailing the individual compounds of the BTEX index (Fig. 1b), benzene and toluene remained constant with time, while *m*- and *p*-xylenes presented the most marked increase. Smoker individuals did not smoke during their work and smoking was only allowed outdoor, thus it is expected that tobacco smoke substances did not noticeably contribute to VOC determined in the room air samples.

Table 1 summarizes the characteristics of the two participating populations. Distribution of genders was practically equitable in both groups; individuals were quite young due to the fact that they all were university students. Smokers represented only 30% of the selected populations; the number of cigarettes smoked per day was very similar in the two groups, although exposed individuals smoked for more years than controls. Among the exposed group, 13 individuals (35.2%) were exposed for less than 150 h, 10 (29.4%) for 150–500 h, and 11 (35.4%) for more than 500 h. They all wore gloves and waterproof overall as security clothing, but only 17 (50%) used cellulose protective mask. Frequencies of XRCC1 *194Trp* and *399Gln* variant alleles obtained (0.030 and 0.288, respectively) were in the range described by Tuimala et al. (2004), Angelini et al. (2005) and Wilding et al. (2005). XRCC3 *241Met* variant allele frequency observed (0.282) was slightly lower than those previously reported by Hu et al. (2001), Mateuca et al. (2005) and Wilding et al. (2005). Variant APE1

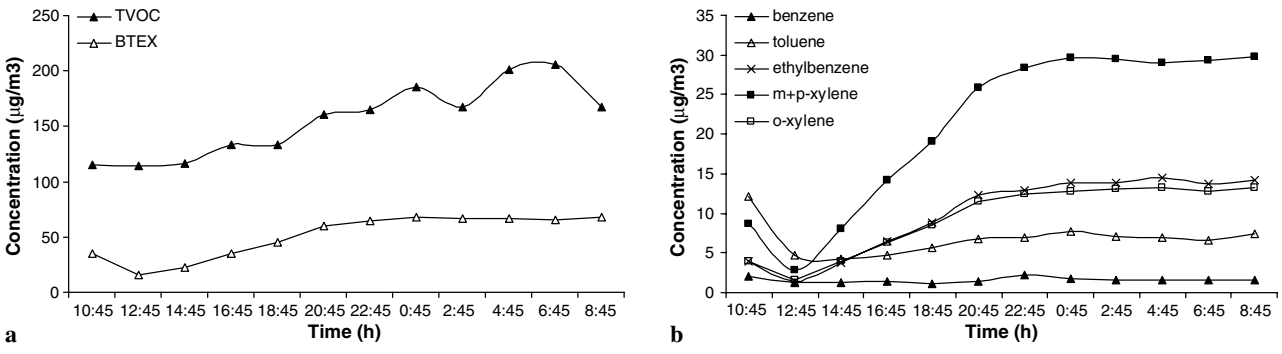

Fig. 1. Concentrations of TVOC and BTEX (a) and of the individual BTEX compounds (b) in the room air where volunteers performed autopsies and cleaning of the oil-contaminated birds.

Table 1
Characteristics of the study populations

	Exposed	Controls
No. of subjects	34	35
Time of exposure (h)[a]	341.4 ± 266.6	
Age[a]	29.6 ± 7.3	24.4 ± 4.3
Gender		
Females	16	17
Males	18	18
Tobacco smoking		
Smokers	11	11
Non-smokers	23	24
Cigarettes per day[a]	11.3 ± 6.5	12.3 ± 7.9
Years smoking[a]	12.4 ± 7.7	8.9 ± 6.0
XRCC1 codon 194		
Arg/Arg	31	32
Arg/Trp	2	3
Trp/Trp	0	0
XRCC1 codon 399		
Arg/Arg	18	21
Arg/Gln	11	10
Gln/Gln	4	4
XRCC3 codon 241		
Thr/Thr	19	15
Thr/Met	13	18
Met/Met	2	2
APE1 codon 148		
Asp/Asp	20	13
Asp/Glu	14	15
Glu/Glu	0	7

[a] Mean ± SD.

148Glu allele was observed at a similar frequency (0.311) than that obtained by Hu et al. (2001).

Multifactorial analysis of variance was applied in the evaluation of the genotoxicity tests results, introducing the variables exposure, age, gender and tobacco consumption for the total population, and analysed genotypes also for the two populations separately. The introduction of the variable use of protective mask complicated excessively the analysis, so it was not included in the multifactor analysis, although higher MN frequency (16.82 ± 1.01 vs. 15.71 ± 0.75) and significantly higher TL (one-way ANOVA test) (48.59 ± 0.16 μm vs. 45.02 ± 0.21 μm) was observed in the individuals that did not wear it, as compared with the individuals that did use mask.

The distribution of TL and MN frequencies in the two populations analysed are shown in Fig. 2, where it can be seen that distributions in the exposed population are displaced to higher values for the two parameters. Table 2 gathers results obtained in the comet assay and MN tests, in the exposed and control groups and in the total population. Overall, there was a small but statistically significant increase in comet tail lengths amongst the exposed subjects as compared to the controls, but no such difference was observed in the frequencies of micronucleated cells. Subgroup analysis of the comet tail length data also showed several other significant differences. Higher levels of DNA damage were observed in males than in females of

Table 2
Results of comet assay and MN test in the exposed, control, and total populations

	N	Exposed	*N*	Controls	*N*	Total
Comet assay (mean TL ± SE (μm))						
Total	34	46.80 ± 0.13[a]	35	43.95 ± 0.07	69	45.36 ± 0.08
Males	18	47.87 ± 0.17	18	43.43 ± 0.10	36	45.65 ± 0.10
Females	16	45.61 ± 0.21[b]	17	44.50 ± 0.11	33	45.04 ± 0.12[b]
⩽25 years	15	45.12 ± 0.23	29	43.91 ± 0.08	44	44.33 ± 0.09
>25 years	19	48.14 ± 0.15[c]	6	44.13 ± 0.18	25	47.18 ± 0.13[c]
Non-smokers	23	47.20 ± 0.17	24	44.04 ± 0.08	47	45.59 ± 0.09
Smokers	11	45.97 ± 0.23[d]	11	43.75 ± 0.14[d]	22	44.86 ± 0.14[d]
Micronucleus test (mean MN ± SE)						
Total	34	16.26 ± 0.63	35	15.17 ± 0.68	69	15.71 ± 0.47
Males	18	16.61 ± 0.71	18	14.22 ± 0.73	36	15.41 ± 0.55
Females	16	15.88 ± 1.08	17	16.18 ± 1.12	33	16.03 ± 0.77
⩽25 years	15	15.53 ± 1.07	29	15.00 ± 0.78	44	15.18 ± 0.63
>25 years	19	16.84 ± 0.73	6	16.00 ± 1.39	25	16.64 ± 0.64
Non-smokers	23	16.43 ± 0.80	24	15.00 ± 0.82	47	15.70 ± 0.58
Smokers	11	15.91 ± 1.01	11	15.55 ± 1.28	22	15.73 ± 0.80

[a] $P < 0.01$, significant difference with regard to controls.
[b] $P < 0.01$, significant difference with regard to males.
[c] $P < 0.01$, significant difference with regard to the younger group.
[d] $P < 0.01$, significant difference with regard to non-smokers.

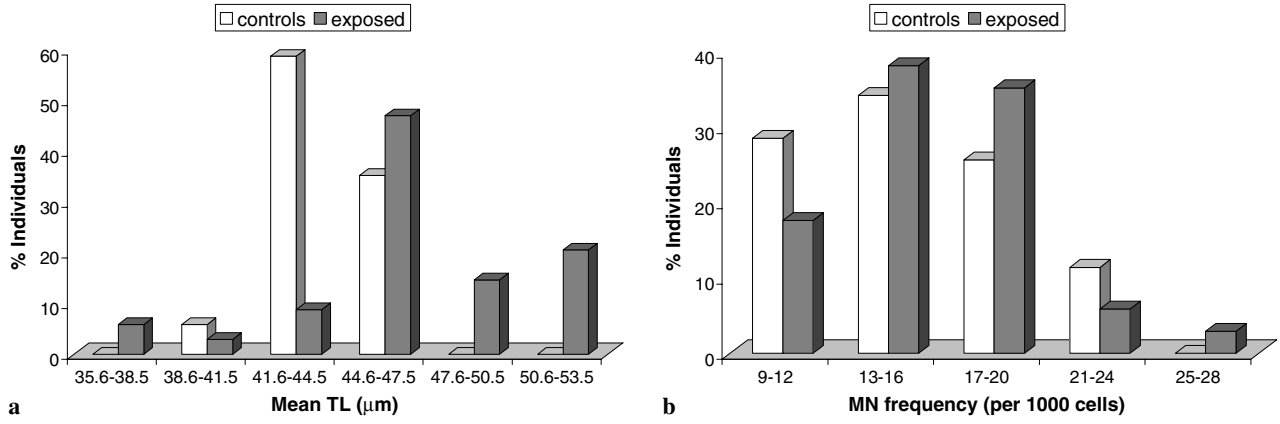

Fig. 2. Controls and exposed distributions of tail length (TL) (a) and micronucleus (MN) frequency (b).

both the exposed and total populations, but not in the control population.

An age group effect ($\leqslant$25 years vs. $\geqslant$25 years) in the comet assay was also observed in the exposed and total populations and the correlation analysis of comet tail length on years of age was also significant ($r = 0.464$, $P < 0.01$). Tobacco consumption unexpectedly decreased TL values both in the control and exposed groups. No effect of gender, age or smoking habits was detected in the MN test results.

By dividing the exposed population into three groups according to time of exposure (Fig. 3), increasing TL values were observed with extending time of exposure, being the differences among all groups statistically significant. Correlation coefficient obtained for association between TL in exposed subjects and time of exposure was $r = 0.376$, significant at the level of 0.05. Nevertheless, no effect of this variable was obtained in the MN test.

Table 3 shows results obtained for the effect of the DNA repair polymorphisms studied on the genotoxicity tests applied. Exposed individuals carrying XRCC1 *194Trp* variant allele presented significantly lower TL level than wild-type homozygotes. The same behaviour was observed in controls carrying XRCC1 *399Gln* variant allele; however, the contrary was observed in exposed subjects, i.e. lower DNA damage in wild-type homozygous subjects. With regard to XRCC3 codon 241 polymorphism, no differences in DNA damage were observed between the two

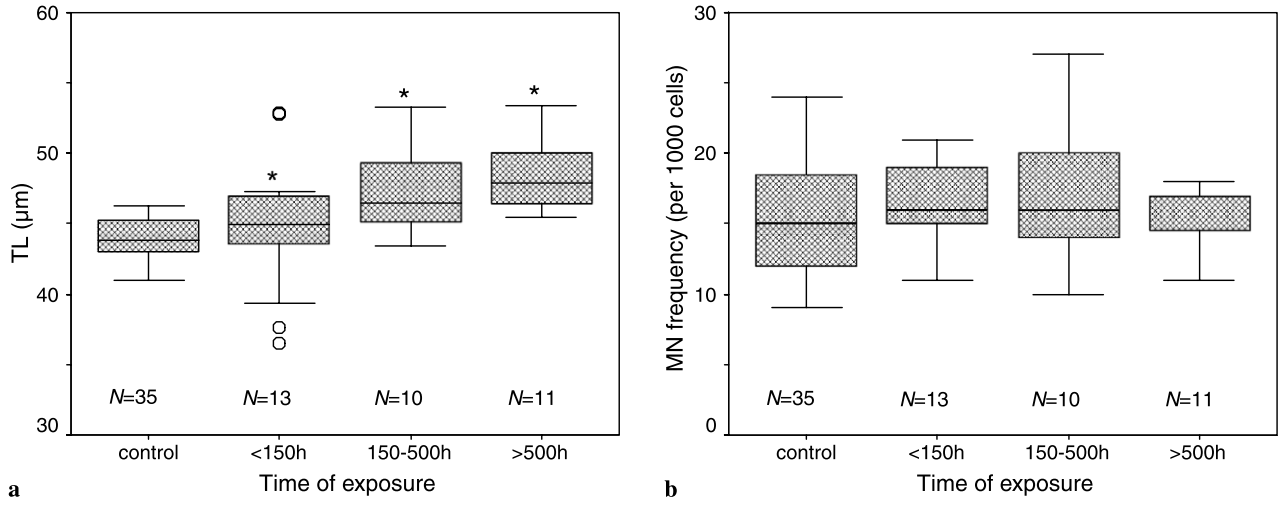

Fig. 3. Boxplots showing comet assay (a) and micronucleus test (b) results according to time of exposure. *o*: outliers, *$P < 0.01$, significant difference with regard to controls and all other exposed groups, according to multiple comparison Bonferroni's test.

Table 3
Effect of the selected DNA repair polymorphisms on DNA damage and MN frequency

Gene	Polymorphism	Genotype	*N*	Exposed	*N*	Controls
Comet assay (mean TL ± SE (μm))						
XRCC1	codon 194	*Arg/Arg*	31	47.49 ± 0.14	32	43.89 ± 0.07
		Arg/Trp + Trp/Trp	2	37.91 ± 0.31[a]	3	44.56 ± 0.31
	codon 399	*Arg/Arg*	18	46.62 ± 0.17	21	44.05 ± 0.09
		Arg/Gln + Gln/Gln	15	47.26 ± 0.22[a]	14	43.81 ± 0.12[a]
XRCC3	codon 241	*Thr/Thr*	19	45.49 ± 0.17	15	43.72 ± 0.11
		Thr/Met + Met/Met	15	48.47 ± 0.21	20	44.12 ± 0.09
APE1	codon 148	*Asp/Asp*	20	47.31 ± 0.17	13	43.51 ± 0.12
		Asp/Glu + Glu/Glu	14	46.08 ± 0.22[a]	22	44.21 ± 0.09[a]
Micronucleus test (mean MN ± SE)						
XRCC1	codon 194	*Arg/Arg*	31	16.16 ± 0.64	32	15.28 ± 0.68
		Arg/Trp + Trp/Trp	2	14.50 ± 1.50	3	14.00 ± 4.04
	codon 399	*Arg/Arg*	18	16.50 ± 0.67	21	15.67 ± 0.96
		Arg/Gln + Gln/Gln	15	15.53 ± 1.09	14	14.43 ± 0.92
XRCC3	codon 241	*Thr/Thr*	19	16.26 ± 0.63	15	13.80 ± 0.83
		Thr/Met + Met/Met	15	16.27 ± 1.21	20	16.20 ± 0.98
APE1	codon 148	*Asp/Asp*	20	16.75 ± 0.87	13	14.23 ± 1.09
		Asp/Glu + Glu/Glu	14	15.57 ± 0.88	22	15.73 ± 0.88

[a] $P < 0.01$, significant difference with regard to wild-type homozygous genotype.

Table 4
Interactions found between analysed variables in the comet assay in the control and exposed populations (multifactor analysis of variance)

Source of variation	Sum of squares	df	Mean square	*F*-ratio	Significance level
Control population					
XRCC3*APE1	491.913	1	491.913	26.906	0.0001
Residual	63,533.136	3475	18.283		
Total	6,829,020.970	3500			
Total (corrected)	67,928.761	3499			
Exposed population					
Age*XRCC1-399	530.208	1	530.208	11.048	0.001
Smoking*XRCC3	236.883	1	236.883	4.936	0.026
XRCC1-194*XRCC1-399	864.914	1	864.914	18.023	0.0001
XRCC1-399*XRCC3	574.481	1	574.481	11.971	0.001
XRCC1-399*APE1	426.840	1	426.840	8.894	0.003
Residual	161,871.525	3373	47.990		
Total	7,661,462.630	3400			
Total (corrected)	212,840.040	3399			

established groups. Carriers of APE1 *148Glu* variant allele showed significantly lower TL values than wild-type homozygotes in the exposed population, but significantly increased TL values in the control group. No statistically significant effects of the polymorphisms were found in the micronucleus frequency data.

As shown in Table 4 for the comet assay, in the control population only interaction between XRCC3 and APE1 polymorphisms was found. However, interactions between age and XRCC1 codon 399 polymorphism, smoking and XRCC3 polymorphism, both polymorphisms of XRCC1, polymorphisms of XRCC1 codon 399 and XRCC3, and polymorphisms of XRCC1 codon 399 and APE1 were observed. No interaction could be found in the MN test.

4. Discussion

In the last two decades there have been numerous catastrophes involving oil tankers all around the world, like *Exxon Valdez* (Canada, 1989), *Braer* (Scotland, 1993), *Haven* (Italy, 1996), *Sea Empress* (Wales, 1996), *Nakhodka* (Japan, 1997) and *Erika* (France, 1999). Several epidemiological studies have been conducted to determine the consequences of the oil spills on human health. Most of them evaluated acute toxicity indicators such as headache, low back pain, leg pain, and symptoms of eyes and throat (Attias et al., 1995; Lyons et al., 1999; Morita et al., 1999), or psychological symptoms such as depression, anxiety or posttraumatic stress (Palinkas et al., 1992, 1993; Lyons et al., 1999). Baars (2002) estimated the carcinogenic and teratogenic risk associated with dermal exposure to *Erika* oil to be negligible. So far, to the best of our knowledge the only study that evaluated the genotoxicity related to oil exposure is the one by Cole et al. (1997), who reported no increase in the levels of DNA adducts or HPRT somatic mutations in the group exposed to *Braer* oil, although this results come from a rather low number of individuals (7 controls and 20 exposed).

The *Prestige* accident caused the spill of lots of heavy oil off the Galician shores, affecting a wide coastal zone and great number of organisms, including numerous bird species. During several months volunteers carried out cleaning tasks both of coast and birds contaminated by the oil. These were collected and transported to Wild Fauna Recovery Centres, especially dedicated to the cleaning and care of live birds, and to performing autopsies on dead birds. All individuals involved in this work wore protective equipment that assured protection against dermal exposure to the oil. Nevertheless, only half of them used protective masks, so exposure by inhalation was considered highly probable. That is why the exposure was evaluated by analysing VOC in environmental air samples. Levels of VOC determined were in the range of those obtained in urban environments of low contaminated cities, such as La Coruña (Spain) (Fernández-Martínez et al., 2001; Fernández-Villarrenaga et al., 2004b). Even in the moments when higher VOC values were registered, they kept below levels observed in highly contaminated cities, such as Athens (Rappenglück et al., 1998). These higher values coincided with night samples due to the absence of ventilation, since all the doors and windows that during day were open and maintained the room intensely aerated, were closed during night. Legislation on VOC in environment is scarce or practically inexistent. Benzene is the only VOC regulated because of its toxicological characteristics, since it has been classified by the IARC as carcinogenic to humans (group 1) (IARC, 1987). Levels of benzene obtained in this study did not exceed the threshold limit value of 1.6 mg/m^3 determined by the American Conference of Governmental Industrial Hygienists (ACGIH, 2005) or the mean annual value of 5 μg/m^3 established by the European Union (Directive 2000/69/EC). Anyway, the determinations done did not reach the time interval demanded by the European Directive.

Results obtained in the present study showed significantly increased DNA damage in the group of individuals engaged in the cleaning and autopsy of oil-contaminated birds, all of them wearing waterproof overall and gloves. Nevertheless, no significant increase in MN frequency has been obtained in the exposed population. Our results agree

with others reported in several populations exposed to low levels of VOC for the comet assay (Binková et al., 1996; Pitarque et al., 1999; Lam et al., 2002) but not for cytogenetic tests (Bolognesi et al., 1997; Parry et al., 1997; Pitarque et al., 1999; Carere et al., 2002). It is considered that, for chronic exposures, cytogenetic techniques reflect cumulative damage while comet assay provide information about recent repairable exposure levels (Maluf and Erdtmann, 2000). Therefore, handling of *Prestige* oil-contaminated birds induced DNA damage that was mostly repaired and did not become fixed as structural chromosomal alterations.

Gender differences detected in the DNA damage in the exposed population, being higher in males than in females, had been previously described in healthy control populations (Betti et al., 1994, 1995; Bajpayee et al., 2002), or in individuals exposed to benzene (Lam et al., 2002) and to ionizing radiations (Wojewódzka et al., 1998). In spite of the mean age of the populations analysed being rather low and of restricted ranges, an age effect was observed in the comet assay, as was reported by Betti et al. (1995), Wojewódzka et al. (1998) and Poli et al. (1999), but only in the exposed group. This effect confined to the exposure group could be due to its wider age distribution, together with the more equitable distribution of individuals between the two age groups, since in the control group there were only 6 individuals in the $\geqslant 25$ years age group. As for tobacco consumption, usually smokers present higher DNA damage than non-smokers (Betti et al., 1994, 1995; Rojas et al., 1996; Piperakis et al., 1998; Dhawan et al., 2001). Nevertheless, our results both in the control and exposed populations were surprisingly opposite, i.e., statistically higher TL in non-smokers. These data are similar to those observed by Wei et al. (2000) and Wang et al. (2003). The reason for the lower DNA damage in non-smokers could be related to a higher proficiency in DNA repair processes, since they are stimulated as a consequence of chronic exposure to tobacco carcinogens (Wang et al., 2003). This increased DNA repair capacity could result from an adaptation due to the increased demand for repair (Wei et al., 2000). Nevertheless, these results must be interpreted cautiously because of the small size of the smokers groups ($N = 11$ both in controls and exposed). Recently, it has been reported that only heavy smokers (daily consumption >30 cigarettes) exhibited a significant increase in MN frequency (Bonassi et al., 2003). Agreeing with this, smokers included in this study (daily consumption <30 cigarettes/day) did not present higher MN frequency than non-smokers. Although no significant gender or age effect could be detected in this work in the MN test, higher frequencies were obtained both in females and in the older age groups, as previously described (Fenech, 1998; Bonassi et al., 2001).

Use of protective mask is highly recommended in the presence of substances that can penetrate in the organism by inhalation. In this study, as previously mentioned, only half of the exposed individuals used protective mask, and presented higher values of DNA damage and MN frequency than inhalation-protected subjects. This fact supports the importance of security measures when handling hazardous substances, adequate to each particular exposure.

Susceptibility to exogenous and endogenous genotoxins may be related to genetic polymorphisms of DNA repair proteins, resulting in altered efficiency of removing DNA lesions. Thus, the integrated application of biomarkers of effect and susceptibility provide a valuable tool for human biomonitoring of environmental or occupational exposures. As recommended by Norppa (2004), the effect of genetic polymorphisms on exposure-related genotoxicity results has been analysed in comparison with the unexposed population, in order to distinguish exposure–genotype interactions from genotype effect on baseline levels of the biomarkers. Although no effect of the studied DNA repair polymorphisms could be detected in the MN test, some interesting effects were observed in the comet assay.

XRCC1 protein participates in base excision repair (BER) and recombinational repair pathways (Thompson and West, 2000). XRCC1 codon 194 polymorphism has been demonstrated not to impair DNA repair ability (Takanami et al., 2005), but published data to date are consistent with the association between XRCC1 *194Trp* variant and reduced risk of various types of cancer (for review see Goode et al., 2002), and recently individuals carrying the variant allele showed a decreased level of chromosome-type breaks (Tuimala et al., 2004). Due to the low number of XRCC1 *194Trp* variant allele carriers included in this study (2 exposed and 3 controls), no conclusive results could be obtained in the analysis of this polymorphism.

Several studies demonstrating an association between the XRCC1 *399Gln* variant allele and cancer risk have been published, although conflicting reports demonstrating no clear association are also available (Goode et al., 2002). Nevertheless, this polymorphism has been reported to influence the ability of XRCC1 to repair DNA (Qu et al., 2005), and the repair rate of irradiation-induced DNA damage was found to be almost 2-fold higher in wild-type homozygote individuals, as compared with variant allele homozygotes (Vodicka et al., 2004). Increased DNA damage has been obtained in our exposed population for carriers of the variant *399Gln* allele, but decreased DNA damage in the control group. This is indicative of an exposure-specific genotype effect, since the effect observed in the controls probably represents the influence on the baseline level of the biomarker.

XRCC3 is involved in the homologous recombinational pathway of DNA double-strand break repair; its gene product interacts with Rad51 (Johnson and Jasin, 2001). XRCC3 *241Met* allele was related to higher DNA adduct levels in leukocytes (Matullo et al., 2001), but did not alter oxidative or radiation-induced DNA damage repair rate (Vodicka et al., 2004). XRCC3 *Thr241Met* was not consis-

tently associated with cancer risk either (Goode et al., 2002). Agreeing to these results, no effect of this genetic polymorphism on exposure-associated DNA damage was observed in this study.

APE1 participates in the BER pathway by incising the phosphodiester bond immediately 5′ to an abasic lesion, and has been suggested to be one of the rate-limiting steps in the BER pathway under a variety of situations (Kelley and Parsons, 2001). APE1 *148Glu* variant allele has been shown to be significantly associated to ionizing radiation-induced mitotic delay in peripheral lymphocytes (Hu et al., 2001). Nevertheless, no association has been found between APE1 codon 148 polymorphism and lung cancer risk (Misra et al., 2003), and APE1 *148Glu* variant has not been found to have impact on the DNA binding capacity or endonuclease activity of the protein (Hadi et al., 2000). As for XRCC1 codon 399, different results in both populations analysed have been obtained for the effect of APE1 codon 148 polymorphism on the comet assay. Increased DNA damage has been observed in the controls carriers of the *148Glu* variant allele, and decreased DNA damage in the exposed, suggesting again an exposure–genotype interaction.

Interactions between variables included in this work have been only found in the comet assay. Agreeing with our data, interactions have been previously found between age and XRCC1 codon 399 polymorphism (Rossit et al., 2002), tobacco consumption and XRCC3 polymorphism (Stern et al., 2001; Matullo et al., 2001), both polymorphisms of XRCC1 gene (Joseph et al., 2005) and polymorphisms of XRCC1 codon 399 and APE1 (Hu et al., 2002). Nevertheless, interaction between tobacco smoking and XRCC1 codon 399 polymorphism, widely documented (Duell et al., 2000; Matullo et al., 2001; Lei et al., 2002; Olshan et al., 2002), was not observed probably due to the low number of smokers examined.

In summary, our results indicate that exposure to *Prestige* oil during cleaning and autopsies of contaminated birds induces DNA damage, as evaluated by means of comet assay, but does not increase MN frequency. Individuals carrying XRCC1 *399Gln* or APE1 *148Glu* variant alleles might be considered at higher risk for DNA damage related to this exposure, although cautious interpretation is required because the relatively small sample size does not allow definitive conclusions to be reached. These results must be confirmed in further studies actually running, that will enlarge sample sizes and include several groups of individuals exposed to *Prestige* oil, engaged in different cleaning labours.

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