Annals of Internal Medicine | ORIGINAL RESEARCH

Health Changes in Fishermen 2 Years After Clean-up of the *Prestige* Oil Spill

Gema Rodríguez-Trigo, MD; Jan-Paul Zock, PhD; Francisco Pozo-Rodríguez, MD; Federico P. Gómez, MD; Gemma Monyarch, MSc; Laura Bouso, MSc; M. Dolors Coll, PhD; Héctor Verea, MD; Josep M. Antó, MD; Carme Fuster, PhD; and Joan Albert Barberà, MD, for the SEPAR (Sociedad Española de Neumología y Cirugía Torácica)-Prestige Study Group*

Background: In 2002, the oil tanker *Prestige* spilled more than 67 000 tons of bunker oil, heavily contaminating the coast of northwestern Spain.

Objective: To assess respiratory effects and chromosomal damage in clean-up workers of the oil spill 2 years after the exposure.

Design: Cross-sectional study.

Setting: Fishermen cooperatives in coastal villages.

Participants: Local fishermen who were highly exposed ($n = 501$) or not exposed ($n = 177$) to oil 2 years after the spill.

Measurements: Respiratory symptoms; forced spirometry; methacholine challenge; markers of oxidative stress (8-isoprostane), airway inflammation (interleukins, tumor necrosis factor-α, and interferon-γ), and growth factor activity in exhaled breath condensate; and chromosomal lesions and structural alterations in circulating lymphocytes.

Results: Compared with nonexposed participants, persons exposed to oil were at increased risk for lower respiratory tract symptoms (risk difference, 8.0 [95% CI, 1.1 to 14.8]). Lung function did not significantly differ between the groups. Among nonsmoking participants, exposed individuals had higher exhaled 8-isoprostane levels than nonexposed individuals (geometric mean ratio, 2.5 [CI, 1.7 to 3.7]), and exposed individuals with lower respiratory tract symptoms had higher 8-isoprostane levels than those of exposed individuals without symptoms. Exposed nonsmoking participants also had higher levels of exhaled vascular endothelial growth factor (risk difference, 44.8 [CI, 27.9 to 61.6]) and basic fibroblast growth factor (risk difference, 16.0 [CI, 3.5 to 28.6]). A higher proportion of exposed participants had structural chromosomal alterations (risk difference, 27.4 [CI, 10.0 to 44.8]), predominantly unbalanced alterations. The risk for elevated levels of exhaled 8-isoprostane, vascular endothelial growth factor, and basic fibroblast growth factor and structural chromosomal alterations seemed to increase with intensity of exposure to clean-up work.

Limitations: The clinical significance of exhaled biomarkers and chromosomal findings are uncertain. The association between oil exposure and the observed changes may not be causal. The findings may not apply to spills involving other types of oil or to different populations of oil spill workers.

Conclusion: Participation in clean-up of a major oil spill was associated with persistent respiratory symptoms, elevated markers of airway injury in breath condensate, and chromosomal damage.

Primary Funding Source: Instituto de Salud Carlos III.

Ann Intern Med. 2010;153:489-498. www.annals.org

For author affiliations, see end of text.

* For members of the SEPAR-Prestige Study Group, see the **Appendix** (available at www.annals.org).

This article was published at www.annals.org on 24 August 2010.

More than 2.2 billion metric tons of oil is shipped by tanker every year around the world by a fleet comprising more than 11 000 oil tankers. Between 1974 and 2008, more than 9000 tanker incidents were reported, among which 348 resulted in spills of more than 700 tons of oil (1). Oil spills cause great public concern, especially among people living in affected coastal areas, and large numbers of volunteers are mobilized to clean up the oil sediment.

Consequences of oil spills are usually evaluated in terms of environmental damage, effects on marine species, and economic losses, but relatively little is known about the effects of oil exposure on humans. Acute exposure to aromatic hydrocarbons, which are common constituents of oil, are known to cause respiratory symptoms (2). Certain volatile organic oil compounds, in particular benzene, are carcinogenic in humans and have been associated with hematologic cancer (3). Exposure to polycyclic aromatic hydrocarbons can damage the skin and mucous membranes and have been implicated in the pathogenesis of skin tumors (4).

In November 2002, the oil tanker *Prestige* foundered and spilled more than 67 000 tons of bunker oil, heavily contaminating the coast of Galicia in northwestern Spain. The spilled oil contained aromatic hydrocarbons (including benzene), saturated hydrocarbons, heavy metals, resins, and asphaltenes (5). More than 300 000 volunteers participated in clean-up activities; among them, local fishermen

See also:

Print

Editors' Notes 490
Glossary 490
Editorial comment. 540
Summary for Patients. I-28

Web-Only

Appendix
Appendix Tables
Appendix Figures
Conversion of graphics into slides

Context

Oil spills are ecological disasters, but their health effects on humans are not well known.

Contribution

This study found that Spanish fishermen who participated in the clean-up of a coastal oil spill had a higher prevalence of respiratory symptoms, higher levels of markers suggestive of airway injury in exhaled breath condensate, and chromosomal alterations in lymphocytes than did those who did not participate in clean-up activities.

Caution

The clinical significance of the marker and chromosomal findings is not known. The study does not prove that oil exposure caused the abnormalities.

Implication

Participation in clean-up of a major oil spill seemed to have adverse health effects. The clinical significance of the findings is not known.

—The Editors

were a large and highly exposed group. Studies of persons who participated in clean-up activities either as volunteers or as paid workers during the active period of clean-up showed that exposure to oil was associated with genomic damage (6–8), and a questionnaire that we distributed to fishermen showed increased rates of respiratory symptoms 1 to 2 years after participating in clean-up (9).

We sought to follow up those observations in this study of longer term health effects of the *Prestige* oil spill. Specifically, we evaluated changes in lung function; assessed respiratory markers of oxidative stress and airway inflammation in exhaled breath condensate (EBC) (10); and assessed chromosomal damage, a biomarker of increased risk for cancer (11, 12), 22 to 27 months after the spill in fishermen who had been highly exposed to oil during the clean-up work. We hypothesized that exposure to spilled oil would be associated with persistent abnormalities in lung function, inflammatory and oxidative changes in the airways, and evidence for genotoxicity similar to those reported in other occupational exposures to oil and its components (13–16).

METHODS

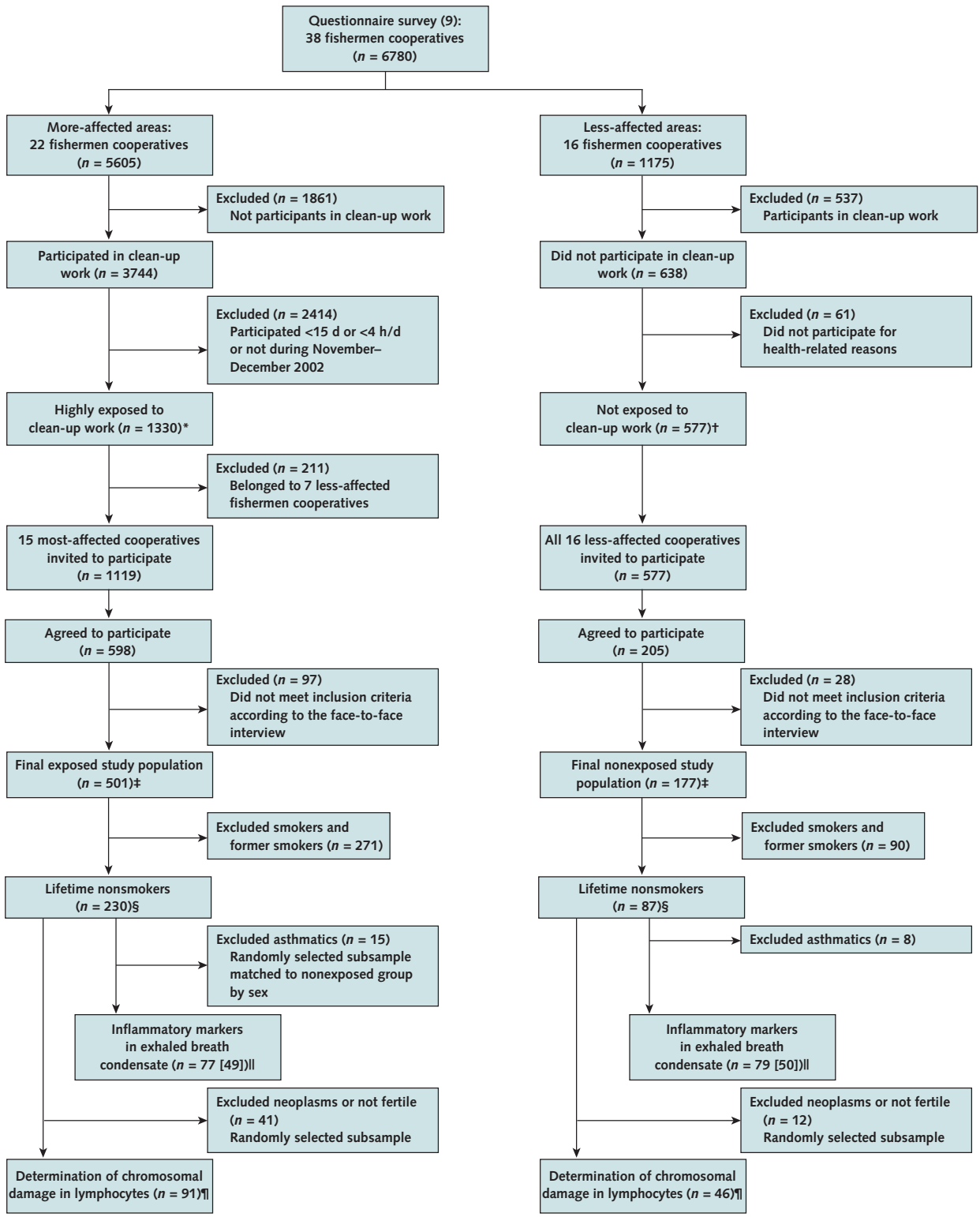
Design and Participants

Study participants were fishermen who had taken part in a previous questionnaire survey that included qualitative and quantitative information about participation in clean-up activities (9). Using this self-reported information, we distinguished exposed from nonexposed individuals (**Figure**). Exposed individuals ($n = 1119$) were members of fishermen cooperatives in heavily affected areas of the Atlantic coast who had participated at least 15 days in clean-up activities, for 4 or more hours per day, including November and December 2002, when exposure presumably was greatest. Nonexposed fishermen ($n = 577$) were members of cooperatives in areas of the Cantabrian coast (which was less affected by the oil spill) who did not participate in clean-up activities for reasons other than those related to health. Among the 598 (53%) exposed and 205 (35%) nonexposed fishermen who agreed to participate in the study, 97 exposed and 28 nonexposed individuals reported inconsistencies in details of clean-up work in a subsequent interview and were excluded from this analysis,

Glossary

Aberrant metaphase: Metaphase with some chromosomal lesion or alteration.
Acentric chromosome/acentric fragments: Chromosome with no centromere.
Balanced chromosomal alterations: Exchange of segments between chromosomes so that no genetic material is lost or gained.
Banded chromosome: Chromosome that is clearly distinguishable from the others by showing darker or lighter regions obtained with banding techniques.
Banded metaphases: All chromosomes of the cell phase are banded.
Banding techniques: Technical procedures that produce banding patterns on metaphase chromosomes.
Chromatid: One of 2 replicated arms of a chromosome.
Chromatid break: Discontinuity of a single chromatid in which there is a clear misalignment of 1 of the chromatids.
Chromatid gap: Nonstaining region of a single chromatid in which there is minimal misalignment of the chromatid.
Chromosomal alteration: Change of chromosome number or structure.
Chromosomal damage: Chromosomes with some lesion or structural alteration.
Chromosomal lesions: Chromosomes with gaps or breaks.
Chromosomal unbalances: Chromosomes with loss or gain of genetic material.
Chromosome: Structure in which genes are located within the cell, consisting of a highly compacted stretch of DNA with associated proteins.
Chromosome break: Discontinuity at the same locus in both chromatids of a single chromosome.
Chromosome gap: Nonstaining region at the same locus in both chromatids of a single chromosome in which there is minimal misalignment of the chromatids.
Chromosome preparation: Extension of a cell suspension on a slide.
Cytogenetic: Pertaining to chromosomes.
Deletion: Mutation due to loss of large chromosomal region.
Destain: To remove the color from a chromosome preparation.
G-banding: Chromosome staining by Giemsa resulting in characteristic patterns of light and dark bands along the chromosome.
Genotoxic effects: DNA damage produced by a toxic agent.
Karyotype: Chromosome complement of a cell or organism; often represented by an arrangement of metaphase chromosomes according to their lengths and the positions of their centromeres.
Leishman stain: Stain used in nonbanding technique.
Marker chromosomes: Structurally abnormal chromosome that cannot be identified or characterized by conventional banding cytogenetics.
Metaphase: Stage of cell division when chromosomes are aligned at the center of the cell before separation.
Ring chromosome: Chromosome abnormality in which a ring forms after breakage of both the long and the short arms.
Structural chromosomal alteration: Significant change of chromosome structure.
Translocation: Exchange of segments between chromosomes.
Unbalanced chromosomal alterations: Exchange of segments between chromosomes, with loss or gain of genetic material.
Uniform stain: Chromosomes stained by methods that do not produce bands.

Figure. **Study flow diagram.**



* Participated in clean-up activities for at least 15 days, for 4 or more hours per day on average, including November and December 2002. † Did not participate in clean-up activities for non–health-related reasons. ‡ Met the inclusion criteria both at the questionnaire survey and at the face-to-face interview. § Participants reported never having smoked both at the questionnaire survey and at the face-to-face interview. || 8-Isoprostane was measured in all participants indicated; the numbers in brackets indicates those for whom additional analyses of cytokines and growth factors were done. ¶ Participants reported having children (which proved their fertility) and had no history of malignant neoplasms.

leaving 501 exposed and 177 nonexposed persons in the final study population (**Figure**).

The study was performed between September 2004 and February 2005, 22 to 27 months after the spill and almost 2 years after most of the exposed participants came into contact with the oil (**Appendix Figure 1**, available at www.annals.org, shows the timing of events). A face-to-face interview was performed and outcome measures were obtained on the same day at the fishermen cooperative in a mobile unit that traveled to participants' coastal villages. Because the coastal area affected by the oil spill was known, nurses obtaining the measures were not blinded to exposure status. The project was approved by the Ethics Committee on Clinical Research of Galicia, and all participants provided written informed consent.

Interview and Clinical Testing

All participants completed a second interviewer-led questionnaire on respiratory symptoms and medication use, smoking habits, participation in clean-up activities, and characteristics of these activities. Items in this questionnaire were the same as those in the previous survey (9). Participants underwent spirometry testing for FEV_1 and FVC measurement and methacholine challenge; bronchial hyperresponsiveness was defined as a 20% decrease in FEV_1 associated with a methacholine dose of 2 mg or less. Participants also had serum total IgE measurement and skin-prick testing for 19 common and occupational allergens to help distinguish intrinsic (atopic) from extrinsic (environmental) causes of symptoms. Atopy was defined as a positive reaction to at least 1 of the tested allergens (**Appendix Table 1**, available at www.annals.org).

Assessment of Biomarkers in EBC

We used EBC to assess respiratory biomarkers of oxidative stress and inflammation. Samples were obtained by using an EcoScreen condenser (Jaeger, Würzburg, Germany) following current recommendations (17), through breathing at normal frequency and tidal volume until a total expired volume of 180 L was achieved. After collection, the condensing device was centrifuged at 4 °C, and the resultant EBC volume was distributed in 1-mL aliquots and rapidly frozen in liquid nitrogen. All samples were lyophilized and stored at −80 °C before analysis.

We measured 8-isoprostane in a subsample of the population by using an enzyme immunoassay after resuspension with 400 μL of assay buffer. The subgroup comprised all 79 nonexposed individuals who were nonasthmatic and lifetime nonsmokers (75% women) and 77 exposed individuals randomly selected from 230 exposed individuals who also were nonasthmatic and lifetime nonsmokers, matched by sex to the nonexposed group (**Figure**). 8-Isoprostane is a well-known stable product of local oxidative stress (18). We excluded smokers because of associations between smoking and markers of oxidative stress and inflammation (19).

Among 49 exposed participants and 50 nonexposed participants for whom sufficient EBC samples remained after 8-isoprostane testing, we also measured 10 cytokines and growth factors (interleukin-1β, 2, 4, 6, and 8; tumor necrosis factor-α; interferon-γ; vascular endothelial growth factor [VEGF]; monocyte chemotactic protein-1; and basic fibroblast growth factor [bFGF]) that are representative of Th1/Th2 inflammation (17, 20) and airway remodeling (21). Investigators who obtained these measurements were blinded to exposure status and used the Cytometric Bead Arrays Flex System (BD Biosciences, Erembodegem, Belgium) after resuspension with 100 μL of human soluble protein buffer.

Assessment of Chromosomal Damage

We assessed chromosomal damage in circulating lymphocytes. This measure of harm is often used in environmental studies and is an early marker of genotoxicity that has been associated with an increased risk for cancer (22–24). The assessment was conducted in a preselected subsample of lifetime nonsmoking participants without a history of cancer. We again excluded smokers because of associations between smoking and chromosomal damage; we also excluded participants who did not have children because infertile individuals may have an impaired ability to produce gametes. Thus, 91 exposed and 46 nonexposed participants were included (**Figure**). Assessment of chromosomal damage included evaluation of chromosomal lesions (chromatid gaps and breaks) and structural chromosomal alterations (deletions, acentric fragments, translocations, and marker chromosomes) (25).

Peripheral blood samples were obtained, and within 48 hours, lymphocytes were cultured for 72 hours in RPMI-1640 medium (GIBCO Invitrogen Cell Culture, Invitrogen, Carlsbad, California), according to standard procedures. All cultures were performed in duplicate. Chromosome preparations were uniformly stained with Leishman stain (1:4 in Leishman buffer) in order to detect gaps and breaks. For each participant, at least 100 randomly selected metaphases were investigated. In addition, the same preparations were destained and reexamined after G-banding to further detect break-points involved in chromosomal lesions and to characterize structural chromosomal alterations. At least 25 banded metaphases were karyotyped in each participant. The analyses were carried out independently by 2 trained evaluators who were blinded to exposure status. All aberrant metaphases were checked by 2 observers, and agreement was reached in cases of discordance. We verified that the number of evaluated metaphases did not differ between exposed and nonexposed participants.

Statistical Analysis

Differences in characteristics between participants and nonparticipants and between exposed and nonexposed participants were evaluated by using chi-square tests for categorical variables and *t* tests for continuous variables. Dif-

Table 1. **Participant Characteristics**

Characteristic	All Participants (n = 678)			Lifelong Nonsmokers (n = 317)		
	Exposed (n = 501)	Nonexposed (n = 177)	P Value*	Exposed (n = 230)	Nonexposed (n = 87)	P Value*
Women, *n (%)*	141 (28.1)	80 (45.2)	<0.001	109 (47.4)	64 (73.6)	<0.001
Mean age (SD), *y*						
All participants	44.7 (11.4)	47.3 (10.6)	0.007	46.5 (11.6)	51.4 (9.4)	0.001
Women	51.0 (8.9)	51.3 (9.3)	0.85	53.0 (8.3)	53.9 (6.7)	0.48
Men	42.1 (11.3)	44.0 (10.6)	0.15	40.6 (11.1)	44.5 (12.2)	0.13
Smoking status, *n (%)*			0.49			
Former	118 (23.6)	34 (19.2)		–	–	–
Current smokers	153 (30.5)	56 (31.6)		–	–	–
Ever had asthma, *n (%)*	34 (6.8)	10 (5.6)	0.59	15 (6.5)	6 (6.9)	0.90
Participation in clean-up work						
Median days of clean-up work (range)	87 (15–429)	0	–	90 (15–429)	0	–
Median hours per day of clean-up work (range)	6 (4–18)	0	–	6 (4–14)	0	–
Median types of clean-up activity (range), *n*†	5 (1–10)	0	–	5 (1–9)	0	–
Used facemask often or always, *n (%)*	166 (33.1)	0	–	81 (35.2)	0	–

* Obtained from chi-square tests for categorical variables and t tests for continuous variables.
† The most common clean-up activities were gathering oil from coastal rocks (83%), transporting the gathered oil (81%), gathering oil from beaches (73%), gathering oil from the sea (67%), cleaning work clothes or boots that were used during the gathering of oil (45%), and cleaning boats used for gathering oil (43%).

ferences in categorical health outcomes between exposed and nonexposed persons were expressed as adjusted absolute risk differences, estimated from multivariable generalized linear models with the binomial family with identity link and regular variance estimates based on the expected information matrix, controlling for sex and smoking where applicable. If the models did not converge, sex as a covariate was removed. Differences in percentage of predicted lung function (26) between the 2 groups were evaluated by using multivariable linear regression analyses adjusted for pack-years smoked. Because the concentration of 8-isoprostane in EBC approximated a log-normal distribution, log-transformed values of this variable were used throughout in analyses. Thus, group means were expressed as geometric means, and differences in 8-isoprostane level between groups were expressed as adjusted geometric mean ratios obtained from multivariable linear regression analysis of the log-transformed concentration adjusted for sex. Levels of each of the 10 markers measured in EBC were dichotomized by using the lower limit of detection provided by the manufacturer as the cutoff. Potential dependence of the presence of chromosomal lesions and structural alterations between metaphases within individuals was evaluated by using the correlation matrix from generalized estimating equation analysis. Because no dependence could be demonstrated (correlation coefficient <0.01), associations between exposure to clean-up work and chromosomal damage at the individual level were determined by using generalized linear models as described. Dose–response relationships were investigated for major study outcomes in analyses by using 3 increasing categories of exposure intensity. The P value for linear trend was obtained from adjusted regression models that included the respective exposure index as a continuous variable. Analyses were done by using Stata SE, version 10.0 (StataCorp, College Station, Texas).

Role of the Funding Source

The study was supported by grants from Instituto de Salud Carlos III/European Regional Development Fund, Sociedad Española de Neumología y Cirugía Torácica (SEPAR), and Centro de Investigación en Red de Enfermedades Respiratorias. The sponsors had no role in study design, data collection, data analysis, or data interpretation or in the writing of the report.

RESULTS

Study participants were more likely than nonparticipants in both the exposed and nonexposed groups to be female and never-smokers ($P < 0.001$); age distribution was similar. Nonexposed participants reported lower respiratory tract symptoms in the questionnaire survey (9) more often than nonparticipants (30% vs. 19%; $P = 0.003$), whereas the prevalence of lower respiratory tract symptoms was similar in exposed participants and nonexposed participants (37% vs. 38%; $P = 0.80$).

A higher proportion of participants exposed to clean-up work were men and were younger than those who were not exposed (**Table 1**). Female participants were 8 years older than men on average. Exposed and nonexposed persons did not significantly differ in smoking history, although a higher proportion of men than women were current smokers (39.8% vs. 12.8%) and former smokers (28.7% vs. 9.5%). Geometric mean serum total IgE levels

Table 2. **Associations Between Exposure to Clean-up Work and Respiratory Outcomes**

Variable	All Participants (n = 678)			Lifelong Nonsmokers (n = 317)		
	Exposed (n = 501)	Nonexposed (n = 177)	Risk Difference (95% CI)*	Exposed (n = 230)	Nonexposed (n = 87)	Risk Difference (95% CI)†
Lower respiratory tract symptoms, *n (%)*‡						
All types of symptoms	132 (26.6)	37 (20.9)	8.0 (1.1 to 14.8)	51 (22.3)	14 (16.1)	8.8 (0.1 to 17.4)
Asthma-like symptoms	101 (20.2)	32 (18.1)	4.1 (−2.4 to 10.5)	41 (17.8)	13 (14.9)	5.0 (−3.1 to 13.1)
Bronchitis-like symptoms	64 (12.9)	17 (9.6)	4.2 (−0.4 to 8.7)§	19 (8.3)	4 (4.6)	3.7 (−2.0 to 9.4)‖
Nasal symptoms, *n (%)*	128 (25.7)	42 (23.9)	1.4 (−5.9 to 8.7)	55 (23.9)	22 (25.6)	−0.9 (−11.5 to 9.7)
Use of inhaled medication, *n (%)*	41 (8.2)	9 (5.1)	2.9 (−1.1 to 7.0)	17 (7.4)	7 (8.0)	−1.0 (−7.4 to 5.4)
Use of oral medication, *n (%)*	28 (5.6)	11 (6.2)	−0.7 (−4.8 to 3.5)	12 (5.2)	3 (3.4)	1.8 (−2.9 to 6.6)
Mean FEV_1 (SD), *% predicted*¶	101.3 (16.5)	101.5 (17.0)	−0.2 (−2.9 to 2.5)**	103.6 (15.5)	105.0 (17.4)	−1.2 (−5.2 to 2.8)**
Mean FVC (SD), *% predicted*¶	98.7 (14.1)	99.7 (14.7)	−0.9 (−3.3 to 1.5)**	98.7 (14.2)	100.3 (16.7)	−1.6 (−5.3 to 2.1)**
Bronchial hyperresponsiveness, *n (%)*††	79 (18.2)	24 (15.5)	3.4 (−2.1 to 8.9)	30 (15.2)	7 (9.1)	6.1 (−2.1 to 14.2)‖

* Adjusted for sex and smoking status.
† Adjusted for sex.
‡ Wheeze with breathlessness, wheeze apart from colds, or nocturnal attacks of shortness of breath (asthma-like symptoms) and chronic cough or chronic phlegm (bronchitis-like symptoms).
§ Adjusted for smoking status only (the fully adjusted model did not converge).
‖ Unadjusted estimate (the fully adjusted model did not converge).
¶ Based on 670 measurements for all participants and 312 measurements for lifelong nonsmokers.
** Difference (regression coefficient) in predicted lung function, adjusted for pack-years of smoking in analysis of all participants.
†† Methacholine dose of 2 mg or less causing a 20% decrease in FEV_1. Based on 589 measurements for all participants and 275 measurements for lifelong nonsmokers.

were 32 IU/mL in exposed participants and 24 IU/mL in nonexposed participants ($P = 0.021$); atopy (a positive reaction to at least 1 skin prick antigen) was present in 22.7% and 17.0%, respectively ($P = 0.12$). After sex, age, and smoking status were controlled for, the difference in serum total IgE level between exposed and nonexposed participants lost statistical significance ($P = 0.086$).

Participants exposed to oil had an increased risk for lower respiratory tract symptoms (adjusted risk difference, 8.0 [95% CI, 1.1 to 14.8]), a finding also evident in the subsample of nonsmokers (**Table 2**). No statistically significant differences were found between exposed and nonexposed participants in nasal symptoms, medication use, or lung function. In sensitivity analyses of all associations reported in **Table 2** that were restricted to nonasthmatics, associations between exposure status and respiratory health outcomes remained similar in magnitude and direction (data not shown).

Exposed participants had statistically significantly higher concentrations of 8-isoprostane in EBC than nonexposed participants (geometric mean ratio, 2.5 [CI, 1.7 to 3.7]) (**Table 3**), and a higher proportion of exposed participants had measurable VEGF levels (risk difference, 44.8 [CI, 27.9 to 61.6]) and bFGF (risk difference, 16.0 [CI, 3.5 to 28.6]); differences between groups in other biomarkers were not statistically significant. Exposed participants with lower respiratory tract symptoms had higher 8-isoprostane levels in EBC than did those without symptoms (geometric mean, 34 vs. 11 pg/mL; adjusted geometric mean ratio, 3.2 [CI, 1.5 to 6.8]), a difference not found in nonexposed participants (geometric mean, 7.3 vs. 5.4 pg/mL; geometric mean ratio, 1.3 [CI, 0.7 to 2.4]) (**Appendix Figure 2**, available at www.annals.org).

There were no statistically significant differences between exposed and nonexposed participants in chromosomal lesions, whereas a higher proportion of exposed participants had structural chromosomal alterations (adjusted risk difference, 27.4 [CI, 10.0 to 44.8]) (**Table 4**), primarily chromosomal imbalances (translocations, acentric fragments, deletions, and markers). Twelve exposed participants and 1 nonexposed participant had metaphases with multiple structural chromosomal alterations when evaluated by using uniform stain.

The risk for several study outcomes increased with the degree of exposure to clean-up work (**Appendix Table 2**, available at www.annals.org). A statistically significant linear trend was seen for 8-isoprostane concentration and measurable VEGF or bFGF levels in EBC, as well as structural chromosomal alterations in lymphocytes, when the number of hours per day and the number of different clean-up activities was evaluated.

DISCUSSION

This study of health effects among fishermen who participated in clean-up of the *Prestige* oil spill about 2 years earlier confirms previously reported findings of an increase in respiratory symptoms and newly demonstrates increased 8-isoprostane levels and growth factor activity in EBC and more structural chromosomal alterations in circulating lymphocytes among fishermen exposed to the oil.

Compared with unexposed fishermen, a greater proportion of persons who voluntarily participated in clean-up activities of the oil spill had lower respiratory tract symptoms 2 years later. Consistent with the results of our first cross-sectional survey (9), the increase in respiratory symptoms was not explained by an increased prevalence of

chronic respiratory diseases in the exposed group. We could not demonstrate reductions in lung function values associated with exposure to clean-up work, consistent with a previous study that failed to demonstrate changes in airflow among residents of a coastal area affected by the *Braer* oil spill (27, 28) and inconsistent with another study showing lower lung function values in workers involved in the *Tasman Spirit* oil spill clean-up (29). A subgroup of lifetime nonsmokers had a non–statistically significant higher risk for bronchial hyperresponsiveness in response to methacholine challenge, suggesting that compounds of spilled oil might act as respiratory irritants that increase bronchial reactivity, at least in persons not chronically exposed to cigarette smoke. This finding is consistent with previously demonstrated associations between various aromatic hydrocarbons present in spilled oil and airway inflammation and hyperresponsiveness (30, 31).

The previous respiratory symptom findings are reinforced by the new observation of increased 8-isoprostane levels and growth factor activity in EBC of a subsample of nonasthmatic, nonsmoking exposed participants; the increased 8-isoprostane levels in this subgroup seem to be related to some measures of intensity of exposure. High levels of 8-isoprostane in EBC are considered to reflect local oxidative stress (32), and increased concentrations have been reported in a variety of inflammatory airway diseases (18, 19, 33, 34), in cigarette smokers (19), and in some occupational exposures (35). Of note, 8-isoprostane levels were associated with the presence of lower respiratory tract symptoms in exposed participants. These findings suggest that among the fishermen who participated in clean-up activities after the *Prestige* oil spill, exposure to oil products over days to a few months might have contributed to respiratory oxidative changes that were still measurable 2 years later. The association between exhaled 8-isoprostane levels and respiratory symptoms also suggests that oil-induced oxidative stress underlies the increased respiratory morbidity in exposed fishermen. Although we acknowledge that the clinical meaning of the measures is unclear, our findings suggest a potential role for measuring EBC biomarkers in epidemiologic studies.

The growth factors bFGF and VEGF were also increased in EBC in an even smaller subgroup of nonasthmatic, nonsmoking exposed participants. Basic fibroblast growth factor is involved in angiogenesis and stimulates the proliferation, migration, and differentiation of epithelial cells and fibroblasts. In asthma, epithelial injury by inflammation products or environmental agents can stimulate the release of a range of growth factors, including bFGF, that are active on fibroblasts and smooth-muscle cells, leading to airway remodeling (21). Accordingly, we speculate that increased bFGF levels in EBC might reflect an ongoing process of airway-wall remodeling. Regarding VEGF, little is known about its origins in biological lung fluids. It has been associated with asthma (36) and lung cancer (37), suggesting stimulated angiogenesis. In our study, the presence of VEGF in EBC might be related to airway remodeling; the origin and pathobiological significance of this finding merit further investigation.

We also detected an increased risk for structural chromosomal alterations in circulating lymphocytes among exposed workers, which had a dose-dependent relationship with some measure of intensity of exposure; frequency of chromosomal alterations in nonexposed persons was within

Table 3. **Respiratory Biomarkers in Exhaled Breath Condensate of Exposed and Nonexposed Participants Without Asthma Who Are Lifelong Nonsmokers**

Biomarker		Geometric Mean Level (95% CI), *pg/mL*		Geometric Mean Ratio (95% CI)*
		Exposed (*n* = 77)	Nonexposed (*n* = 79)	
8-Isoprostane		14	5.6	2.5 (1.7 to 3.7)
Biomarker	**Lower Limit of Detection, *pg/mL***	**Participants With Levels Above the Limit of Detection, *n (%)***		**Risk Difference (95% CI)***
		Exposed (*n* = 49)	Nonexposed (*n* = 50)	
Interleukin-1β	2.3	2 (4)	2 (4)	0.1 (−6.8 to 6.9)
Interleukin-2	11.2	7 (14)	4 (8)	4.0 (−6.9 to 14.8)
Interleukin-4	1.4	8 (16)	8 (16)	1.5 (−10.7 to 13.8)
Interleukin-6	1.9	0 (0)	0 (0)	NE
Interleukin-8	1.2	6 (12)	3 (6)	3.5 (−5.3 to 12.2)
Tumor necrosis factor-α	0.7	2 (4)	3 (6)	−0.5 (−7.5 to 6.5)
Interferon-γ	1.8	13 (27)	8 (16)	11.4 (−2.9 to 25.7)
Vascular endothelial growth factor	4.5	29 (59)	7 (14)	44.8 (27.9 to 61.6)
Monocyte chemotactic protein-1	1.3	15 (31)	10 (20)	9.0 (−6.5 to 24.6)
Basic fibroblast growth factor	3.4	11 (22)	3 (6)	16.0 (3.5 to 28.6)

NE = not estimable.
* Adjusted for sex.

Table 4. **Associations Between Exposure to Clean-up Work and Chromosomal Lesions and Structural Alterations in Lymphocytes of Lifelong Nonsmoking Participants***

Chromosomal Lesions†	Exposed Participants		Nonexposed Participants		Risk Difference (95% CI)‡
	Total (n = 91), n (%)	Metaphases With Chromosomal Lesions (n = 9520), n	Total (n = 46), n (%)	Metaphases With Chromosomal Lesions (n = 4859), n	
Absent	42 (46)	0	26 (57)	0	0.0 (reference)
Present	49 (54)	84	20 (43)	32	10.8 (−7.4 to 28.9)
Gaps	31 (34)	44	15 (33)	18	5.4 (−14.0 to 24.7)
Chromatid gap	27 (30)	38	11 (24)	13	9.9 (−9.4 to 29.2)
Chromosome gap	6 (7)	7	5 (11)	5	−4.4 (−20.8 to 12.1)
Breaks	31 (34)	45	13 (28)	14	10.9 (−8.2 to 29.9)
Chromatid break	17 (19)	21	8 (17)	8	5.7 (−12.9 to 24.2)
Chromosome break	18 (20)	27	7 (15)	7	9.9 (−8.9 to 28.7)
Structural Chromosomal Alterations§	**Exposed Participants**		**Nonexposed Participants**		**Risk Difference (95% CI)‡**
	Total (n = 91), n (%)	Metaphases With Structural Chromosomal Alterations (n = 2448), n	Total (n = 46), n (%)	Metaphases With Structural Chromosomal Alterations (n = 1285), n	
Absent	27 (30)	0	25 (54)	0	0.0 (reference)
Present	64 (70)	136	21 (46)	27	27.4 (10 to 44.8)
Balanced abnormalities	11 (12)	12	7 (15)	7	7.1 (−13.3 to 27.4)‖
Unbalanced abnormalities	62 (68)	125	17 (37)	20	31.3 (13.4 to 49.1)
Deletions	29 (32)	32	8 (17)	9	27.6 (7.3 to 48.0)
Translocations	24 (26)	29	4 (9)	4	33.3 (14.7 to 51.8)‖
Rings	8 (9)	8	0 (0)	0	NE
Acentric fragments	18 (20)	25	0 (0)	0	NE
Markers	30 (33)	37	9 (20)	9	27.8 (8.3 to 47.2)

NE = not estimable.
* Participants reported never having smoked both at the questionnaire survey and at the face-to-face interview, had children (proven fertility), and had no history of cancer.
† Detected by using uniform stain.
‡ Adjusted for sex.
§ Detected by using G-banded karyotypes.
‖ Unadjusted estimate (fully adjusted model did not converge).

the normal range (22). Chromosomal damage in circulating lymphocytes is an early marker of genotoxicity associated with increased risk for cancer (22–24). Genotoxicity studies, particularly those focusing on structural chromosomal alterations, are standard ways to assess risk for cancer in potentially toxic occupational or environmental exposures (12, 23). Genotoxic studies in volunteers exposed to oil spills are scarce (38). It is well-known that chromosomal damage is increased in cigarette smokers (39) and is associated with occupational exposure to benzene (13–16, 40) or coal combustion products (41), both of which contain aromatic hydrocarbons that are an important constituent of the oil spilled by the *Prestige* tanker. Genome damage related to early exposure has been shown in clean-up workers of the *Prestige* oil spill during the clean-up (6, 8); the chromosomal alterations we analyzed provide additional information on more persistent adverse health effects (11, 12).

We did not anticipate finding increased chromosomal damage, in particular unbalanced alterations, 2 years after exposure. Little information exists regarding the cytogenetic effects induced by acute exposure to chemical clastogens (42). Persistent unstable chromosome alterations in lymphocytes 20 years after acute benzene exposure have been reported (42). The precise pathways involved in chromosomal damage remain to be elucidated, but possible mechanistic explanations for the findings include persistence of genotoxic effects of acute exposure (43); persistence of chemical compounds leading to continuous exposure; genotoxic effects on bone marrow progenitor cells; and dysfunction in DNA repair proteins or alterations in epigenetic factors induced by exposure to oil.

The clinical implications of chromosomal damage in individuals exposed to oil while cleaning up spills are unknown. Chromosomal damage is a characteristic feature of cancer cells and is crucial for tumor pathogenesis (44). An increased frequency of chromosomal alterations in circulating lymphocytes has been associated with augmented cancer risk (45). The distribution of chromosomal damage is assumed to be similar in different tissues (11). Accordingly, we speculate that chromosomal damage detected in circulating lymphocytes might reflect a more general increased risk for cancer. Because the possibility of a higher risk for cancer in exposed workers cannot be excluded, a surveillance program in the target population would be appropriate. Follow-up studies to evaluate persistent respiratory

health effects, chromosomal damage, and the development of cancer in these individuals for longer periods are currently under way.

Our study has limitations. First, its design cannot establish that any of the observed associations are causal, and the findings cannot be extrapolated to other populations of clean-up workers or to the general population living in the area of the oil spill. In addition, the findings cannot be extrapolated to spills of other types of oil. For example, the *Prestige* tanker contained bunker C oil, whereas oil spilled in the 2010 U.S. Deepwater Horizon disaster is crude oil. The main components of the oil spills are presumably similar, but proportions of those components (for example, volatile organic compounds, polycyclic aromatic hydrocarbons, hydrogen sulfide gas, and heavy metals) are probably different, as might be dispersants to break up the oil slick and the proportion of oil that evaporates and could be inhaled by humans. Findings from this study therefore cannot predict what effects individuals exposed to other oil spills, such as that in the Gulf of Mexico might experience.

In summary, 2 years after participating in clean-up efforts of the *Prestige* oil spill, exposed fishermen had increased prevalence rates of respiratory symptoms and biomarkers of pulmonary oxidative stress and growth factor activity, suggesting persistent airway injury. In addition, they had more structural chromosomal abnormalities in circulating lymphocytes. Our findings indicate that exposure to oil sediments, even for short periods, may have detrimental health effects. To fully understand the importance and nature of these effects, further longitudinal and mechanistic research in similar episodes is warranted. Because, unfortunately, oil spills will most likely occur again, it is crucial that the authorities responsible for organizing clean-up operations take appropriate measures to guarantee the health protection of persons involved in the clean-up activities and to establish registries to systematically assess possible adverse health outcomes in exposed workers over time.

From Complexo Hospitalario Universitario A Coruña, A Coruña, Spain; Hospital Clínico San Carlos and University Hospital 12 de Octubre, Madrid, Spain; Centre for Research in Environmental Epidemiology (CREAL), Municipal Institute of Medical Research (IMIM-Hospital del Mar), Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Autonomous University of Barcelona, and Pompeu Fabra University, Barcelona, Spain; and Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Bunyola, Mallorca, Spain.

Acknowledgment: The authors thank the participating fishermen, the staff of their cooperatives, and the efforts made by A. Devesa; the SEPAR-Prestige data collection team (Y. Torralba, A. Souto, M. Rodríguez-Valcárcel, and L. Vázquez-Rey) for their outstanding work; the staff of Complexo Hospitalario Universitario A Coruña (S. Lamela, E. Rodríguez, M. Garea, and M. Saleta) for their support; D. Macfarlane for assistance with data management and reviewing the manuscript; A. Espinosa for her contribution to the statistical analyses; A. Serrano-Mollar and G. Gay for analysis of EBC; A. López-Rodríguez and C. Rodríguez-Escudero, formerly of Servizo Galego de Saúde, for their valuable collaboration; and J.L. Alvarez-Sala and J. Ancochea, past presidents of SEPAR, for their initiative and continuous support.

Grant Support: By Instituto de Salud Carlos III/European Regional Development Fund (grants PI03/1685 and PI05/0548), SEPAR, and Centro de Investigación en Red de Enfermedades Respiratorias. The Instituto de Salud Carlos III/European Regional Development Fund also provided grants to Dr. Rodríguez-Trigo (grant BAE 06/90018) and Dr. Zock (grant Miguel Servet 01-3058).

Potential Conflicts of Interest: Disclosures can be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M10-1030.

Reproducible Research Statement: *Study protocol and statistical code:* Available from Dr. Zock (e-mail, jpzock@creal.cat). *Data set:* Not available.

Requests for Single Reprints: Joan Albert Barberà, MD, Servei de Pneumologia, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain; e-mail, jbarbera@clinic.ub.es.

Current author addresses and author contributions are available at www.annals.org.

References

1. **The International Tanker Owners Pollution Federation.** Oil Tanker Spill Statistics: 2008. London: The International Tanker Owners Pollution Federation; 2009.

2. **Lyons RA, Temple JM, Evans D, Fone DL, Palmer SR.** Acute health effects of the Sea Empress oil spill. J Epidemiol Community Health. 1999;53:306-10. [PMID: 10396538]

3. **International Agency for Research on Cancer.** IARC Monographs on the Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans (Supplement 7). Lyon: International Agency for Research on Cancer; 1998.

4. **Agency for Toxic Substances and Disease Registry.** Toxicological profile for fuel oils. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 1995.

5. **Rodríguez-Trigo G, Zock JP, Isidro Montes I.** [Health effects of exposure to oil spills]. Arch Bronconeumol. 2007;43:628-35. [PMID: 17983548]

6. **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. [PMID: 16814914]

7. **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. [PMID: 17055056]

8. **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. 2008;653:117-23. [PMID: 18495522]

9. **Zock JP, Rodriguez-Trigo G, Pozo-Rodriguez F, Barbera JA, Bouso L, Torralba Y, et al; SEPAR-Prestige Study Group.** Prolonged respiratory symptoms in clean-up workers of the prestige oil spill. Am J Respir Crit Care Med. 2007;176:610-6. [PMID: 17556713]

10. **Antczak A, Górski P.** Markers of pulmonary diseases in exhaled breath condensate. Int J Occup Med Environ Health. 2002;15:317-23. [PMID: 12608619]

11. **Norppa H, Bonassi S, Hansteen IL, Hagmar L, Strömberg U, Rössner P, et al.** Chromosomal aberrations and SCEs as biomarkers of cancer risk. Mutat Res. 2006;600:37-45. [PMID: 16814813]

12. **Au WW.** Usefulness of biomarkers in population studies: from exposure to susceptibility and to prediction of cancer. Int J Hyg Environ Health. 2007;210:239-46. [PMID: 17174154]

13. **Holecková B, Piesová E, Sivikova K, Dianovsky J.** Chromosomal aberrations in humans induced by benzene. Ann Agric Environ Med. 2004;11:175-9. [PMID: 15627321]
14. **Tompa A, Jakab MG, Major J.** Risk management among benzene-exposed oil refinery workers. Int J Hyg Environ Health. 2005;208:509-16. [PMID: 16325561]
15. **Roma-Torres J, Teixeira JP, Silva S, Laffon B, Cunha LM, Méndez J, et al.** Evaluation of genotoxicity in a group of workers from a petroleum refinery aromatics plant. Mutat Res. 2006;604:19-27. [PMID: 16431152]
16. **McHale CM, Lan Q, Corso C, Li G, Zhang L, Vermeulen R, et al.** Chromosome translocations in workers exposed to benzene. J Natl Cancer Inst Monogr. 2008:74-7. [PMID: 18648008]
17. **Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, et al; ATS/ERS Task Force on Exhaled Breath Condensate.** Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J. 2005;26:523-48. [PMID: 16135737]
18. **Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ.** Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. Am J Respir Crit Care Med. 1999;160:216-20. [PMID: 10390403]
19. **Montuschi P, Collins JV, Ciabattoni G, Lazzeri N, Corradi M, Kharitonov SA, et al.** Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. Am J Respir Crit Care Med. 2000; 162:1175-7. [PMID: 10988150]
20. **Idali F, Wikén M, Wahlström J, Mellstedt H, Eklund A, Rabbani H, et al.** Reduced Th1 response in the lungs of HLA-DRB1*0301 patients with pulmonary sarcoidosis. Eur Respir J. 2006;27:451-9. [PMID: 16507843]
21. **Fedorov IA, Wilson SJ, Davies DE, Holgate ST.** Epithelial stress and structural remodelling in childhood asthma. Thorax. 2005;60:389-94. [PMID: 15860714]
22. **Hagmar L, Strömberg U, Bonassi S, Hansteen IL, Knudsen LE, Lindholm C, et al.** Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res. 2004;64:2258-63. [PMID: 15026371]
23. **Norppa H.** Cytogenetic biomarkers and genetic polymorphisms. Toxicol Lett. 2004;149:309-34. [PMID: 15093278]
24. **Rossner P, Boffetta P, Ceppi M, Bonassi S, Smerhovsky Z, Landa K, et al.** Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. Environ Health Perspect. 2005;113:517-20. [PMID: 15866756]
25. **Shaffer LG, Slovak ML, Campbell LJ.** ISCN 2009. An International System for Human Cytogenetic Nomenclature: Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. Basel: Karger; 2009.
26. **Roca J, Sanchis J, Agusti-Vidal A, Segarra F, Navajas D, Rodriguez-Roisin R, et al.** Spirometric reference values from a Mediterranean population. Bull Eur Physiopathol Respir. 1986;22:217-24. [PMID: 3730638]
27. **Campbell D, Cox D, Crum J, Foster K, Christie P, Brewster D.** Initial effects of the grounding of the tanker Braer on health in Shetland. The Shetland Health Study Group. BMJ. 1993;307:1251-5. [PMID: 8281057]
28. **Campbell D, Cox D, Crum J, Foster K, Riley A.** Later effects of grounding of tanker Braer on health in Shetland. BMJ. 1994;309:773-4. [PMID: 7950562]
29. **Meo SA, Al-Drees AM, Meo IM, Al-Saadi MM, Azeem MA.** Lung function in subjects exposed to crude oil spill into sea water. Mar Pollut Bull. 2008;56:88-94. [PMID: 18031764]
30. **Jang AS, Choi IS, Koh YI, Park CS.** Volatile organic compounds contribute to airway hyperresponsiveness. Korean J Intern Med. 2007;22:8-12. [PMID: 17427638]
31. **Podechard N, Lecureur V, Le Ferrec E, Guenon I, Sparfel L, Gilot D, et al.** Interleukin-8 induction by the environmental contaminant benzo(a)pyrene is aryl hydrocarbon receptor-dependent and leads to lung inflammation. Toxicol Lett. 2008;177:130-7. [PMID: 18289803]
32. **Koutsokera A, Loukides S, Gourgoulianis KI, Kostikas K.** Biomarkers in the exhaled breath condensate of healthy adults: mapping the path towards reference values. Curr Med Chem. 2008;15:620-30. [PMID: 18336277]
33. **Barreto M, Villa MP, Olita C, Martella S, Ciabattoni G, Montuschi P.** 8-Isoprostane in exhaled breath condensate and exercise-induced bronchoconstriction in asthmatic children and adolescents. Chest. 2009;135:66-73. [PMID: 18753466]
34. **Biernacki WA, Kharitonov SA, Barnes PJ.** Increased leukotriene B4 and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. Thorax. 2003;58:294-8. [PMID: 12668789]
35. **Pelclová D, Fenclová Z, Kacer P, Kuzma M, Navrátil T, Lebedová J.** Increased 8-isoprostane, a marker of oxidative stress in exhaled breath condensate in subjects with asbestos exposure. Ind Health. 2008;46:484-9. [PMID: 18840939]
36. **Leung TF, Wong GW, Ko FW, Li CY, Yung E, Lam CW, et al.** Analysis of growth factors and inflammatory cytokines in exhaled breath condensate from asthmatic children. Int Arch Allergy Immunol. 2005;137:66-72. [PMID: 15832052]
37. **Beinert T, Binder D, Oehm C, Ziemer S, Priem F, Schweigert M, et al.** Increased levels of vascular endothelial growth factor in bronchoalveolar lavage of patients with bronchial carcinoma effect of tumour activity and oxidative stress due to radio-chemotherapy? Eur J Med Res. 1999;4:328-34. [PMID: 10471544]
38. **Cole J, Beare DM, Waugh AP, Capulas E, Aldridge KE, Arlett CF, et al.** Biomonitoring of possible human exposure to environmental genotoxic chemicals: lessons from a study following the wreck of the oil tanker Braer. Environ Mol Mutagen. 1997;30:97-111. [PMID: 9329634]
39. **Littlefield LG, Joiner EE.** Analysis of chromosome aberrations in lymphocytes of long-term heavy smokers. Mutat Res. 1986;170:145-50. [PMID: 3713724]
40. **Zhang L, Eastmond DA, Smith MT.** The nature of chromosomal aberrations detected in humans exposed to benzene. Crit Rev Toxicol. 2002;32:1-42. [PMID: 11846214]
41. **Celik M, Donbak L, Unal F, Yüzbasioglu D, Aksoy H, Yilmaz S.** Cytogenetic damage in workers from a coal-fired power plant. Mutat Res. 2007;627: 158-63. [PMID: 17178253]
42. **Forni A.** Benzene-induced chromosome aberrations: a follow-up study. Environ Health Perspect. 1996;104 Suppl 6:1309-12. [PMID: 9118911]
43. **Sprent J, Tough DF.** Lymphocyte life-span and memory. Science. 1994;265: 1395-400. [PMID: 8073282]
44. **Fröhling S, Döhner H.** Chromosomal abnormalities in cancer. N Engl J Med. 2008;359:722-34. [PMID: 18703475]
45. **Bonassi S, Norppa H, Ceppi M, Strömberg U, Vermeulen R, Znaor A, et al.** Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. Carcinogenesis. 2008;29:1178-83. [PMID: 18356148]

Current Author Addresses: Dr. Rodríguez-Trigo: Servicio de Neumología, Hospital Clínico San Carlos, Profesor Martin Lago s/n, 28040 Madrid, Spain.
Drs. Zock and Antó and Ms. Bouso: Centre for Research in Environmental Epidemiology (CREAL), Doctor Aiguader, 88, 08003 Barcelona, Spain.
Dr. Pozo-Rodríguez: Servicio de Neumología y Unidad de Epidemiología Clínica, Hospital 12 de Octubre, Avenida de Córdoba s/n, 28041 Madrid, Spain.
Drs. Gómez and Barberà: Servei de Pneumologia, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain.
Ms. Monyarch and Drs. Coll and Fuster: Unidad de Biología Celular y Genética Médica, Facultad de Medicina, Universidad Autónoma de Barcelona, Campus de Bellaterra, 08193 Cerdanyola del Vallés, Barcelona, Spain.
Dr. Verea: Servicio de Neumoloxía, Complexo Hospitalario Universitario A Coruña, Xubias de Arriba 84, 15006 A Coruña, Spain.

Author Contributions: Conception and design: G. Rodríguez-Trigo, J.P. Zock, F. Pozo-Rodríguez, F.P. Gómez, H. Verea, J.M. Antó, C. Fuster, J.A. Barberà.
Analysis and interpretation of the data: G. Rodríguez-Trigo, J.P. Zock, F. Pozo-Rodríguez, F.P. Gómez, G. Monyarch, M.D. Coll, H. Verea, J.M. Antó, C. Fuster, J.A. Barberà.
Drafting of the article: G. Rodríguez-Trigo, J.P. Zock, F. Pozo-Rodríguez, F.P. Gómez, M.D. Coll, J.M. Antó, C. Fuster, J.A. Barberà.
Critical revision of the article for important intellectual content: G. Rodríguez-Trigo, J.P. Zock, F. Pozo-Rodríguez, F.P. Gómez, M.D. Coll, H. Verea, J.M. Antó, C. Fuster, J.A. Barberà.
Final approval of the article: G. Rodríguez-Trigo, J.P. Zock, F. Pozo-Rodríguez, F.P. Gómez, G. Monyarch, M.D. Coll, H. Verea, J.M. Antó, C. Fuster, J.A. Barberà.
Provision of study materials or patients: G. Rodríguez-Trigo, J.P. Zock, F. Pozo-Rodríguez, F.P. Gómez, H. Verea.
Statistical expertise: J.P. Zock, F. Pozo-Rodríguez.
Obtaining of funding: G. Rodríguez-Trigo, J.P. Zock, F. Pozo-Rodríguez, H. Verea, J.M. Antó, C. Fuster, J.A. Barberà.
Administrative, technical, or logistic support: G. Rodríguez-Trigo, F. Pozo-Rodríguez, G. Monyarch, H. Verea.
Collection and assembly of data: G. Rodríguez-Trigo, F. Pozo-Rodríguez, F.P. Gómez, G. Monyarch, L. Bouso, H. Verea, C. Fuster.

Appendix: Members of the SEPAR-Prestige Study Group

Chairs

J.A. Barberà, F. Pozo-Rodríguez, H. Verea (Spanish Society of Pulmonology and Thoracic Surgery, SEPAR).

Investigators

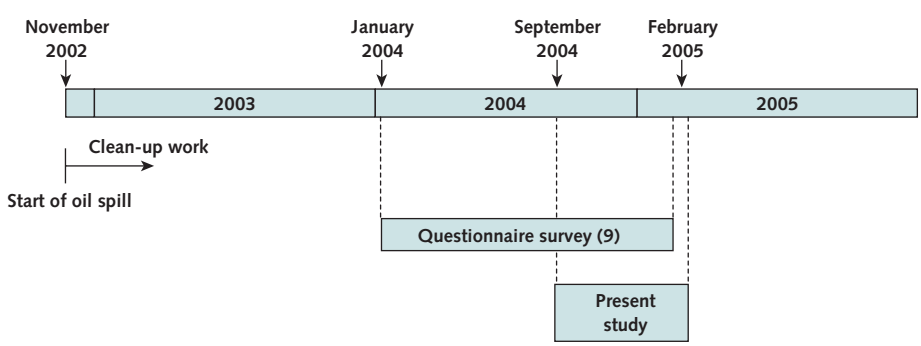
J.P. Zock, J.M. Antó, L. Bouso (Centre for Research in Environmental Epidemiology and Municipal Institute of Medical Research, Barcelona); G. Rodríguez-Trigo (Complexo Hospitalario Universitario A Coruña, A Coruña, and Hospital Clínico San Carlos, Madrid); F.P. Gómez, Y. Torralba, F. Burgos (Hospital Clínic-IDIBAPS, Barcelona); C. Fuster, G. Monyarch (Autonomous University of Barcelona, Barcelona).

Collaborators

L. Vázquez, L. Rodríguez-Valcárcel, A. Souto, M. Blanco (Complexo Hospitalario Universitario A Coruña, A Coruña); A. Serrano-Mollar, O. Bulbena (Institute of Biomedical Investigations of Barcelona [IIBB-CSIC], Barcelona); J. Tò (Hospital Clínic-IDIBAPS, Barcelona); M.D. Coll, J. Egozcue† (Autonomous University of Barcelona, Barcelona); E. Toubes (Complexo Hospitalario Universitario de Ourense, Ourense); I. Isidro (National Silicosis Institute, Oviedo); A. Palacios (Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela); M. Suárez (Complexo Hospitalario Universitario de Vigo, Vigo).

†Deceased.

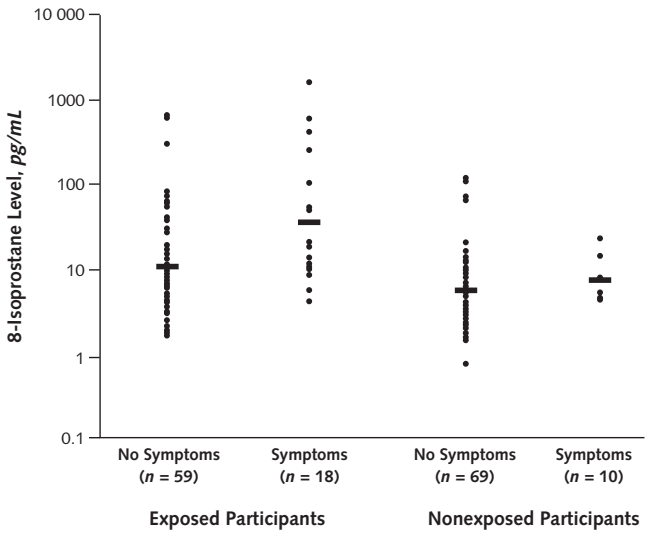
Appendix Figure 1. **Timeline of the study relative to the oil spill and clean-up events.**



Appendix Table 1. Allergens Included in Skin-Prick Testing

House dust mites: *Dermatophagoides pteronyssinus* and *D. farinae*
Cat: *Felis domesticus*
Dog: *Canis familiaris*
Grass pollen mix: *Gramineae* spp., Bermuda grass (*Cynodon dactylon*), timothy grass (*Phleum pratense*), and spreading pellitory (*Parietaria judaica*)
Tree pollen: birch (*Betula nigra*) and European pear (*Pyrus communis*)
Molds: *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Mucor* spp., and *Alternaria alternata*
Soybean (*Glycine max*) and whitefish mix

Appendix Figure 2. 8-Isoprostane levels in exhaled breath condensate of exposed and nonexposed participants, by presence of lower respiratory tract symptoms.



Bars indicate the geometric mean of the 8-isoprostane level. Lower respiratory tract symptoms were wheeze with breathlessness, wheeze apart from colds, nocturnal attacks of shortness of breath, chronic cough, or chronic phlegm.

Appendix Table 2. Associations Between the Degree of Exposure to Clean-up Activities and Selected Health Outcomes

Exposure Characteristic	Lower Respiratory Tract Symptoms		Bronchial Hyperresponsiveness*		8-Isoprostane Level†			Detectable VEGF or bFGF†‡		Chromosomal Lesion§		Structural Chromosomal Alterations‖	
	Participants, *n/N (%)*	RD (95% CI)‖	Participants, *n/N (%)*	RD (95% CI)¶	Participants, *n*	GM Level, *pg/mL*	GMR (95% CI)**	Participants, *n/N (%)*	RD (95% CI)††	Participants, *n/N (%)*	RD (95% CI)††	Participants, *n/N (%)*	RD (95% CI)††
Nonexposed	177/37 (20.9)	0.0 (reference)	77/7 (9.1)	0.0 (reference)	79	5.6	1.0 (reference)	50/8 (16)	0.0 (reference)	46/20 (43)	0.0 (reference)	46/21 (46)	0.0 (reference)
Exposed													
Overall duration													
15–100 d	283/78 (27.6)	8.5 (0.8 to 16.1)	114/23 (20.2)	11.1 (1.3 to 20.9)	41	14	2.5 (1.6 to 4.1)	29/15 (52)	34.9 (14.1 to 55.6)	45/25 (56)	12.5 (−8.4 to 33.5)	45/35 (78)	33.7 (15.0 to 52.4)
>100 d	213/54 (25.4)	7.3 (−0.7 to 15.3)	84/7 (8.3)	−0.8 (−9.5 to 8.0)	36	14	2.4 (1.5 to 4.0)	20/14 (70)	53.7 (31.6 to 75.8)	46/24 (52)	9.1 (−11.7 to 29.9)	46/29 (63)	20.2 (−0.4 to 40.8)
P value‡‡		0.14		0.43			<0.001		<0.001		0.41		0.064
Daily duration													
4–5 h	197/55 (27.9)	9.2 (0.9 to 17.5)	91/15 (16.5)	7.4 (−2.6 to 17.4)	42	11	2.0 (1.3 to 3.3)	28/21 (75)	58.3 (39.9 to 76.7)	53/28 (53)	9.7 (−10.1 to 29.4)	53/35 (66)	22.8 (3.2 to 42.3)
6–8 h	299/77 (25.8)	7.1 (−0.5 to 14.7)	107/15 (14.0)	4.9 (−4.3 to 14.1)	35	18	3.2 (1.9 to 5.2)	21/8 (38)	18.2 (−5.6 to 42.1)	38/21 (55)	12.7 (−9.9 to 35.3)	38/29 (76)	33.3 (13.9 to 52.7)
P value‡‡		0.13		0.65			<0.001		0.008		0.26		0.001
Number of types of clean-up work													
1–4	200/54 (27.0)	7.3 (−0.9 to 15.5)	82/15 (18.3)	9.2 (−1.3 to 19.7)	39	12	2.2 (1.4 to 3.6)	24/14 (58)	41.5 (19.2 to 63.7)	40/26 (65)	21.5 (0.6 to 42.4)	40/26 (65)	21.2 (0.8 to 41.6)
5–9	296/78 (26.4)	8.5 (0.9 to 16.0)	116/15 (12.9)	3.8 (−5.0 to 12.7)	38	16	2.8 (1.7 to 4.6)	25/15 (60)	43.9 (22.5 to 65.2)	51/23 (45)	1.6 (−19.0 to 22.1)	51/38 (75)	33.6 (14.5 to 52.6)
P value‡‡		0.076		0.87			<0.001		<0.001		0.95		0.002
Used facemask during clean-up													
Always	164/46 (28.0)	9.7 (0.9 to 18.6)	71/11 (15.5)	6.4 (−4.2 to 17.0)	25	15	2.6 (1.5 to 4.6)	15/11 (73)	55.8 (31.2 to 80.4)	29/14 (48)	5.1 (−18.1 to 28.2)	29/21 (72)	28.0 (6.3 to 49.6)
Sometimes	332/86 (25.9)	7.1 (−0.2 to 14.4)	127/19 (15.0)	5.9 (−3.1 to 14.8)	52	14	2.5 (1.6 to 3.8)	34/18 (53)	37.1 (17.8 to 56.5)	62/35 (56)	13.9 (−5.8 to 33.7)	62/43 (69)	27.1 (8.3 to 45.9)
P value‡‡		0.16		0.38			<0.001		<0.001		0.17		0.008

bFGF = basic fibroblast growth factor; GM = geometric mean; GMR = geometric mean ratio; RD = risk difference; VEGF = vascular endothelial growth factor.
* In lifelong nonsmokers.
† Measured in exhaled breath condensate.
‡ VEGF level ≥4.5 pg/mL or bFGF level ≥3.4 pg/mL.
§ Detected in lymphocytes.
‖ Compared with nonexposed participants, adjusted for sex and smoking status.
¶ Compared with nonexposed participants, unadjusted.
** For exposed vs. nonexposed participants, adjusted for sex.
†† Compared with nonexposed participants, adjusted for sex.
‡‡ For linear trend.