보건학박사 학위논문

Long-Term Health Effects of the Hebei Spirit Oil Spill

허베이스피리트호 유류유출사고로 인한 중장기 건강영향

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ABSTRACT

Long-Term Health Effects of the Hebei Spirit Oil Spill

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Background: Leakage of the oil tanker Hebei Spirit in December 2007 resulted in the spill of 12,547 kL of crude oil onto only 5 miles off the Taean county, and polluted more than 1,052 km of west coastline of Republic of Korea. The crude oils mainly contain various volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals. These compounds might have caused a variety of short- and long-term adverse health effects on humans.

Objectives: The purpose of this dissertation was to longitudinally investigate long-term health effects in adults and children up to 5 years after the Hebei Spirit oil spill.

Chapter 1 aimed to assess oxidative stress in adults 1 year after the spill.

Chapter 2 aimed to follow up the levels of oxidative stress in adults living high-exposed area and to assess whether the effects are remaining 3 and 4 years after the spill.

Chapter 3 aimed to longitudinally assess asthma symptoms in children 1, 3, and 5 years after the spill.

Chapter 4 aimed to longitudinally assess lung function changes in children 1, 3 and 5 years after the spill.

Materials and Methods: In 2009, 1 year after the spill, a cohort of 9,246 adults and 828 school students was studied in the initial survey. Out of this initial cohort, two follow-up surveys of the adults in only the high-exposed area were conducted, once in 2010 (3 years after the spill) with 1,257 adults and the other in 2012 (4 years after the spill) with 1,158 adults, respectively. For children, two follow-up surveys were conducted 3 years after the spill (2011) with 760 school students and 5 years after the spill (2013) with 783 school students.

In cases of the oxidative stress of the adults (Chapter 1 and Chapter 2), total durations of clean-up work and the levels of urinary metabolites of polycyclic aromatic hydrocarbons (PAHs), including 1-hydroxypyrene (1-OHP) and 2-naphthol (2-NAPH), were used as surrogates of exposure to oil. Oxidative stress was measured using urinary levels of malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG), as indicators of oxidative lipid peroxidation and oxidative DNA damage, respectively.

In cases of the respiratory health of the children (Chapter 3 and Chapter 4),

the distance from oil-contaminated coastline to home and/or school and ambient cumulative levels of volatile organic compounds (VOCs), such as benzene, toluene, ethylbenzene, and xylene, for 4 days right after the spill based on modeling technique were used as surrogates of exposure to oil. Respiratory health was measured using standardized questionnaire for asthma symptoms and spirometry with parameters of the lung function, such as the percent predicted forced expiratory volume in 1 second (FEV_1%).

Results: Chapter 1. Levels of oxidative stress biomarkers were significantly increased for longer involvement in clean-up work 1 year after the spill (MDA, *p*-trend < 0.0001; 8-OHdG, *p*-trend < 0.0001). Level of 1-OHP had a significant positive correlation with the total duration of clean-up work involvement, with a higher level found in those who participated in clean-up for > 100 days. Increasing levels of 1-OHP were significantly associated with increased MDA and 8-OHdG after adjusting for covariates, while the strength of association weakened as time passed since the last participation in clean-up work. The association remained statistically significant up to 1 year after the last clean-up work.

Chapter 2. When high exposed group within 2 km from the oilcoastline was followed up, the levels of 8-OHdG slightly increased over time. Geometric mean of 8-OHdG 1 year, 3 years, and 4 years after the spill was 5.6, 5.8, and 6.3 μg/g creatinine, respectively, after adjusting for age, gender, smoking, and education levels. The levels of 1-OHP and total duration of clean-up work involvement did not show a significant positive relation to the levels of 8-OHdG 3 years and 4 years after the spill.

Chapter 3. When analysed in cross-sectional design, asthma symptoms were significantly more prevalent among children who resided in area and attended school closer to the accident point and exposed to higher cumulative BTEX levels, 1 year, 3 years, and up to 5 years after the spill. These relationships were observed more clearly among preschool children upon accident. When analysed in longitudinal design, there was a significant association between the oil-exposure and the asthma symptoms. The strength of the associations with the oil-exposure decreased slightly over time.

Chapter 4. VOCs, in order of evaporated total mass from the oil spill, were xylene (mean: 11.2 $mg/m^3 \cdot 4$ d), toluene (10.1), ethylbenzene (6.2), and benzene (2.3). Percent predicted FEV_1 decreased significantly over time, after 1 year (100.7), 3 years (96.3), and up to 5 years (94.6). In cross-sectional design, percent predicted FEV_1 1 year and 3 years after the spill was significantly associated with xylene, toluene, ethylbenzene, and total VOCs. Percent predicted FEV_1 5 years after the spill was marginally significantly associated with ethylbenzene, xylene, and TVOCs. In longitudinal design, percent predicted FEV1 was significantly associated with benzene, toluene, ethylbenzene, xylene, and TVOCs.

Conclusions: For adults, the results suggested that oil exposure from prolonged clean-up activitiy induced oxidative stress up to at least 1 year after the last exposure (Chapter 1). When the high-exposed group was followed up 3 and 4 years after the spill, the levels of oxidative DNA damage remained as increased over time. However, the relationships between oil-exposure and oxidative stress were not significant (Chapter 2).

For children, the results showed that residing near the oil spill site and being exposed to BTEX during initial period likely led to increased asthma symptom risks. These associations were persistent up to 5 years after the spill (Chapter 3). In addition, exposure to VOCs during the initial period likely led to undergrowth of lung function. These deficits were persistent without catch-up during the subsequent 5 years after the spill (Chapter 4).

In summary, long-term health effects from oil spill were observed up to 5 years after the spill in both adults and children. The excess health risk became less apparent over time, however, and parts of the adverse health effects seemed to be reversible over time. Guidelines for preventive management, therapeutic interventions, and a continued surveillance of adults and children are required. Lessons learned should guide current and future responses to oil spill disasters.

Keywords: Clean-up work, Environmental disaster, Long-term health, Longitudinal, Oil spills, Oxidative DNA damage, Oxidative lipid peroxidation, Pulmonary function, Respiratory health, Wheeze

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Ⅰ. BACKGROUND

Hebei Spirit oil spill

About the accident

On the morning (about 7 o'clock) of December 7th, 2007, an anchored Hong Kong-registered crude oil tanker Hebei Spirit, which was carrying about 209,000 tons, collided with a crane barge, as the towing ropes were cut, 5 miles off the shore of Taean County on the west coast in Republic of Korea (latitude 36-52-00N, longitude 126-02-09E).

Source: Korea Coast Guard

Figure 1. Hong Kong tanker Hebei Spirit (left) and Samsung crane ship (right)

After the collision, an estimated 10,900 tons (about 12,547 kL) of crude oil spilled into the sea from the punctured three of the five tanks on the Hebei Spirit. The size of the holes at the three tanks was 30 × 3 cm at the No.1 tank, 160 × 10 cm at the No.3 tank, and 200 × 160 cm at the No.5 tank, and the name of the oil was UAE Upper Zakum Crude, Kuwait Export Crude and Iranian Heavy Crude, respectively. Most of the oil from the No.3 and No.5 tank, which relatively large holes were made, was spilled out within about 4.5 hours, while the oil spill from

the No.1 tank, which hole was relatively small, was stopped at the night of December 8.

Source: Korea Coast Guard

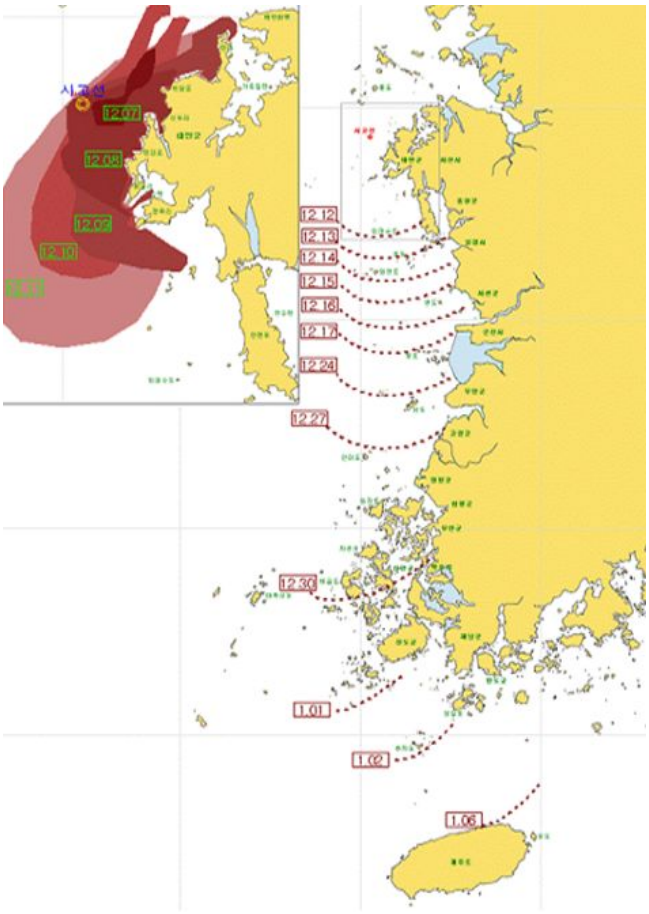
Figure 2. No.1 tank (left) and No.3 tank (right)

The spilled oil was then rapidly extended over several hundred kilometres of coastlines during 30 days by northwesterly winds and currents driven. The oil firstly reached the coast (Gureumpo, Euihang-ri, Sowon-myeon) of Taean County at 9:10 p.m., 14 hours after the accident. About 35 km interval of the coast of Taean County (from Hakampo to Pado-ri) was the most severely contaminated by a thick oil layer just four days after the accident. The oil finally reached the western coastline of the Korean peninsula with a range in spread of 1,052 km or 3,000 ha including Chungnam, Jeonbuk, Jeonnam, and as far as Jeju island.

So far, the spill was confirmed as the largest oil spill contamination to occur in Korean history.

Source: Taean County

Figure 3. A beach in Taean one day after the Hebei Spirit oil spill



Source: Korea Coast Guard

Figure 4. Distribution of oil during 30 days after the Hebei Spirit oil spill

Oil composition and toxicity

Crude oils contain many thousands of different hydrocarbons. All oils contain the same compounds and molecular structures, but differences among oils are caused by differences in quantities of those compounds in the oils. The analyses of the Hebei Spirit crude oil mad by the Korean Ocean Research & Development Institute revealed 53% saturated hydrocarbons, 17% polar compounds, and 30% aromatic hydrocarbons.

Very little information is available for most of the chemical compounds in oil. Many compounds in oil hardly know their toxic effects on human health, while the aromatic hydrocarbons are responsible for much of the toxicity. Therefore, among all oil compounds, three groups of the aromatic hydrocarbons must be highlighted with regard to the human health risk, namely volatile organic compounds (VOCs), polycyclic aromatic compounds (PAHs), and heavy metals (Perez-Cadahia et al. 2007).

VOCs

Several of these compounds present in the Hebei Spirit oil are classified by the International Agency for Research on Cancer (IARC) as possible human carcinogens (group 2B: toluene, ethylbenzene, and styrene) or human carcinogens (group 1: benzene). The components have been proven to induce respiratory (Elliott et al. 2006; Rumchev et al. 2004; Yoon et al. 2010), cytotoxic (Anderson et al. 1996), hematotoxic, and immunotoxic effects (Robinson et al. 1997).

PAHs and alkylated PAHs

PAHs and alkylated PAHs are much more persistent, can bioaccumulate, potentially cause skin and lung cancer, and have reproductive and developmental toxic effects. Several PAHs and alkylated PAHs compounds contained in the Hebei Spirit oil are classified as human carcinogens (group 1: benzo[a]pyrene), possible human carcinogens (group 2A: benzo[a]anthracene and dibenz[a,h]anthracene) and probable human carcinogens (group 2B: naphthalene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene , benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, and chrysene) by the IARC. It has been clearly demonstrated that the components are carcinogenic in humans and animals, small quantities of these compounds can induce malignant tumors that affect mainly to the skin and other epithelial tissues (IARC 1983). PAHs and alkylated PAHs have great affinity for the nucleophilic center of big macromolecules like proteins, RNA, and DNA. They place inside the DNA structure forming covalent links that constitute a key process in chemical carcinogenesis. Another important damaging effect of PAHs and alkylated PAHs concern to the endocrine system. Some of them act like endocrine disruptors. For example, benzo[a]pyrene behaves as a xenoestrogen (Tsai et al. 2004).

Heavy metals

They are highly toxic substances at medium and at long term, because they are involved the frequent accumulative processes. Some heavy metals in the Hebei Spirit oil are classified as human carcinogens (group 1: Al, Cr, As, S, and Cd), possible human carcinogens (group 2A: Pb) and probable human carcinogens (group 2B: Ni, V, and C) by the IARC. Heavy metals also have carcinogenic

properties. Association of metals with endocrine and genotoxic effects after an oil spill was reported (Perez-Cadahia et al. 2008a).

Table 1. Composition ratio in aromatic hydrocarbons of the Hebei Spirit crude oil

Type of compounds	Iranian Heavy Crude	Kwait Export Crude	UAE Upper Zakum	Ratio (%)
BTEX	9,937	8,673	9,782	53-65%
US EPA Priority 16 PAHs	277	130	236	1%
Alkylated PAHs	6,763	4,511	8,259	33-45%
Heavy metals	167	72	62	0.6%

Unit: μg/g (ppm)

Source: Marine Environments Risk Research (2008) in Korea Ocean Research & Development Institute

Table 2. Compounds of aromatic hydrocarbons of the Hebei Spirit crude oil

BTEX	16 US EPA-PAHs	Alkylated PAHs	Heavy metals
Benzene	Naphthalene	C1-Naphthalene	C
Toluene	Acenaphthylene	C2-Naphthalene	S
Ethylbenzene	Acenaphthene	C3-Naphthalene	V
m,p-Xylene	Fluorene	C4-Naphthalene	Cr
o-Xylene	Phenanthrene		Co
C3-benzene	Anthracene	C1-Fluorene	Ni
	Fluoranthene	C2-Fluorene	Cu
	Pyrene	C3-Fluorene	Zn
	Benz[a]anthracene		As
	Chrysene	C1-Phenanthrene	Cd
	Benzo[b]fluoranthene	C2-Phenanthrene	Pb
	Benzo[k]fluoranthene	C3-Phenanthrene	Hg
	Benzo[a]pyrene	C4-Phenanthrene	Al
	Indeno[1,2,3-cd]pyrene		Fe
	Dibenzo[a,h]anthracene	C1-Dibenzothiophene	
	Benzo[ghi]perylene	C2-Dibenzothiophene	
		C3-Dibenzothiophene	
		C1-Chrysene	
		C2-Chrysene	
		C3-Chrysene	

Source: Marine Environments Risk Research (2008) in Korea Ocean Research & Development Institute

Atmospheric concentration

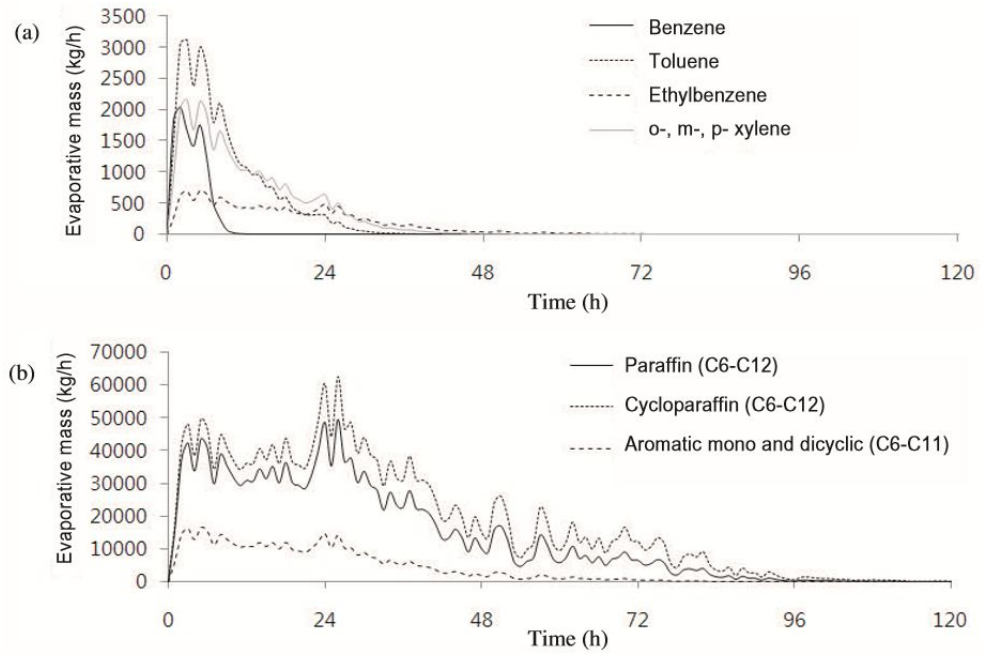
Direct measurements of ambient exposure at the time of the accident were not performed due to a lack of systemic preparedness against an oil spill disaster from a health standpoint. Alternatively, the theoretical models of dispersion, volatilization, emulsification, dissolution and mechanical removals of the crude oil were used, which can estimate the physico-chemical characteristics and amount of volatilization that resulted from the oil spill. The meteorological status information, the amount of crude oil discharged around the time of the accident, and the amount of oil removed from the clean-up work were input into the CALPUFF (Kim et al. 2012) and BOX model. This then enabled a simulation of the ambient diffusion, thereby allowing an estimate of the concentration of ambient VOCs, PAHs, and alkylated PAHs.

VOCs

It has been known that most of the volatile organic compounds included in the spilled oil were benzene, toluene, ethylbenzene, meta- (m-), para- (p-) and orth- (o-) xylene. It was expected that these substances were released to the atmosphere in a short period of time after the oil spill because of their high volatility. The actual modeling result showed that almost all the benzene which is highly volatile was to be volatilized within 24 hours and most of the toluene, ethylbenzene, m-, p- and o-xylene were to be volatilized within 48 hours.

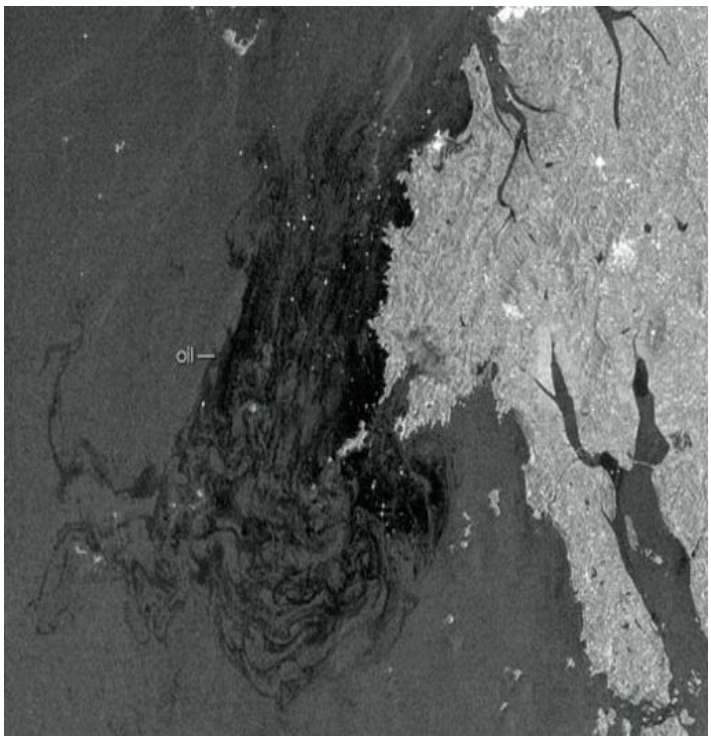
The highest ambient concentrations of benzene estimated by the simulation were several ppm near the accident area and several ppb around the coastline.

Ethyl-benzene were a maximum concentration of several hundred ppb around the accident site and several ppb in the Taean area.



Source: Kim et al. (2012)

Figure 5. Evaporation rate of (a) toxic VOCs and (b) light compounds of crude oil with time



Source: European Space Agency

Figure 6. Satellite view 4 days after the Hebei Spirit oil spill

PAHs

The concentration change of PAHs were divided into a few patterns depending on the vapor pressure. According to the simulation, most of naphthalene that existed in the highest content and was the most volatile among the 16 PAHs was completely volatilized within 15 days after the accident and the concentration was drastically decreased. Meanwhile, acenaphthylene, acenaphthene, fluorene and phenanthrene were to be volatilized almost all within one month. The rest, which are less volatile, were volatilized continuously for more than 2 months.

The highest level of naphthalene and pyrene was 1 ppb and 10^{-6} ppb in areas close to the accident site, while was several tens of ppt and 10^{-7} ppb in land areas, respectively.

Alkylated PAHs

The quantity of alkylated PAHs discharged at the accident was 300 to 400 times more than that of PAHs. The concentrations of alkylated PAHs was still high even 1 to 2 years after the spills.

Table 3. Estimation of total duration of volatilization

	VOCs	PAHs	Alkylated PAHs
Overall	0−4 days	Several months	Several years

To summarize, for VOCs, about four days are sufficient as the total modeling time to understand the exposure through the atmosphere, while more than several months are required for the modeling of PAHs. In case of the alkylated PAHs, the some components could be evaporated during considerably long terms like several years. Oil that contains lighter smaller molecules is less viscous, and evaporate neighborhood in a short time. Given that VOCs, roughly 20 percent of the oil, may evaporate within the four days right after the spill, it is logical that workers participating clean-up activity and children living proximity to oil spill site at this time may be at the greatest risk of exposure to these evaporated chemicals. In addition, workers participating clean-up for a long period may be continuously exposed to toxic oil components.

Populations at risk

Clean-up workers

The Taean County received the most severe damage, which is mainly agricultural and fishing region, 530.8 km of coastline, and an area of 503 km^2 with a recorded population of 63,939 people as of 2006.

Following the accident, local residents, military personnel, governmental employees, and many volunteers, i.e., members from civic groups, schools and universities, companies, communities, and families and individuals, throughout the country worked to clean up the spilled oil. A total of 2,122,296 days (556,343 residents, 1,233,393 volunteers, 152,695 military personnel, 32,356 polices, 17,394 maritime polices, 53,431 professional workers, and 76,684 local government personnel) were estimated as the total number of days dedicated by participants to the clean-up as of July 4th, 2008.

Source: Taean County

Figure 7. Pictures of clean-up activity

Among them, local residents participated in the clean-up efforts for a long period, mostly several months, whereas volunteers participated for a range of a few hours to days. Main reason of participating in clean-up for a long-term among local residents was that they lost their job, such as fishing and tourism for an indefinite period. The residents started to clean the spilt oil the day after the accident, December 8th, 2007 and the volunteers followed two days after, on December 9th, 2007. Unfortunately, they were not able to properly protect themselves against oil spill contaminants, because they lacked appropriate safety wear, especially at the initial period.

Clean-up efforts related to oil spills can pose numerous hazards to the physical health of individuals and communities.

Community members

Among residents, there are vulnerable populations, such as pregnant women, children, persons with disabilities and preexisting conditions, fishermen, low income and medically under-served populations, and so on. Among those vulnerable populations, children constitute a relatively large proportion of the population. Children are at particular risk for effects from environmental exposures as compared with adults. They breathe in more air per unit of body mass, their bodies detoxify many chemicals less effectively. So, they could affect health effects from massive oil spills without clean-up activity. Evidence is lacking regarding the health effect of oil spills in children.

Theoretical pathway

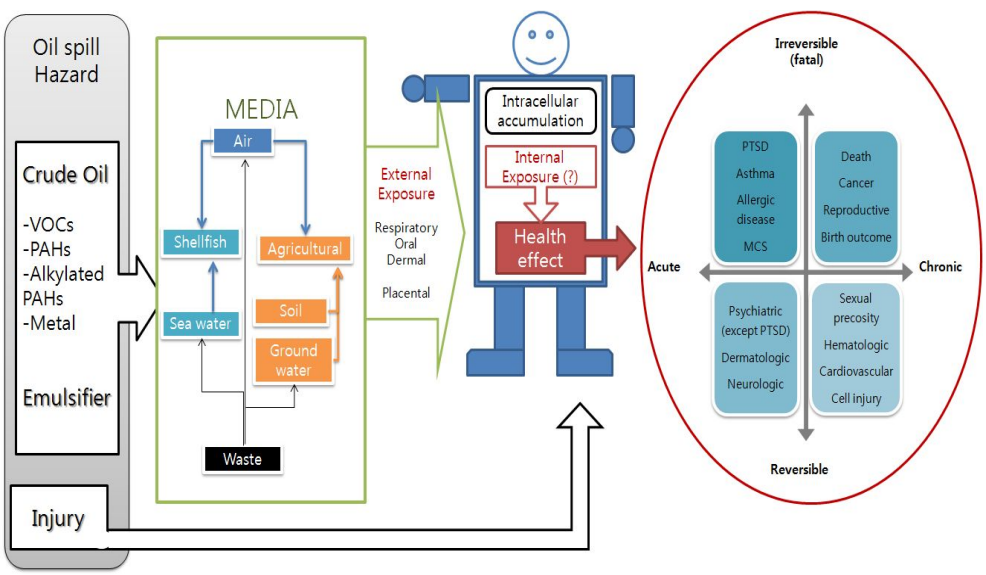
Nothing or limited information is known about toxicity and health effects of the oil spill currently. Even in the cases of the oil components of which toxicity is known, only the toxicity as a single substance is known, but the toxicity mechanism in a mixture, such as the spilled oil, is little studied. In case of the long-term health effects, little is known. Only Prestige oil spill research team (2002, Spain) reported a few studies related to respiratory health and genotoxic alteration throughout epidemiological investigation up to now.

Theoretically, various types of the oil components contaminate various media, such as the air, sea and soil. The oil components are exposed to human body through the contaminated media or through the agricultural or fishery products growing in the contaminated media. Since various media and agricultural or fishery products are polluted, there are various pathways through which the oil components were exposed to human body including the respiratory organs, digestive organs, skin contact and placenta. Some of the oil components can be accumulated in human body. Those components can affect health by the internal exposure in a body, even after the complete elimination in the environment (Figure 1).

Theoretically, health effects by large-scale chemical exposure, such as oil spill, can be divided into four categories (reversible vs irreversible and acute vs chronic) depending on the time of the disease occurrence and the recovery possibility. First is the acute and reversible case that health effect is generated at the initial stage of the accident, and is recovered as time passes. Second is the acute and irreversible case that health effect is generated at the initial stage of the accident, but continues

for a considerable period of time. Third is the chronic and reversible case, health effect may be discovered not at the initial stage of the accident but after several months or years, and soon recovered or diluted over time. Fourth is the chronic and irreversible case, health effect occur months or years after the accident, and continue for the lifetime.

Therefore, this study about long-term health effect of the oil spill considered the possibilities mentioned above.



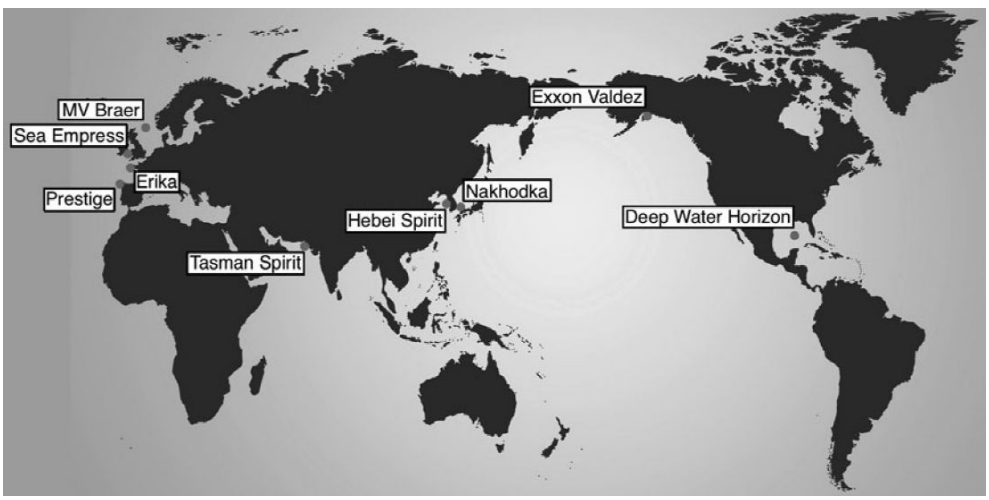
Source: Taean Environmental Health Center

Figure 8. Theoretical pathway of exposure and health effects of oil spills

Literature review

Global oil spills

The human health effects of exposures during oil spills have been studied following only 9 major oil spills over the past half century.



Source: D'Andrea and Reddy (2014a)

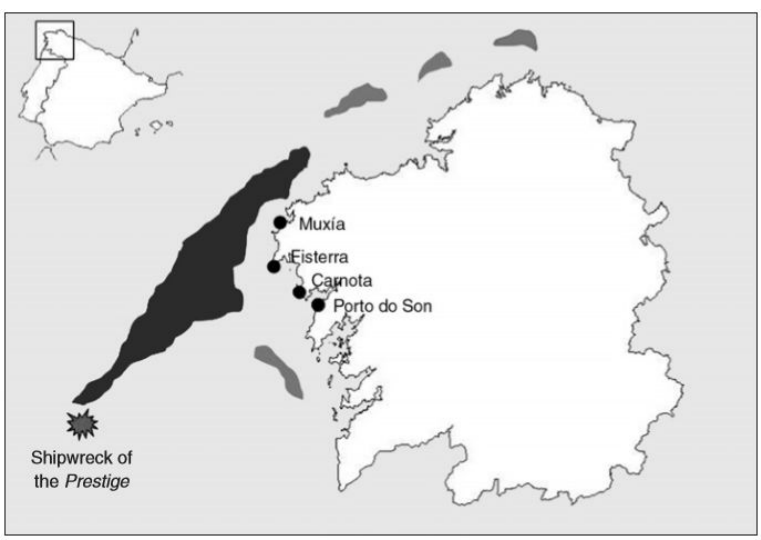
Figure 9. Location of major spill disasters around the world with studies investigating potential effects on human health

Table 4. Oil spills reported health effects

Acute	Long-term
Exxon Valdez oil spill (1989)	Prestige oil spill (2002)
MV Braer oil spill (1993)	Hebei Spirit oil spill (2007)
Sea Empress oil spill (1996)	Deep Water Horizon oil spill (2010)
Nakhodka oil spill (1997)	
Erika oil spill (1999)	
Prestige oil spill (2002)	
Tasman Spirit oil spill (2003)	
Hebei Spirit oil spill (2007)	
Deep Water Horizon oil spill (2010)	

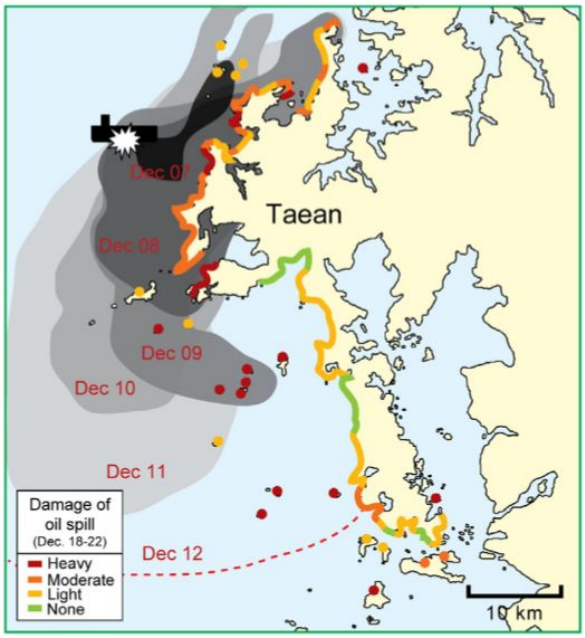
Acute and long-term health effects were divided as less than 1 year and over 1 year after the spill, respectively.

Understanding the effects of oil spills on human health has improved over the past 20 years since the Exxon Valdez spill. Much of the information about the short-term physical health effects of exposure comes from studies of nine major oil spills. Most of these studies were cross-sectional and investigated the short-term, and used only questionnaires to measure acute subjective symptoms. Meanwhile, relatively little information about the long-term physical health effects of exposure comes from only Prestige oil spill studies before the Hebei Spirit oil spill worldwide. Long-term health impact assessment of the Deepwater Horizon oil spill in the Gulf of Mexico in April in 2010 is currently underway.



Source: Rodríguez-Trigo et al. (2007)

Oil spill: Prestige oil spill (2002)
Spillage: 67,000 ton
The closet distance off coast: 130 miles (200 km) off the Galicia, Spain



Source: Hong et al. (2014)

Oil spill: Hebei Spirit oil spill (2007)
Spillage: 10,900 ton
The closet distance off coast: 5 miles (8 km) off the Taean, Republic of Korea

Source: D'Andrea and Reddy (2014a)

Oil spill: Deep Water Horizon oil spill (2010)
Spillage: 680,000 ton
The closet distance off coast: 50 miles (80 km) off the Louisiana, USA

Figure 10. Oil spills reported long-term health effects

Literature review of acute health effects

Table 5. Summary of literature on acute health effects

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
Exxon Valdez March 24, 1989 Alaska, United States 37,000 ton	Mental health	1 year	Cross-sectional	371 Euro-Americans and 188 Alaskan Natives	CES-D score	Depression ↑	Palinkas et al. (1992)
	Mental health	1year	Cross-sectional	437 exposed residents and 162 controls	CES-D score National Institute of Mental Health Diagnostic Interview Schedule	Anxiety disorder, PTSD, and depression ↑ Women and younger ↑	Palinkas et al. (1993)
	Mental health	1 year	Cross-sectional	371 Euro-Americans and 188 Alaskan Natives	Diagnostic Interview Schedule	PTSD ↑	Palinkas et al. (2004)
MV Braer January 5, 1993 Shetland,	Health: Acute physical	8 days	Cross-sectional	420 exposed residents and 92 controls	Questionnaire Tests of peak expiratory flow	Headache, throat irritation, and itchy eyes ↑ Urinary toluene in exposed	Campbell et al. (1993)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
Scotland 85,000 ton					Blood tests Levels of urinary biomarkers of exposure	residents ↑ No significant differences in blood and PEF tests	
	Health: Acute physical	5 months	Longitudinal	A follow-up 344 of the 420 exposed residents and 77 of the 92 controls	Same as for Campbell et al. (1993)	Poor health, deteriorating health, and breathlessness on exertion ↑ Throat, eye irritation, and headaches with time ↓ No significant differences in biomarkers	Campbell et al. (1994)
	Health: Respiratory	3−12 days	Cross-sectional	44 (at 3 days) and 56 (at 9−12 days) children	Peak expiratory flow rate	Normal range in PEF levels	Crum (1993)
Sea Empress February 15, 1996 Milford Haven, United Kingdom 72,000 ton	both Mental health and Health: Acute physical	7 weeks	Cross-sectional	539 exposed residents and 550 controls	Questionnaires of acute symptoms Hospital Anxiety and Depression Scale SF-36 scores	Mental health including anxiety and depression ↑ Quality of life by SF-36 ↓ Toxicologically related symptoms including headache, sore eyes, sore	Lyons et al. (1999)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
						throat ↑	
	Mental health	7 weeks	Cross-sectional	794 exposed residents and 791 controls	Questionnaires of acute symptoms Hospital Anxiety and Depression Scale	Perceived health and financial risks -> anxiety and depression ↑	Gallacher et al. (2007)
Nakhodka January 2, 1997 Oki Island, Japan 6,000 ton	Health: Acute physical	18 days	Cross-sectional	282 exposed residents	Home interview Levels of urinary biomarkers of exposure (n=97) Personal air monitors during clean-up (n=4)	Duration of clean-up activities ↑ -> lower back pain, leg pain, headaches, and itchy eyes and throat irritation ↑ Slight increases in urinary biomarker of toluene No increases in urinary biomarker of benzene	Morita et al. (1999)
Erika December 12, 1999 Brittany, France 20,000 ton	Health: Risk assessment	4 months	Health risk assessment		PAHs exposure levels based on 5 exposure scenarios Cancer-risk analysis	PAH residues on rocky soil ↑ About 10^{-5} per lifetime in the highest estimated cancer risk	Dor et al. (2003)
	Health:	2 weeks	Health risk		PAH and benzene	Risk of acute eye irritation	Baars (2002)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
	Risk assessment	2 months 6 months	assessment		exposure levels based on extrapolation from measured values Risk analysis	and dermatitis in workers with skin exposure from cleaning oil-contaminated birds ↑ Negligible in long-term cancer risks	
Prestige November 19, 2002 Galicia, Spain 63,000 ton	Health: Acute physical	7 months	Cross-sectional	799 exposed clean-up workers (265 paid workers, 266 volunteers, 133 seamen, and 135 bird cleaners)	Structured telephone interview	Headache and throat and respiratory symptoms in seamen ↑ Clean-up activity ↑ -> headache, nausea, vomiting, and dizziness, and throat and respiratory symptoms ↑	Suarez et al. (2005)
	Health: Acute physical	7 months	Cross-sectional	Same as for Suarez et al. (2005)	Same as for Suarez et al. (2005) Odds ratios based on logistic regression	Itchy eyes, nausea, vomiting, dizziness, headaches, throat, and respiratory symptoms in uninformed workers before engaging in clean-up rather than informed workers ↑	Carrasco et al. (2006)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
	Health: Cytogenetic	7 months 11 months	Cross-sectional	34 exposed men and 34 controls	Volatile organic compounds in air samples DNA repair genetic polymorphisms (XRCC1 codons 194 and 399, XRCC3 codon 241 and APE1 codon 148) Comet assay Micronucleus (MN) test	DNA damage (by comet assay) related to time of exposure ↑ Not cytogenetic damage (by MN test) Suggested exposure–genotype interactions (by comet assay) No e ect of the DNA repair genetic polymorphisms by MN test	Laffon et al. (2006)
	Health: Cytogenetic and Endocrine	4 months	Cross-sectional	68 exposed individuals (25 volunteers that collaborated for 1 week, 20 hired manual workers, and 23 hired high-pressure cleaner	Volatile organic compounds in environment Heavy metal in blood Sister chromatid exchanges (cytogenetic damage) Plasmatic prolactin and cortisol levels (endocrine toxicity)	Cytogenetic damage in exposed individuals Influenced by age, sex, tobacco consumption and GSTM1 polymorphism Alterations in hormonal status (endocrine disruptor) Using protective devices -> preventing the effects related to the exposure	Perez-Cadahia et al. (2007)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
				workers) and 42 control individuals.	GST genetic polymorphisms (GSTM1 and GSTT1 deletion polymorphisms, GSTP1 Ala105Val)		
	Health: Cytogenetic and Endocrine	4 months	Cross-sectional	179 exposed individuals (60 college student volunteers working for 5 days, 59 manual workers working for 4 months, and 60 high-pressure machine workers working for 3 months)	Heavy metals (blood concentrations of aluminium, cadmium, nickel, lead and zinc) Genotoxic parameters (sister chromatid exchanges, micronucleus test and comet assay) Endocrine parameters (plasmatic concentrations of prolactin and cortisol)	Found possible associations between blood concentrations of heavy metals and genotoxic or endocrine alterations (lead & comet assay, aluminium, nickel, and cadmium & cortisol concentrations) Higher concentrations of prolactin and cortisol in women rather than men	Perez-Cadahia et al. (2008a)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
	Health: Cytogenetic	4 months	Cross-sectional	159 exposed individuals (59 volunteers working for 5 days, 53 manual workers working four months, and 47 high-pressure machines workers working for 3 months) and 60 non-exposed individuals	Genotype analysis for the role of polymorphisms in genes involved in metabolism and DNA repair Micronucleus test	Micronucleus (MN) frequency ↑ and proliferation index ↓ in individuals with longer time of exposure Age : significant predictor of MN frequency CYP1A1 3'-UTR, EPHX1 codons 113 and 139, GSTP1, GSTM1 and GSTT1 metabolic polymorphisms, and XRCC3 codon 241 and XPD codon 751 repair polymorphisms influenced cytogenetic damage levels	Perez-Cadahia et al. (2008b)
	Mental health	1 year	Cross-sectional	938 residents	SCL-36 (mental health questionnaire)	Great social support, high satisfaction with economic aid, or evasive coping strategies -> mental health	Sabucedo et al. (2009)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
						scores ↑	
	Mental health	1 year	Cross-sectional	938 residents (compared across three zones of proximity)	Exposure Status Scale SCL-36 (mental health questionnaire) SF-36 (perception of health and functional capacity)	Somatization, anxiety, obsessive-compulsive disorder, and hostility as more proximity ↑ Closer to spill -> perception of physical health and functional capacity ↓	Sabucedo et al. (2010)
Tasman Spirit July 26, 2003 Karachi, Pakistan 37,000 ton	Health: Acute physical	3 weeks	Cross-sectional	216 exposed residents and 184 controls	Questionnaire on acute symptoms	Physical symptoms including ocular, respiratory, skin symptoms, headache, irritability, fever, and fatigue among exposed group ↑ Wheezing and shortness of breath ↑ Distance from oil spill site ↑ -> symptom-specific prevalence odds ratios ↓	Janjua et al. (2006)
	Health: Hematological	1 month	Cross-sectional	100 exposed residents and workers	Hematological and biochemical parameters	Lymphocyte and eosinophil levels ↑ Found ALT elevations in	Khurshid et al. (2008)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
				No control group	Liver and renal function tests	some residents The other tests within normal range	
	Health: Respiratory	1 year	Longitudinal	20 male workers and 31 controls	Spirometry	Immediately after the spill, lower FVC, FEV_1, FEF_{25-75}%, MVV in exposed group 1 year after the spill, similar between exposed and controls	Meo et al. (2008)
Hebei Spirit December 7, 2007 Taean, Republic of Korea 10,900 ton	Health: Acute physical	7−14 days	Cross-sectional	846 clean-up workers	Questionnaire on acute symptoms	Skin lesions, eye, neurovestibular, respiratory symptoms with exposure ↑	Sim et al. (2010)
	Health: Acute physical	2−8 weeks	Cross-sectional	288 residents (urine samples : 154 residents and 39 controls)	Questionnaire on subjective physical symptoms Urinary metabolites of VOCs, PAHs, and heavy metals	Days of work, degree of skin contamination, and levels of some exposure biomarkers ↑ -> physical symptoms ↑	Cheong et al. (2011)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
	Health: Acute physical	2−3 weeks	Cross-sectional	565 volunteers (urine samples : 105 volunteers)	Questionnaire on subjective physical symptoms Collected urine samples before and after the clean-up work (metabolites of VOCs and PAHs)	Longer clean-up work -> physical symptoms ↑ Levels of urinary exposure biomarkers after clean-up work ↑ t,t-muconic acid associated with dermal irritation	Ha et al. (2012)
	Health: Acute physical	1−2 months	Cross-sectional	2,624 military personnel	Questionnaire on acute symptoms including neurologic, respiratory, dermatologic, and ophthalmic symptoms	Most acute symptoms ↑ with clean-up work duration Acute symptoms ↓ with personal protective equipment	Gwack et al. (2012)
	Health: Acute physical	1 year	Cross-sectional	442 clean-up workers	Continuation and duration of symptoms	Eye (9.7 mo), headaches (8.4 mo), skin (8.3 mo), neuro-vestibular (6.9 mo), back pain (1.8 mo), and respiratory symptoms (2.1 mo)	Na et al. (2012)
	Mental health	8 weeks	Cross-	1,361 children	Korean versions of	Closest distance to the school	Ha et al.

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
		4−5 months	sectional	(234 in 8 weeks and 1127 in 4−5 months)	the Children's Depression Inventory State Anxiety Inventory for Children	from the contaminated coastline -> depression ↑ No differences in anxiety with distance	(2013)
	Mental health	7 months	Cross-sectional	993 residents	Post-traumatic stress (PTS) Depression Suicidal ideation Anxiety	Prevalence of PTS (19.5%), depression (22.0%), suicidal ideation (2.3%), and anxiety (4.2%) PTS and suicidal ideation in fishery ↑	Choi et al. (2016)
Deep Water Horizon April 20, 2010 Gulf of Mexico, United States 680,000 ton	Mental health	4 months	Cross-sectional	452 residents	Telephone and face-to-face interviews assessing concerns and direct impact	Disruption to participants' lives, work, family, and social engagement -> anxiety, depression, and post-traumatic stress ↑	Osofsky et al. (2011)
	Mental health	4 months	Cross-sectional	94 residents (71 indirectly impacted and 23 directly	Psychological distress (mood, anxiety), coping, resilience, neurocognition, and	No significant differences in psychological distress between communities Significant depression and	Grattan et al. (2011)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
				exposed; 47 income stability and 47 spill-related income loss)	perceived risk	anxiety both communities Spill-related income loss -> psychological distress ↑	
	Mental health	5.5 months	Cross-sectional	469 residents	Cluster sampling methodology	Depression (15.4−24.5%) and anxiety (21.4−31.5%) Quality of life and social context outcomes ↓	Buttke et al. (2012)
	Mental health	1 year	Cross-sectional	93 residents	Profile of Mood States Impact of Event Scale Connor-Davidson Resilience Scale	Sustain spill-related income loss -> persisted psychological disruption	Morris et al. (2013)
	Health: Hematological	0−31 months	Cross-sectional	117 exposed subjects and 130 controls	Using medical chart	Platelet counts ↓ in exposed Hemoglobin and hematocrit levels ↑ in exposed ALP, AST, and ALT ↑ in exposed	D'Andrea and Reddy (2013)
	Health:	0−31	Cross-	117 exposed	Same as for	Toward the upper limit of	D'Andrea and

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
	Hematological	months	sectional	subjects	(D'Andrea and Reddy 2013)	normal: hemoglobin (65%), AST (15%), and ALT (31%)	Reddy (2014b)
	Mental health	1 year	Cross-sectional	812 residents	Telephone survey assessing self-perceptions (economic and social, resilience, coping, and depressive and PTSD symptoms)	Perceived most participants as resilient (91%) Associated between lower perceived resilience and ongoing depressive and PTSD symptoms Depressive and PTSD symptoms ↑ with economic impact	Shenesey and Langhinrichsen-Rohling (2015)
	Mental health	Pre-oil spill (0−7 months) and 5−8 months	Longitudinal (compare pre-hurricane exposure with again post-oil spill exposure)	1,577 children and adolescents	Posttraumatic stress disorder (PTSD)	PTSD symptoms ↑ with oil exposure Found interactive effect with preexisting PTSD symptoms from previous hurricane exposure (evidence of cumulative risk)	Osofsky et al. (2016)

Literature review of long-term health effects

Table 6. Summary of literature on long-term health effects

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
Exxon Valdez March 24, 1989 Alaska, United States 37,000 ton	Mental health	3 years	longitudinal	Exposed: 118 in 1989, 228 in 1991, and 152 in 1992 Controls: 73 in 1989, 102 in 1991, and 41 in 1992	Desire or expectation to migrate out of the area Social disruption Score on Impact of Events Scale	Psychological stress still remained after 3 years With time ↓ A desire or expectation to migrate in exposed ↑	Gill and Picou (1998)
Prestige November 19, 2002 Galicia, Spain 63,000 ton	Mental health	16 months	Cross-sectional	1,350 coastal residents and 1,350 controls	SF-36 (quality of life) Hospital Anxiety and Depression Scale Goldberg Anxiety and Depression Scale	Not differ in mental health A higher frequency of suboptimal scores in mental health scores ↑ SF-36 physical-functioning score with level of exposure ↑	Carrasco et al. (2007)
	Health: Respiratory	Over 1 year	Cross-sectional	6,869 fishermen	Questionnaire about respiratory symptoms	Prevalence of lower tract symptoms among exposed	Zock et al. (2007)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
						members ↑	
	Health: Respiratory and Chromosomal damage	2 years	Cross-sectional	510 exposed fishermen and 177 non-exposed fishermen	Questionnaire about respiratory symptoms Spirometry and methacholine challenge Respiratory marker of oxidative stress (8-isoprostane) Airway inflammation (interleukins, tumor necrosis factor-α, and interferon-γ) Growth factor activity Chromosomal lesions and structural alterations	Lower respiratory tract symptoms in exposed ↑ No differences in lung function 8-isoprostane levels in exposed ↑ Exhaled vascular endothelial growth factor in exposed ↑ Structural chromosomal alterations in exposed ↑	Rodriguez-Trigo et al. (2010)
	Health: Respiratory	5 years	Longitudinal (a follow-up Rodriguez-Trigo et al. (2010))	466 exposed fishermen (participation rate 93%) and 156 non-exposed	Re-interviewed about same respiratory symptom question as in the initial survey by telephone	Prevalence of lower respiratory tract symptoms ↓ in both group But still higher respiratory symptoms in exposed Risk of having persistent	Zock et al. (2012)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
				fishermen (88%)		respiratory symptoms with the degree of exposure ↑	
	Health: Respiratory	6 years	Longitudinal (a follow-up only lifetime nonsmokers Rodriguez-Trigo et al. (2010))	158 exposed (participation rate 69%) and 57 non-exposed (66%) among 230 exposed and 87 non-exposed in baseline	Lung function Bronchial responsiveness testing Respiratory biomarkers in exhaled breath condensate (done identically as in baseline)	Less respiratory symptoms at baseline among loss to follow-up in non-exposed Lung function, bronchial responsiveness, respiratory biomarkers, and growth factors in non-exposed ↑ than in exposed during 4-year follow-up No clear differences with the degree of exposure	Zock et al. (2014)
	Health: Endocrine and Immunological	7 years	Cross-sectional	54 exposed fishermen and 50 controls	Prolactin and cortisol in plasma Lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+16^+$) Circulating cytokines (interleukin (IL) 2, IL4,	Cortisol ↑ in exposed than non-exposed No differences with time of exposure to the oil or using protective clothes Differences in neopterin, $\%CD8^+$,	Laffon et al. (2013b)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
					IL6, IL10, tumour necrosis factor α, and interferon γ) in plasma Neopterin, tryptophan, and kynurenine in serum	$CD4^+/CD8^+$ ratio, and IL4 with using protective mask	
	Health: Genotoxic	7 years	Cross-sectional	54 exposed fishermen and 50 controls (same subjects with Laffon et al. (2013b))	Comet assay (for DNA damage) T-cell receptor (TCR) mutation assay MN frequency	No differences in comet assay, TCR mutation assay, and MN test between exposed and controls MN test ↓ in exposed No evidence of the persistence of genotoxic damage 7 years later	Laffon et al. (2013a)
Hebei Spirit December 7, 2007 Taean, Republic of Korea 10,900 ton	Health	Over 1 year	Burden of disease (BOD)	10,171 individuals BOD of 66,473 populations	Years lived with disability (YLD) Disability adjusted life year (DALY)	14,724 DALYs for the year 2008 YLD of mental health in men rather than women ↑ Higher in asthma and allergies in women ↑ Proximity to the spill ->	Kim et al. (2013)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
						BOD ↑	
	Health: Respiratory	Over 1 year	Cross-sectional	662 children	Skin prick test Pulmonary function test Methacholine bronchial provocation test (MBPT)	Lung function ↓, asthma symptoms ↑, and airway hyper-responsiveness ↑ with children living closer from contaminated coastline	Jung et al. (2013)
	Health: Oxidative stress	Over 1 year	Cross-sectional	671 residents	Urinary metabolites of PAHs (1-hydroxypyrene and 2-naphthol) Oxidative stress biomarkers (malondialdehyde and 8-hydroxydeoxyguanosine)	Levels of oxidative stress biomarkers ↑ according to clean-up duration Positive correlation between urinary metabolites of PAHs and clean-up work duration Association ↓ as time passed since last exposure	Noh et al. (2015)
Deep Water Horizon April 20, 2010 Gulf of Mexico, United States	Mental health	11−26 months	Cross-sectional	1,119 adults	Depression Anxiety Stress Scales (DASS-21) PTSD Checklist (PCL-S)	Reported substantial worsening Psychological distress ↑ living related to the Gulf oil spill	Drescher et al. (2014)

Oil spill Date of oil spill Location Spill size	Part	Time elapsed	Design	Subject size	Methods	Findings	Reference
680,000 ton						Psychological distress ↑ in lower income individuals	
	Health: Physical symptoms	2−4 years	Cross-sectional	2,126 adult women	Physical health symptoms (cough, wheezing, shortness of breath, itchy eyes, skin symptoms, nose symptoms, dizziness, fatigue, sore throat and so on)	Physical health symptoms, such as burning in nose, throat or lungs, sore throat dizziness, and wheezing ↑ with high exposure High economic exposure -> wheezing, headaches, itchy eyes, and runny nose ↑	Peres et al. (In press)

A round-up of indicators

Table 7. Indicators used in oil spills worldwide (excluded mental health)

Exposure assessment	Confounder (effect modifier)	Health assessment
◊ Duration of exposure: type and duration of operation for clean-up workers ◊ Geographical location: individual's proximity to the spill site, location of residence ◊ Perceived exposure: personal protective equipment uses ◊ Environmental exposure levels: direct or indirect measurements VOCs and PAHs levels in environment ◊ Internal exposure levels: metabolites of VOCs and PAHs in urine and heavy metals in blood	◊ Age ◊ Gender ◊ Socioeconomic status ◊ Health status ◊ Genotype: metabolic polymorphisms (CYP1A1 3'-UTR, EPHX1 codons 113 and 139, GSTP1, GSTM1 and GSTT1) and repair polymorphisms (XRCC3 codon 241 and XPD codon 751)	◊ Subjective symptoms (generally in acute health): eye irritation, nasal irritation, headache, nausea, fatigue, cognitive disturbance, back pain, dizziness, skin symptoms, respiratory symptoms, sore throat and so on ◊ Hematological effects: hemoglobin, hematocrit, WBC, platelets, BUN, creatinine, lymphocyte, eosinophil, ALP, AST, ALT and so on ◊ Genotoxic effects: sister chromatid exchanges, comet assay (DNA damage), micronucleus (MN) test, chromosomal lesions and structural alterations, and T-cell receptor (TCR) mutation assay (mutagenicity) ◊ Endocrine and Immunological effects: plasmatic prolactin and cortisol levels, plasma levels of lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+16^+$), plasma levels of circulating cytokines (interleukin (IL) 2, IL4, IL6, IL10, tumour necrosis factor α, and interferon γ), and serum concentrations of neopterin, tryptophan, and kynurenine ◊ Respiratory effects: lung function test (FEV1 and FVC), respiratory symptoms by questionnaire (lower tract, nasal, and wheeze), bronchial hyper-responsiveness test (methacholine challenge), respiratory oxidative stress biomarker (8-isoprostane), airway inflammation (interleukins, tumor necrosis factor-α, and interferon-γ), and growth factor activity in exhaled breath condensate

Overview and scope of research

Study area and participants

The surveys were conducted a total of three times.

Specifically, baseline survey was simultaneously conducted both adults and children in 2009, over 1 year after the spill. After that, the survey was performed alternatively every other year, adults (2010 and 2012) and children (2011 and 2013).

In case of the study area and participants, in adults, first survey was done on both high and low exposed area, which include all Taean County except for downtown (Taean-eup). However, second and third survey were done on only high exposed area (coastal area of the four townships), which residents generally participated in clean-up activity for a long period.

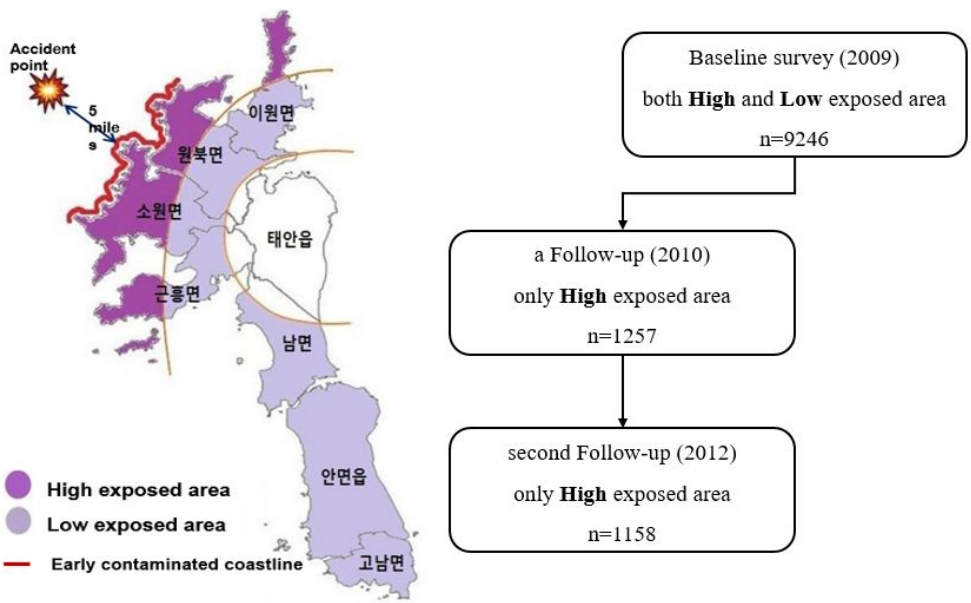


Figure 11. Study area and participants in adults

In children, all surveys (baseline, second, and third survey) were done on both high and low exposed area, but the study area was only four townships, which were relatively limited when compared to adults.

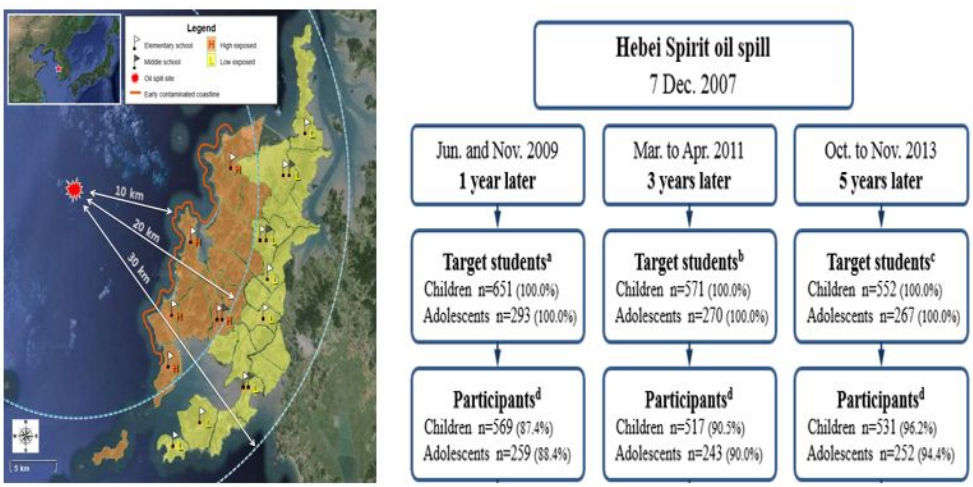


Figure 12. Study area and participants in school students

For in-depth analysis, specifically for analysis of the biological indicators (both biomarkers related to exposure and health), a panel was constituted among the all participated adults (Chapter 1 and Chapter 2). In children, all participants were enrolled (Chapter 3 and Chapter 4).

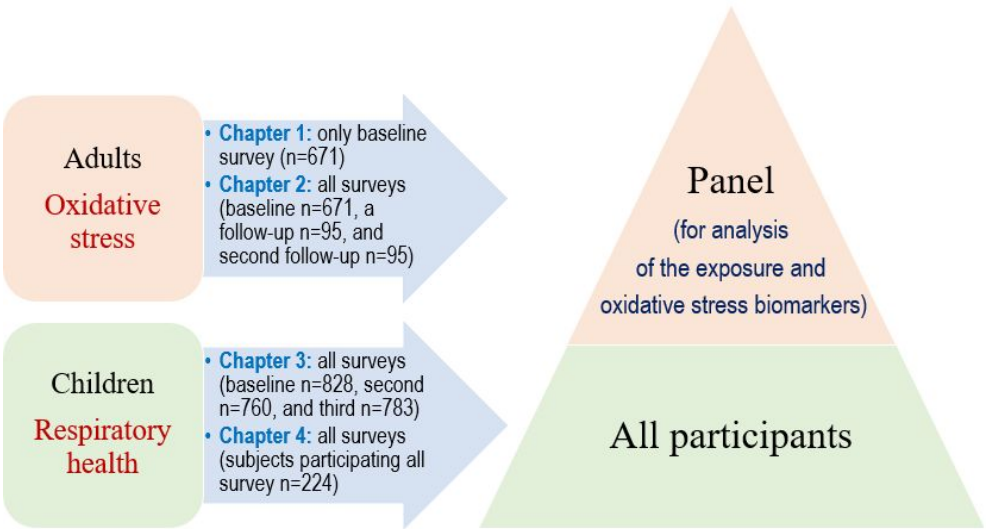


Figure 13. Establishment of each chapter subject

Research scope

The oil spill accident is an unexpected disaster to the people living in the community. The impacts would be wide-reaching and would likely have long-lasting effects on not only physical but also psychological health of populations in more extensive affected regions. The members of the society who directly experienced this disaster have been damaged both economically and psychologically. This combination usually causes mental health deterioration, such as depression, anxiety and psychosocial stress. The psychosocial effects might be due to mental and society exposure of the socioeconomic damage and injury rather than chemical exposure of the oil spill.

Therefore, mental health part was excluded in the research. Finally, the research limited its scope long-term physical health effects of residents within the Taean region as a result of the Hebei Spirit oil spill.

Table 8. Priority within research scope in order of importance

Population at risk	Distinguishing features	At risk	Exposure	Duration	Route	Health
Adult residents	◊ Many residents participated in clean-up activity for a long term. ◊ More participated in clean-up activity as live closer from the oil spill site (coastal area)	◊ Clean-up activity*** ◊ Area (proximity to the accident point)**	◊ PAHs and alkylated PAHs*** ◊ VOCs*** ◊ Heavy metals* ◊ Many other components, which were unknown of their toxicity and health risk*	◊ Several months (PAHs and alkylated PAHs)*** ◊ Initial 4 days (VOCs and light PAHs)*** ◊ Several years (alkylated PAHs and maybe unknown compounds)*	◊ Respiratory organs*** ◊ Skin contact*** ◊ Digestive organs*	◆ **Oxidative stress** ◊ Oxidative DNA damage (8-hydroxydeoxyguanosine) ◊ Lipid proxidation (malondialdehyde)
Child residents†	◊ More vulnerable to toxic oil compounds than adults ◊ Rarely participated in clean-up activity	◊ Area (proximity to the accident point)*** ◊ Clean-up activity*	◊ VOCs*** ◊ PAHs and alkylated PAHs** ◊ Heavy metals* ◊ Many other components, which were unknown of their toxicity and health risk*	◊ Initial 4 days (VOCs and light PAH_S)*** ◊ Several months (heavy PAHs)* ◊ Several years (alkylated PAHs and maybe unknown compounds)*	◊ Respiratory organs*** ◊ Skin contact** ◊ Digestive organs*	◆ **Respiratory health** ◊ Asthma symptoms by questionnaire ◊ Lung function by spirometry

†Children at the time of the accident

***, **, and * in the order of importance

Ⅱ. LONG-TERM HEALTH EFFECTS OF THE HEBEI SPIRIT OIL SPILL IN ADULTS

This chapter has been published in Science of the Total Environment 515-516 (2015) 207-214.

CHAPTER 1.

Oxidative stress biomarkers in long-term participants in clean-up work after the Hebei Spirit oil spill

Introduction

On December 7, 2007, a crane barge owned by Samsung Heavy Industries collided with the anchored Hong Kong-registered crude oil carrier, Hebei Spirit, while being towed by a tug boat. An estimated 12,547 kL of crude oil was released only 5 miles off the western coastline of Korea (Ha et al. 2012) and reached the nearby shore within 14 h after the accident (Sim et al. 2010). Prior to the accident, the coastal villages were typical countryside locales with a relative lack of pollution. During the clean-up period, fishing was banned until September 3, 2008 by the Ministry for Food, Agriculture, Forestry, and Fisheries, Republic of Korea (Taean-gun 2010). Local residents and civil servants were most heavily involved in the clean-up effort for several months following the oil spill (Cheong et al. 2011).

The spilled oil consisted primarily of aliphatic/aromatic hydrocarbons and polar compounds (Sim et al. 2010). Many crude oil components are well-known potential carcinogens and developmental toxicants (Bosch 2003; Goldstein et al. 2011). PAHs particularly, are much more persistent and toxic (Carpenter et al.

2002), and raise health concerns for several decades after exposure (Rotkin-Ellman et al. 2012). These constituents are absorbed mainly via dermal and respiratory routes (Elovaara et al. 1995; VanRooij et al. 1993), and generate reactive oxygen species (ROS) (Yilmaz et al. 2007) via metabolic activation. Excessive production of ROS leads to cellular damage through oxidative stress on lipids in cellular membranes and nucleotides in DNA (Waris and Ahsan 2006).

Studies on potential health effects of Hebei Spirit oil spill were mostly on immediate or acute health effects which included physical and mental symptoms (Cheong et al. 2011; Ha et al. 2012; Lee et al. 2010; Sim et al. 2010). Acute symptoms such as eye symptoms, headaches, skin symptoms, neurovestibular symptoms and respiratory symptoms, persisted for more than 1 to 2 years after one large oil spill accident (Na et al. 2012). Studies on oil spill incidents elsewhere were reported (Morita et al. 1999; Suarez et al. 2005) including recent studies on hematological toxicity (D'Andrea and Reddy 2013) and principal somatic symptoms and complaints (D'Andrea and Reddy 2014b) in relation with the Gulf oil spill accident. However, few previous studies have looked at the health effects of oil spill exposure over prolonged periods (Rodriguez-Trigo et al. 2010; Zock et al. 2007; Zock et al. 2012). Participation in clean-up activity during the Prestige oil spill resulted in prolonged respiratory symptoms that lasted up to 5 years after the spill (Zock et al. 2012). Because of the long lasting nature of oil spill pollution and its health effects, a necessity of long term longitudinal studies on the health effects of oil spill exposures has been suggested (D'Andrea and Reddy 2014a; Ha et al. 2012).

In general, biomarkers are good indicators for estimating exposure and health status. Urinary biomarkers, 1-hydroxypyrene (1-OHP) and 2-naphthol (2-NAPH) were used to represent the levels of PAH exposure from the spilled oil. Urinary 1-OHP has been reported to accurately reflect exposure to PAHs (Nilsson et al. 2004). Urinary 2-naphthol (2-NAPH), a metabolite of naphthalene, is a more sensitive biomarker compared to urinary 1-naphthol for low level inhalation exposure to PAHs (Hecht 2002; Kim et al. 2001). In addition, 1-naphthol is not only a urinary metabolite of naphthalene but also a 1-naphthyl-Nmethylcarbamate (carbaryl, carbamate pesticide), therefore, it cannot be used as a specific biomarker for PAH exposure (Meeker et al. 2007). Since crude oil contains a lot of volatile organic compounds (VOCs) and many of them are genotoxic, their metabolites also could be exposure biomarkers. Referring to preceding modeling results, most of the VOCs have evaporated during first several days of the incident while PAHs have continuously evaporated for a longer period (Kim et al. 2012). In addition, most of VOCs are removed relatively rapidly after exposure while many kinds of PAHs can accumulate and persist in the human body (Moon et al. 2012). Therefore, VOC metabolite was not appropriate as exposure biomarkers to be measured over one year after the oil spill.

Malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) were used to represent the level of oxidative stress. Serum MDA levels were found to predict cardiovascular events (Walter et al. 2004), and urinary 8-OHdG levels have been indicated as a risk factor for atherosclerosis, diabetes, and various cancers (Wu et al. 2004).

This study was conducted to elaborate the association between oil spill exposure and probable indications of oxidative stress and DNA damage in exposed subjects who participated in clean-up that continued for over one year after the accident. The relatively short half life of biomarkers was taken into consideration by including the elapsed time since the last exposure due to clean-up participation.

Materials and Methods

Study population

Following the oil spill accident, volunteers, residents, military personnel, and governmental employees participated in the clean-up work (Ha et al. 2012). Among them, residents worked for an average of several months, whereas volunteers worked for a few hours to a few days during the total clean-up period which lasted for over a year. Nearly all residents of the most affected coastal area participated in an almost daily clean-up effort to rebuild their lives and maintain their households, since their regular occupation was no longer feasible (Cheong et al. 2011). Local civil servants also participated in the clean-up effort for several months.

A cohort was established to examine potential longer-term health effects associated with oil spill exposure more than one year after the accident. The total population of the target region was 37,326 according to the 2009 census data (Taean-gun 2009). A baseline survey was conducted between February and November 2009 (14 to 23 months after the spill) involving 8275 adults (from February to September) and 1026 students (June and November). A total of 699 adults were randomly selected for measurement of 1-OHP, 2-NAPH, MDA and 8-OHdG in their urine. Samples from 671 adults were used for this analysis, after excluding 28 samples for lack of information regarding cleanup (Figure 1).

This study was approved by the Institutional Review Board of Dankook University Hospital, Cheonan, Republic of Korea (DKUH IRB 2009-04-027). Written informed consent was obtained from each participant before enrollment.

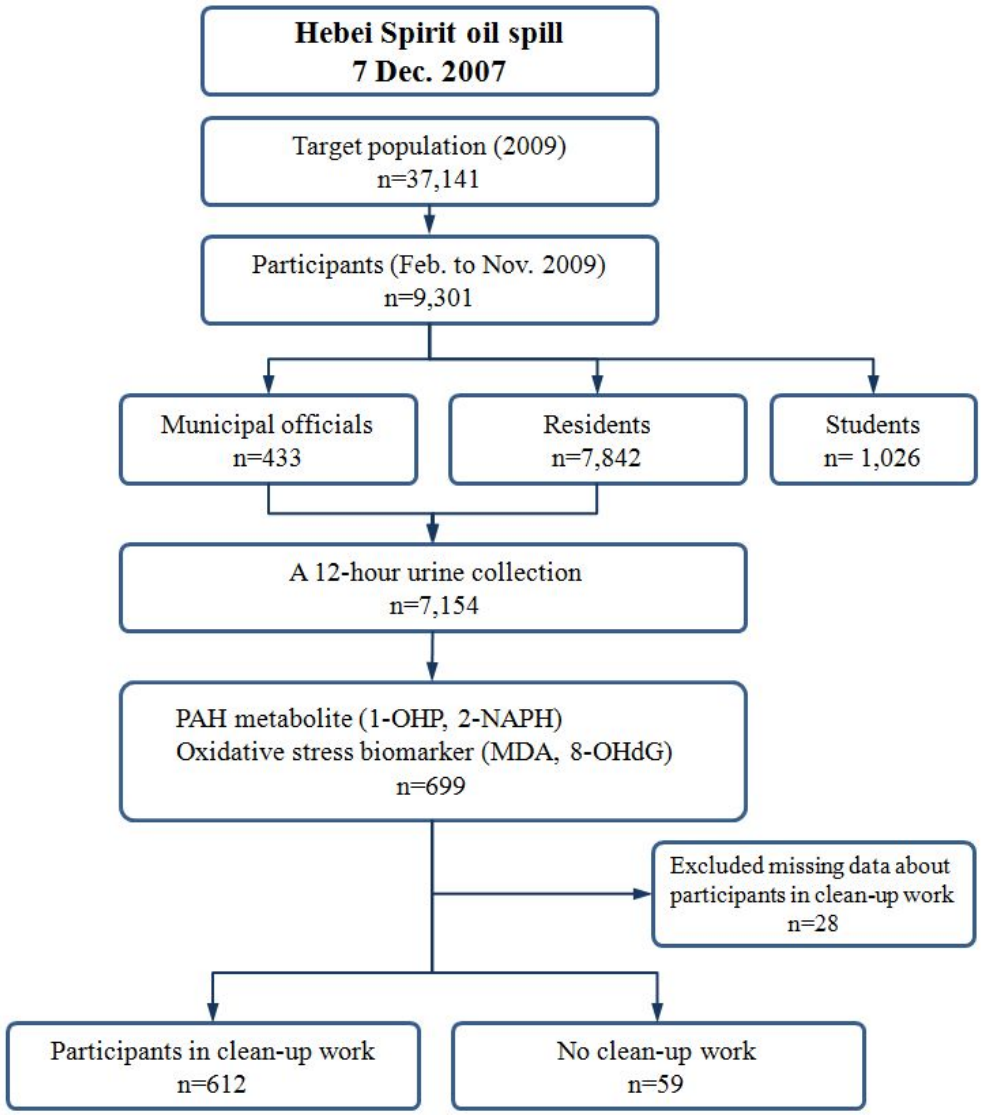


Figure 1. Flow diagram of subject enrollment

Collection of urine sample

A 12-hour urine sample was collected from the time after the last meal to the time of the health examination the next morning (from 7:00 PM to 7:00 AM the next morning). Participants were instructed by expert practitioners who visited their home the day before the examination on how to collect and refrigerate their own urine in the provided urine bag labeled with the subject's identification number. In the morning, all participants were transported to the health examination center by a bus with a portable refrigerator where the collected urine bags were kept. Collected urine samples were divided into aliquots and immediately stored in a freezer at -20ºC until analysis. Later, a polypropylene tube containing 15 mL of the frozen urine was sent to a laboratory for analysis.

Surrogates of the oil spill exposure

Since the ambient measurement data on oil spill exposure at the time of the accident was not available and neither was an appropriate environmental exposure index for the spill according to the complexity of crude oil components, three surrogates for exposure were used: total duration of clean-up participation and the 2 PAH metabolites 1-OHP and 2-NAPH.

Using the questionnaire, the total duration of clean-up work involvement was obtained by determining the number of days the respondent participated in clean-up work for each month between December 2007 and February 2009. The total number of days of clean-up work was calculated by summing the number of days the respondent performed clean-up work.

Two urinary PAH metabolites, 1-OHP and 2-NAPH were measured by HPLC with a fluorescence detector (Agilent 1200 series, Agilent, Santa Clara, CA, USA) (Jongeneelen et al. 1987; Kim et al. 1999). In a dark room, 500 μL of the urine samples was buffered with 100 μL of 2Msodium acetate buffer (pH 5.0). The samples were then hydrolyzed enzymatically for 16 h with 10 μL of β-glucuronidase with sulfatase activity (Sigma, St. Louis, MO, USA) at 37 °C in a shaking dry bath. After hydrolysis, 150 μL of acetonitrile was added and the samples were centrifuged for 10 min at 13,000 revolution/min. About 300 μL of the upper phase was transferred to a vial for analysis with HPLC. A reverse phase column (Zorbax SB-C18, Agilent, Santa Clara, CA, USA) was used to determine 1OHP and 2-NAPH levels, at the flow rate of 1.0 mL/min. The mobile phase was 60% (v/v) acetonitrile for 1-OHP, and 32% (v/v) acetonitrile for 2-NAPH. Excitation/emission wavelengths used in fluorescence detection of 1-OHP and 2-NAPH were 242/388 nm and 227/355 nm, respectively. The limit of detection (LOD) was 0.013 μg/L for 1-OHP and 0.045 μg/L for 2-NAPH. The number (percentage) of the values below LOD was 93 (13.9%) for urinary 1-OHP and 37 (5.5%) for urinary 2-NAPH. For the quality control, the laboratory regularly participated in the German External Quality Assessment Scheme program and was determined to be acceptable.

Oxidative stress biomarkers

Two biomarkers of oxidative stress, MDA and 8-OHdG were used. The level of urinary MDA was determined by measuring thiobarbituric acid reactive substances using HPLC with a fluorescence detector (Agarwal and Chase 2002). The LOD of

urinary MDA was 0.07 nM/mL and the intra-assay coefficient of variation (CV) for the pooled urine sample was 5.25%.

The level of urinary 8-OHdG was determined by an 8-OHdG check competitive enzyme-linked immunosorbent assay (ELISA) kit (Japan Institute for the Control of Aging, Kyoto, Japan), according to manufacturer instructions. The LOD of urinary 8-OHdG was 0.19 ng/mL and the intra-assay CV was 4.18%. All data were within the range of quantification (0.5–200 ng/mL) suggested by the manufacturer. The number (percentage) of the samples below LOD was 3 (0.4%) for urinary MDA and 0 (0%) for urinary 8-OHdG.

Urinary creatinine level was also measured using the creatinine test WAKO (Jaffe method) to adjust for urine concentration.

Time elapsed since the last clean-up work

Given the relatively short half-life of biomarkers, time intervals between the last clean-up participation and health examination were comprised as a variable for analyses. The time intervals were categorized into 0–6, 7–12 and 13 or more months from the last clean-up participation. Non-participants were included in the analysis and all analyses were performed with adjustment for or stratification by the time elapsed since the last clean-up work.

Potential confounders or covariates

Information on potential confounders or covariates was obtained through a questionnaire. Socio-demographic factors, i.e., age (<50, 50–64, ≥65), gender,

education level (<6, 6–11, ≥12 years), other sources of environmental exposure, i.e., whether industrial facilities existed within 2 km from residence (no, yes), distance to main road from residence (<50, 50–99, 100–499, ≥500 m), occupation (agriculture, fishery, others), intake of fishes and shellfishes (≤3 times a month, ≥1 times a week), alcohol consumption (no, yes), and variables related with oil spill exposure, i.e., distance to oil spill point from residence (b16, 16–30, ≥30 km), and duration of clean-up work participation (no clean-up work, 1–29, 30–119, ≥120 days) were considered.

The subject's smoking exposure as a potential confounder was adjusted using the urinary cotinine level. Urinary cotinine levels were analyzed by an ELISA kit (Calbiotech, Spring Valley, CA, USA). The concentration of cotinine was quantified by comparison with the standard curves.

Statistical analyses

For participants that had urinary concentrations of cotinine, PAH metabolites, and oxidative stress biomarkers below LOD, the LOD/2 for each biomarker was substituted. All urinary biomarker concentrations were adjusted to reflect the creatinine level of each respective urine sample and log-transformed to approximate a normal distribution. Statistical methods included a series of t-test, one-way analysis of variance (ANOVA), and simple linear regression analysis. The association between the surrogates of oil spill exposure and oxidative stress biomarkers after adjusting for several covariates were assessed by a multiple generalized linear regression model based on the PROC GLM procedure of the SAS program. Geometric mean (GM) and geometric standard deviation (GSD) and

covariates-adjusted GM (GSD) using least squares mean from a generalized linear model were calculated for all log-transformed urinary biomarkers. Analyses were performed using the SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) with a significance level set at 0.05.

Results

The mean age of study subjects was 57.4 years with a range of 24–79 years. Educational level was b12 years in 71.0% of the residents and most of the subjects (70.0%) worked in the fishing industry. Subjects who did not participate in clean-up activity were 8.8% (59 out of 671). The median level of urinary cotinine was 2.97 μg/g creatinine. Among clean-up work participants, mean clean-up work duration was 70.2 days (ranging from 3 to 350 days) and the mean time interval between the last clean-up exposure and the health examination was 11.8 months (range, 1 to 19 months) (Table 1).

Table 1. Characteristics of the study participants

Characteristic	N (%)
Age (years)	
<50	194 (28.9)
50-64	314 (46.8)
≥65	163 (6.1)
Cotinine (μg/g creatinine)	
Q1 (0.01–1.66)	168 (25.0)
Q2 (1.66–2.97)	167 (24.9)
Q3 (2.97–9.33)	169 (25.2)
Q4 (9.33–7236.74)	167 (24.9)
Alcohol consumption	
No	408 (60.8)
Yes	263 (39.2)
Industrial facilities within 2 km from residence	
No	641 (95.5)
Yes	26 (3.9)
Unknown	4 (0.6)
Distance to main road from residence (m)	
<50	269 (40.1)
50–499	288 (42.9)
≥500	114 (17.0)
Total duration of clean-up work (days)	
0–49	352 (52.5)
50−99	102 (15.2)
100−149	140 (20.9)
≥150	77 (11.5)
Gender	
Male	262 (39.1)
Female	409 (61.0)
Education (years)	
<6	114 (17.0)
6–11	362 (54.0)
≥12	195 (29.1)
Occupation[a]	
Agriculture	337 (50.2)
Fishery	470 (70.0)
Others	147 (21.9)
Unknown	6 (0.9)
Intake of fishes and shellfishes[b]	
2–3 times a week or more	264 (39.3)
1–3 times a month or less	405 (60.4)
Unknown	2 (0.3)
Distance to oil spill point from residence (km)	
<16	241 (35.9)
16–30	237 (35.3)
≥30	193 (28.8)
Time elapsed (months)[c]	
0–6	35 (5.2)
7–12	330 (49.2)
13–20[d]	247 (36.8)
No clean-up work[d]	59 (8.8)

[a]Subject may be duplicated for each item.
[b]One or more among 10 types (oyster, mania clam, mussel, rockfish, blue crab, ray, tiger prawn, small octopus, webfoot octopus, monkfish).
[c]Elapsed time from the last clean-up work to the health examination (months).
[d]Combined 2 groups together in Tables 2, 3, and Figure 2.

Levels of oxidative stress biomarkers were increased by the total duration of clean-up work participation and the increase was observed even over a year after the oil spill (urinary MDA, p-trend < 0.0001; urinary 8-OHdG, p-trend < 0.0001) (Figure 2). Urinary MDA levels showed a 21.6% increase while urinary 8-OHdG levels showed a 27.9% increase per 100 days of clean-up work.

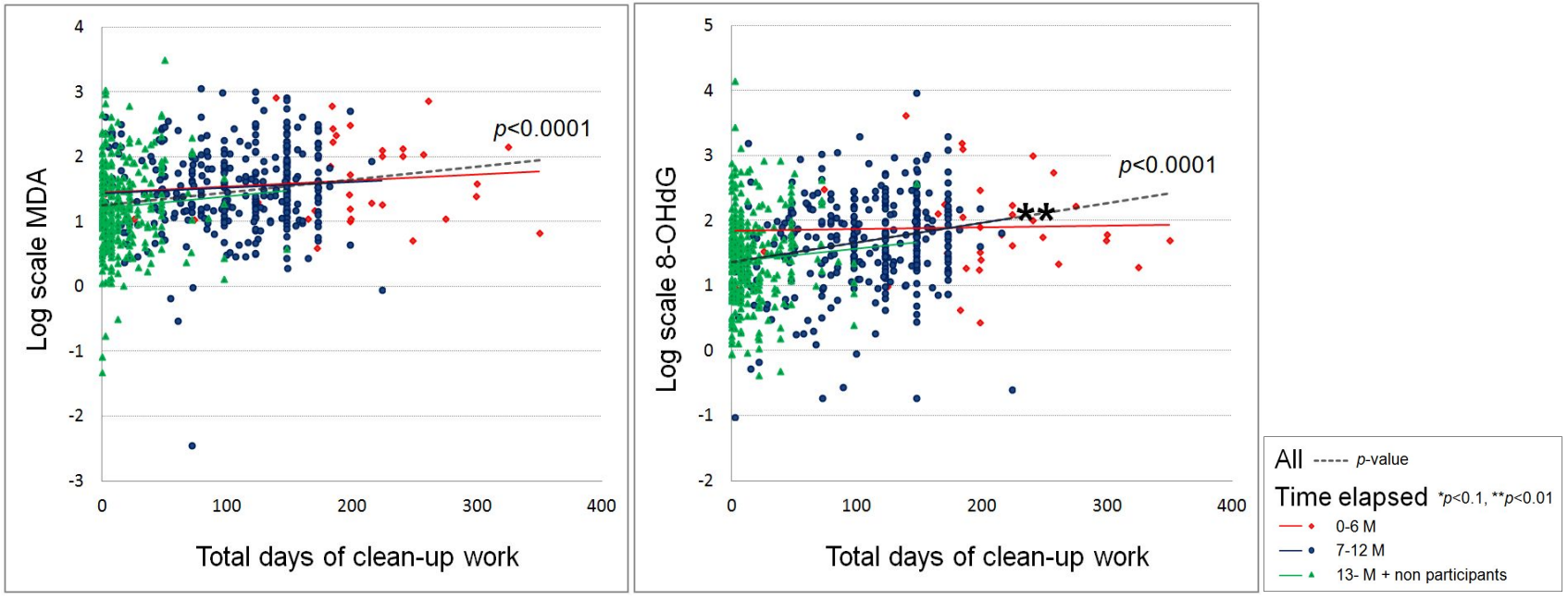


Figure 2. Relationship between total duration of clean-up work and levels of oxidative stress biomarkers over one year after the accident by the time elapsed since last clean-up. The urinary MDA (y=0.002x+1.252; p<0.0001) and 8-OHdG (y=0.003x+1.371; p<0.0001) were associated with the total duration of clean-up work in all subjects. The regression coefficients (p-value) were 0.001 (0.53), 0.001 (0.26), and 0.002 (0.32) for MDA, and 0.000 (0.88), 0.003 (0.0001), and 0.002 (0.28) for 8-OHdG, in groups who participated clean-up works until 0–6 months ago, 7–12 months ago, and 13 or more months ago including non-participants, respectively.

As the time between the last participation in clean-up work and the health examination increased, the total duration of clean-up work involvement decreased (p-trend < 0.0001) (Table 2). Also, two urinary PAH metabolites (p-trend = 0.003), 1-OHP and 2-NAPH, as well as 2 urinary oxidative stress biomarkers (p-trend < 0.0001), MDA and 8-OHdG, were significantly decreased by the time elapsed from last clean-up.

Table 2. Distribution of total duration of clean-up work, levels of urinary PAH metabolites and oxidative stress biomarkers over one year after the Hebei Spirit oil spill by the time elapsed since last clean-up work

		Time elapsed since last clean-up (months)[a]			
	All (n=671)	0–6 (n=35)	7–12 (n=330)	≥ 13 (n=306)[b]	p-Trend
Total clean-up work duration (days)					
Mean ± SD	70.2 ± 67.2	198.7 ± 73.2	106.9 ± 48.9	15.8 ± 20.5	<0.0001
Median (Q1-Q3)	47.0 (7.0–122.0)	198.0 (170.0–240.5)	117.5 (71.5–147.5)	7.0 (2.5–21.5)	
Urinary PAH metabolites					
1-OHP (μg/g creatinine)					0.003
GM (GSD)	0.08 (3.33)	0.14 (2.60)	0.09 (2.78)	0.07 (3.96)	
Median (Q1–Q3)	0.09 (0.04–0.17)	0.10 (0.07–0.21)	0.09 (0.05–0.16)	0.08 (0.02–0.18)	
2-NAPH (μg/g creatinine)					0.003
GM (GSD)	1.89 (4.11)	2.11 (2.95)	2.10 (3.21)	1.66 (5.28)	
Median (Q1–Q3)	1.82 (0.85–4.83)	1.55 (1.05–4.62)	1.86 (0.93–5.11)	1.81 (0.80–4.72)	
Oxidative stress biomarker					
MDA (μmol/g creatinine)					<0.0001
GM (GSD)	4.07 (1.97)	5.15 (1.90)	4.64 (1.90)	3.43 (1.97)	
Median (Q1–Q3)	3.83 (2.68–6.51)	4.21 (2.86–8.48)	4.53 (3.17–7.21)	3.22 (2.23–5.13)	
8-OHdG (μg/g creatinine)					<0.0001
GM (GSD)	4.79 (2.03)	6.69 (2.03)	5.39 (2.02)	4.06 (1.96)	
Median (Q1–Q3)	4.76 (3.01–7.59)	6.06 (4.08–9.44)	5.79 (3.63–8.49)	3.96 (2.66–5.80)	

GM: geometric mean, GSD: geometric standard deviation, PAH: polycyclic aromatic hydrocarbon, 1-OHP: 1-hydroxypyrene, 2-NAPH: 2-naphthol, MDA: malondialdehyde, 8-OHdG: 8-hydroxydeoxyguanosine.

p-Trend estimated by a simple linear regression model using the ordinal scale of the time elapsed since last clean-up work (coded as 1, 2, 3).

[a]Elapsed time from the last clean-up work to the health examination (months).

[b]Included non-participants in clean-up work.

Levels of 1-OHP and 2-NAPH significantly increased according to an increase in the total duration of clean-up work involvement after adjustment for covariates (p-trend = 0.007 for 1-OHP, p-trend = 0.05 for 2-NAPH) (Table 3).

Table 3. Levels of urinary PAH metabolites according to total duration of clean-up work

Urinary PAH metabolites		1-OHP (μg/g cr.)		2-NAPH (μg/g cr.)	
Total duration of clean-up work (days)	n	GM	(95% CI)	GM	(95% CI)
0–49	347	0.07	(0.05–0.09)	1.64	(1.18–2.27)
50–99	102	0.07	(0.05–0.09)	1.88	(1.28–2.76)
100–149	140	0.09	(0.07–0.12)	2.40	(1.66–3.47)
≥150	76	0.11	(0.08–0.15)	1.89	(1.26–2.83)
p-Trend		0.0007		0.05	

PAH: polycyclic aromatic hydrocarbon, 1-OHP: 1-hydroxypyrene, 2-NAPH: 2-naphthol.

Geometric mean (GM) and 95% confidence intervals (CI) calculated using generalized linear model adjusted for age (<50, 50-64, ≥65), gender (male, female), creatinine-adjusted cotinine levels (Q1, Q2, Q3, Q4), alcohol consumption (no, yes), education levels (<6, 6–11, ≥12 years), distance to road (<50, 50–499, ≥500 m), industrial facilities within 2 km (no, yes), and intake of fishes and shellfishes (2–3 times a week or more, 1–3 times a month or less).

p-Trend estimated using the ordinal scale of the total clean-up work duration in the corresponding model.

As shown in Table 4, urinary 1-OHP levels were significantly positively associated with both urinary MDA levels ($p=0.04$) and 8-OHdG levels ($p=0.0009$) after adjustment for covariates, while urinary 2-NAPH levels did not show significant association. To control for the confounding effect of the time elapsed since the last clean-up on the relationships between PAH metabolites and oxidative stress levels, analyses were performed with stratification according to the elapsed time. Significant positive associations between 1-OHP and MDA or 8-OHdG were observed in the group with an interval of 12 months or less since the last clean-up, but not in those with 13 months or more. Significant positive association between 2-NAPH and MDA appeared only within a 6 month interval since the last clean-up ($p=0.04$). When the time elapsed since the last clean-up was additionally adjusted to control for possible residual confounding effects in each stratum, the results were not materially affected as shown in model 2 (Table 4).

Table 4. Association between levels of urinary PAH metabolites and oxidative stress biomarkers over one year after the Hebei Spirit oil spill by the time elapsed since last clean-up work (n=665)

				Time elapsed since last clean-up (months)[a]					
	All (n=665)[b]			0–6 (n=35)		7–12 (n=328)		≥13 (n=302)[c]	
	β (SE)	p-Value		β (SE)	p-Value	β (SE)	p-Value	β (SE)	p-Value
In relation with MDA (μmol/g cr.)									
1-OHP (μg/g cr.)	0.044 (0.022)	0.04	Model 1	0.281 (0.101)	0.01	0.088 (0.035)	0.01	0.003 (0.028)	0.93
			Model 2	0.282 (0.105)	0.01	0.081 (0.035)	0.02	0.007 (0.028)	0.81
2-NAPH (μg/g cr.)	0.030 (0.018)	0.10	Model 1	0.194 (0.090)	0.04	0.047 (0.031)	0.13	0.016 (0.023)	0.49
			Model 2	0.194 (0.093)	0.05	0.044 (0.031)	0.16	0.017 (0.023)	0.46
In relation with 8-OHdG (μg/g cr.)									
1-OHP (μg/g cr.)	0.075 (0.022)	0.0009	Model 1	0.343 (0.139)	0.02	0.151(0.037)	<0.0001	0.020 (0.028)	0.47
			Model 2	0.323 (0.139)	0.03	0.143 (0.037)	0.0001	0.023 (0.028)	0.42
2-NAPH (μg/g cr.)	0.026 (0.019)	0.17	Model 1	0.003 (0.133)	0.98	0.043(0.033)	0.20	0.024 (0.023)	0.30
			Model 2	-0.007 (0.132)	0.96	0.038 (0.033)	0.25	0.025 (0.023)	0.28

PAH: polycyclic aromatic hydrocarbon, 1-OHP: 1-hydroxypyrene, 2-NAPH: 2-naphthol, MDA: malondialdehyde, 8-OHdG: 8-hydroxydeoxyguanosine.
Log scale of MDA and 8-OHdG was used as the dependent variable, and log scale of 1-OHP and 2-NAPH was used as the independent variable. Model 1: β (SE) and p-values estimated using a generalized linear model adjusted for age (<50, 50–64, ≥65), gender (male, female), creatinine-adjusted cotinine levels (Q1, Q2, Q3, Q4), alcohol consumption (no, yes), education levels (<6, 6–11, ≥12 years), distance to road (<50, 50–499, ≥500 m), industrial facilities within 2 km of residence (no, yes), intake of fishes and shellfishes (2–3 times a week or more, 1–3 times a month or less). Model 2: in the corresponding model 1, additionally adjusted for the time elapsed since the last clean-up (3 months interval unit). Six observations were excluded for the analysis because of 4 missing values of "industrial facilities within 2 km of residence" and 2 missing values of "intake of fishes and shellfishes".
[a]Elapsed time from the last clean-up work to the health examination (months).
[b]In addition to the corresponding model 1, adjusted for the ordinal scale of the time elapsed since last clean-up work (coded as 1, 2, 3) in the analysis for all.
[c]Included non-participants in clean-up work.

Discussion

The study found a positive exposure–response relationship between oil spill exposure and oxidative stress biomarkers among long-term clean-up participants more than a year after the Hebei Spirit oil spill. The longer the total duration of clean-up work involvement, the higher the levels of 1-OHP and 2-NAPH in the participants' urine. Levels of oxidative stress biomarkers, MDA and 8-OHdG, showed significant positive associations with total clean-up work duration and level of 1-OHP up to around 12 months after the last clean-up work.

Up to now, this is the first epidemiologic study to examine the effects of oil spill exposure on levels of oxidative stress biomarkers. There are few reports assessing biomarkers after oil spills with the exception of reports on chromosomal abnormality and DNA damage (Laffon et al. 2006; Rodriguez-Trigo et al. 2010).

Urinary 8-OHdG levels resulting from oxidative DNA damage, which occurs after ROS invade the nuclear or mitochondrial membrane (Valavanidis et al. 2009) and urinary MDA levels resulting from damage to cell membranes with abundant lipid molecules (Valko et al. 2006) were measured. Increased oxidative stress levels reportedly correlated with many chronic degenerative diseases such as allergic inflammatory diseases (Bartsch and Nair 2004), obesity (Keaney et al. 2003), diabetes (Wu et al. 2004), atherosclerosis (Kobayashi et al. 2011; Wu et al. 2004), and cancer (Jackson and Loeb 2001; Wu et al. 2004).

The present findings showed that continuous or chronic exposure caused by long-running clean-up activity induced sustained oxidative stress damage even up to 12 months after the last exposure. The clean-up activity in the area had actually

continued for over a year. During the clean-up period, clean-up workers might be continuously exposed to various PAHs (Kim et al. 2012). PAHs are major components of crude oil and are much more persistent and bioaccumulative in the environment (Thomas et al. 2007). Most PAHs are highly lipid soluble and can be readily absorbed through the lungs, skin, and gastrointestinal tract, to which clean-up participants might be easily exposed. The absorption of oil components like PAHs by the human body could induce prolonged and/or delayed ROS generation, which could cause oxidative DNA damage and lipid peroxidation.

Two PAH metabolites, 1-OHP and 2-NAPH, have a relatively short metabolic half-life: i.e., several hours (Brzeznicki et al. 1997; Li et al. 2012). Thus, these biomarkers have come to be known as markers for current exposure. However, many kinds of PAHs can accumulate within fat tissue (Moon et al. 2012), particularly those PAHs with high molecular weight found in crude oil and/or under the condition of prolonged exposure (Lu et al. 2002). A previous study reported that continuous exposure to large amounts of PAHs is likely to result in the storage of PAHs in human tissues (Guo et al. 2012). This supports speculation on accumulation and continuous release of PAHs from stored tissues in clean-up participants who were involved for a sustained period of time, i.e., at least >100 days. The 1-OHP level was especially high among those who participated in clean-up work >100 days.

Of the two PAH metabolites, only 1-OHP levels were significantly associated with the levels of oxidative stress biomarkers. This is possibly due to the relatively longer half-life of pyrene, the precursor to 1-OHP, which has high molecular weight and possibly higher accumulation in human tissues, compared to

naphthalene, a precursor of 2-NAPH, with low molecular weight and less accumulation.

In addition, continuous PAH exposure might change the induction of related metabolic enzymes, i.e., long lasting induction, increase of induction efficiency or increased bioactivation (Bartsch et al. 1992; Fazili et al. 2010). Long lasting induction and/or increased efficiency of PAH metabolic enzymes might increase production of metabolites and prolong ROS generation for some time (at least 7–12 months in the present study) after the end of exposure. Even when bodily accumulation of PAHs as a result of long-term clean-up has not yet occurred, the highly induced/activated metabolic enzymes could produce more PAH metabolites from other sources such as diet and smoking rather than spilled oil. The significant positive association between levels of 1-OHP and oxidative stress biomarkers disappeared 13 months or more after the last clean-up participation. This is understandable given the fact that the relatively short duration of clean-up participation within this group (average 15.8 days) might have resulted in little or no PAH accumulation and may have been insufficient to affect the related metabolic enzyme induction.

This results reinforced the view that participation in the clean-up activity is an important factor for oil exposure as well as for adverse acute and longer-term health effects, particularly in cases of prolonged exposure. It is not clear whether the previous report of prolonged respiratory problems (Zock et al. 2012) arose from continuous exposure to oil, or from irreversible change to affected organs due to the initial high exposure levels to toxic chemicals. It may be more reasonable to consider both speculations.

There are some limitations to the present study that warrant consideration. First, the use of an exposure surrogate such as total duration of clean-up work is relatively crude since actual ambient exposure data for each resident could not be established. At the time of the oil spill, ambient exposure assessment could not be performed systematically and a modeling of human exposure levels (Kim et al. 2012) showed that airborne exposure levels were highest in the first weeks, as was the case for the Prestige oil spill (Zock et al. 2007). The urinary PAH metabolites as exposure surrogates for oil exposure, 1-OHP and 2-NAPH, also could not represent the whole spectrum of exposure to complex components of crude oil. Urinary 1-OHP has long been known as a sensitive and specific marker for the assessment of exposure to not only pyrene but other PAHs (Jongeneelen et al. 1986; Nilsson et al. 2004). The urinary excretion of 1-OHP can also be increased by tobacco smoking, frequent intake of charcoal grilled meat, exposure to intense local traffic, and the presence of industries near home. Those factors including the level of urinary cotinine and variables of distance from the road, industrial facilities within 2 km from home, and exposure through intake of contaminated fishes and shellfishes were considered in multiple regression analyses. Another limitation of the present study was the use of 8-OHdG as the sole marker for oxidative DNA damage and the use of MDA as the only marker for lipid peroxidation. It is possible that other biomarkers of oxidative stress may provide additional information not detected in this study. However, the biomarkers used in this study are considered the most promising markers for quantifying in vivo oxidative stress (Wu et al. 2004). 8-OHdG in particular is very stable and is excreted in the urine without being metabolized (Valavanidis et al. 2009). Therefore, the biomarkers

used in the present study might sufficiently represent oxidative stress due to crude oil exposure.

In conclusion, a longer-term dose–response relationship between the severity of spilled oil exposure and the extent of oxidative DNA damage and lipid peroxidation were found in coastal community residents after the Hebei Spirit accident. The present findings support the necessity of exploring the long-term health effects of oil exposure.

CHAPTER 2.

Long-term follow-up of oxidative stress biomarkers up to 4 years after the Hebei Spirit oil spill

Introduction

Hebei Spirit oil spill episode, which oil tanker Hebei Spirit collided with a crane barge, is the largest oil spill to have ever occurred off the coast of Korea. After the collision, an estimated 12,547 kL of crude oil were released only 5 miles off the coast on the morning of December 7, 2007. Health risks could be substantial because the accident occurred in areas densely populated fishing villages, although volume of the crude oils was relatively smaller than the large oil spills of other countries, such as Exxon Valdez oil spill (Peterson et al. 2003) and Gulf oil spill (Goldstein et al. 2011).

Many crude oil components are well known to cause acute and chronic health problems and potential carcinogens (Bosch 2003). These chemicals include volatile organic compounds (VOCs), such as benzene, toluene, xylene, and ethylbenzene, and polycyclic aromatic hydrocarbons (PAHs), and heavy metals. The number of chemically distinct constituents of crude oil, which are among the most complex oragnic mixtures in the world, can reach into the tens of thousands (Klein et al. 2006). Most toxicological research have been conducted on one chemical at a time (Carpenter et al. 2002), and nevertheless, there are still limited information about toxicity and subsequence toxicological effects in long-term of the many oil

components. In addition, in real life people are exposed to mixtures, not single chemical.

The levels of oil chemicals including VOCs and PAHs were extremely high immediately after the accident (Kim et al. 2012). Subsequence clean-up efforts after the accident during several months in most residents and over a year in few residents living high-exposed area were also resulted in high and continuous direct and indirect exposure to oil chemicals from oil contaminated environment, particularly, PAHs, alkylated PAHs, and unknown oil components as a form of mixture of multiple compounds. Even after the active clean-up period, there could be persistent and low-dose exposure indirectly. Residual oils were found even five years after the Hebei Spirit oil spill in some inner part of small bays and mud dominant regions in Taean (Hong et al. 2014). Genotoxicity and endocrine-disruption potentials of the sediment were suggested two years later (Ji et al. 2011) and significant toxicity of PAHs in intertidal sediments was still found five years later (Lee et al. 2013). Similarly, large oil spills of other countries reported unexpected persistence of toxic subsurface oil (Turner et al. 2014) and chronic exposures response to the Exxon Valdez oil spill (Peterson et al. 2003) and highly significant increases in genotoxicity in air samples from the contaminated areas over last four years post the Deepwater Horizon oil spill (Singleton et al. 2016).

From confirmed toxicity in ecosystem, potential toxic effects on residents' health could be remain or newly occur even though several years have passed since the accident. Oil constituents generate reactive oxygen species (ROS) by being absorbed in respiratory tracts, digestive organs, skin contact, or placenta. The excessive production of ROS leads to cell damage described as oxidative stress

(Waris and Ahsan 2006). Excessively produced oxidative stress induce various chronic progressive diseases, such as inflammatory, cardiovascular, and neurodegenerative disorders, and cancer (Walter et al. 2004; Wu et al. 2004). Several studies related to cytogenetic and endocrine biomarkers were released from the Prestige oil spill (Spain, 2002), which were made to evaluate not only acute health effects (Laffon et al. 2006; Perez-Cadahia et al. 2008a; Perez-Cadahia et al. 2008b; Perez-Cadahia et al. 2007) but also persistence of these health effects (Laffon et al. 2013a; Laffon et al. 2013b; Rodriguez-Trigo et al. 2010). These biomarkers include DNA damage by comet assay, chromosomal lesions and structural alteration in circulating lymphocytes, cytogenetic effects by micronucleus test and sister chromatid exchanges, endocrine toxicity by plasmatic prolactin and cortisol levels in plasma, and respiratory marker of oxidative stress measured by 8-isoprostane levels. My preceding study also found that levels of oxidative stress biomarkers significantly associated with the oil spill indicators, such as total duration of clean-up work and PAH metabolites of crude oil components 1 year after the oil spill (Noh et al. 2015). Therefore, the aim of this study was to follow up the levels of oxidative stress biomarker of high-exposed residents and confirm that health risks still remain 3 years and 4 years after the oil spill.

Materials and Methods

Study population

Two follow-up studies were underwent in high-exposed area located in the coastal area of four townships among the seven townships of Taean county (both high-exposed and low-exposed area), in which baseline survey was underwent in 2009 one years after the accident (Figure 1). A baseline survey for a cohort establishment was performed to examine potential long-term health effects associated with exposure to the Hebei Spirit crude oil. Two follow-up surveys were made in three years (October to November, 2010) and four years (February to March, 2012) after the accident. The high-exposed area was located within 2 km of early contaminated coastline from spilled crude oil. The early contaminated coastline was defined as an approximately 35 km seashore from Hagam port to Pado-ri, which was heavily contaminated with a thick layer of spilled oil beginning four days after the accident. Residents living the high-exposed area were enrolled in a voluntary health examination in two follow-up surveys. A self-administered questionnaire with trained interviewer's help or a face-to-face interview for elderly participants was performed and biological samples were collected. Among the 671 residents living in both low-exposed and high-exposed area whose urine samples randomly and measured biomarkers of oxidative stress in baseline survey (Noh et al. 2015), a total of 95 residents living in the high-exposed area, were followed in two surveys three years and four years after the accident (Figure 2). Each study was approved by the Institutional Review Board of Dankook University Hospital, Cheonan, Republic of Korea (DKUH IRB 2009-04-027, DKUH IRB 2011-03-081,

DKUH IRB 2013-09-009). Each time written informed consent was obtained from each participant.

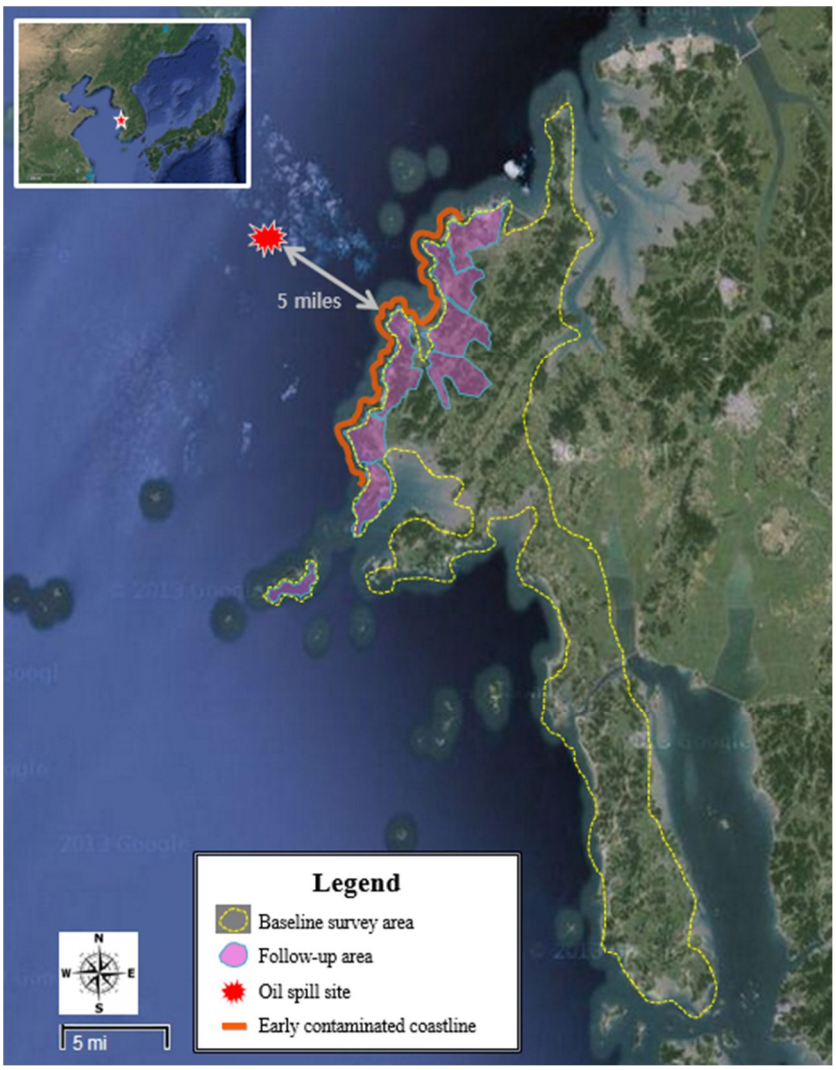


Figure 1. Regional map of follow-up area

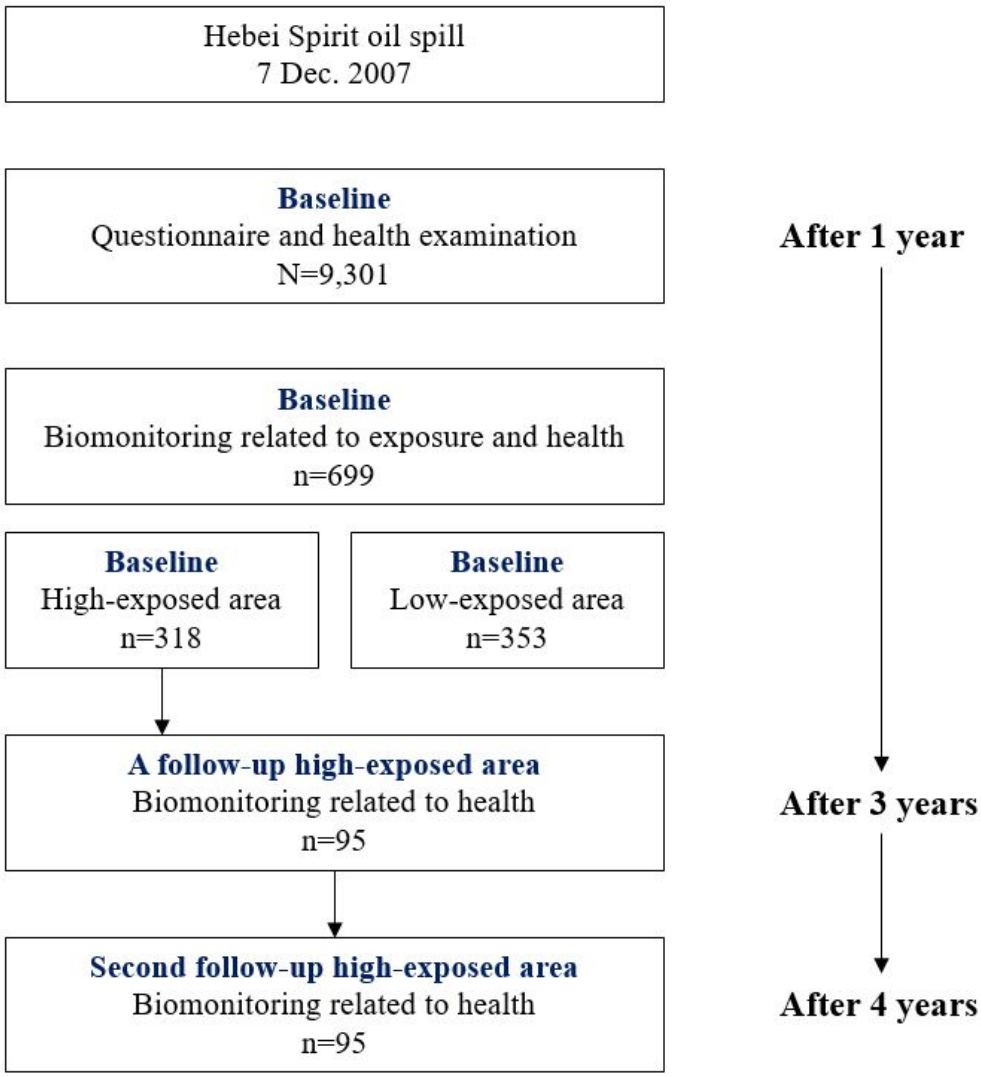


Figure 2. Flow diagram of subject enrolment

Assessment of oxidative stress

Collection of urine samples and analysis procedures and analysist of the levels of oxidative stress were the same as the baseline survey and is detailed in my previous study (Noh et al. 2015). Oxidative stress was assessed using urinary 8-hydroxydeoxyguanosine (8-OHdG) as indicators of oxidative DNA damage.

Assessment of oil exposure

Two exposure indicators were determined that used in my preceding study. One was total number of days of clean-up work in a prolonged period (between December 2007 and February 2009) and another was levels of urinary metabolites of major oil components, 1-hydroxypyrene (1-OHP) at the time of baseline survey, one year after the spills.

Statistical analyses

Urinary creatinine-adjusted 8-OHdG concentrations (μg/g cr), which is biomarker of oxidative DNA damage, was log transformed to approximate to a normal distribution both two follow-up. To assess the relationship between the levels of oxidative stress marker and exposure parameter after adjusting for covariates, a multiple linear regression model was used based on PROC GLM procedure of the SAS. The least square means and standard errors were estimated using the LSMEANS statement, and STDERR option in PROC GLM. Additionally, a mixed linear regression model with an unstructured covariance matrix was used based on PROC MIXED procedure of the SAS to estimate changes levels of oxidative stress

biomarker over time. Age, gender, smoking, and education levels were adjusted as fixed effects, and each participant was treated as a random effect in the models.

Results

Mean age of the study subjects were 57.3 with an age range of 25−69 years. Subjects who did not participate in clean-up activity were 2.1% (2 out of 95). Mean of the total clean-up work duration was 117.7 days (SD: 56.0 days, range, 0 to 325 days). Time elapsed from the oil spills to the surveys was 34−35 months in a follow-up, and 50−51 months in second follow-up. Time elapsed from the last clean-up work to the surveys was 28.9 months (SD: 2.2 months, range, 21 to 34 months) in a follow-up, and 44.8 months (SD: 2.4months, range, 37 to 50 months) in second follow-up (Table 1).

Table 1. Characteristics of the study participants, oil exposure indicators, and oxidative stress biomarker (n=95)

Characteristic	Baseline (n=671) Feb–Aug, 2009	Follow-up the high-exposed area (n=95) Oct–Nov, 2010 (a follow-up) Feb–Mar, 2012 (second follow-up)
Age (years)		
<50	194 (28.9)	11 (11.6)
50–64	314 (46.8)	62 (65.3)
≥65	163 (24.3)	22 (23.2)
Gender		
Male	262 (39.1)	33 (34.7)
Female	409 (61.0)	62 (65.3)
Smoking		
No	586 (87.3)	87 (91.6)
Yes	85 (12.7)	8 (8.4)
Education (years)		
<6	114 (17.0)	17 (17.9)
6–11	362 (54.0)	67 (70.5)
≥12	195 (29.1)	11 (11.6)
Exposure variable		
Area		
High exposed (<2 km)	318	95
Low exposed (≥2 km)	353	-
Total duration of clean-up work (days)		
0–49	352 (52.5)	11 (11.6)
50–99	102 (15.2)	29 (30.5)
100–149	140 (20.9)	37 (39.0)
≥150	77 (11.5)	18 (19.0)
1-OHP (μg/g cr)		
GM (GSD)	0.08 (3.33)	0.09 (2.44)
Median (Q1–Q3)	0.09 (0.04−0.17)	0.09 (0.04–0.15)
Oxidative stress biomarker		
8-OHdG (μg/g cr)		
GM (GSD)	4.79 (2.03)	5.7 (1.8)
Median (Q1–Q3)	4.76 (3.01−7.59)	5.8 (3.8−8.8)

All values at the time of the baseline

High-exposed area (<2 km) and low-exposed area (≥2 km) were divided by distance from early contaminated coastline to residence.

OHP: 1-hydroxypyrene, 8-OHdG: 8-hydroxydeoxyguanosine.

Urinary 8-OHdG levels were significantly higer in residents living high-exposed area than residents living in low-exposed area 1 year after the oil spill ($p<0.0001$). Among the high-exposed area, urinary 8-OHdG levels had slightly increased as time passed, although there were not statistical significance. Geometric mean of the urinary 8-OHdG levels after adjusting for age, gender, smoking, and education levels were 5.6 μg/g cr, 5.8 μg/g cr, and 6.3 μg/g cr, 1 year, 3 years, and 4 years after the spills, respectively (Figure 3).

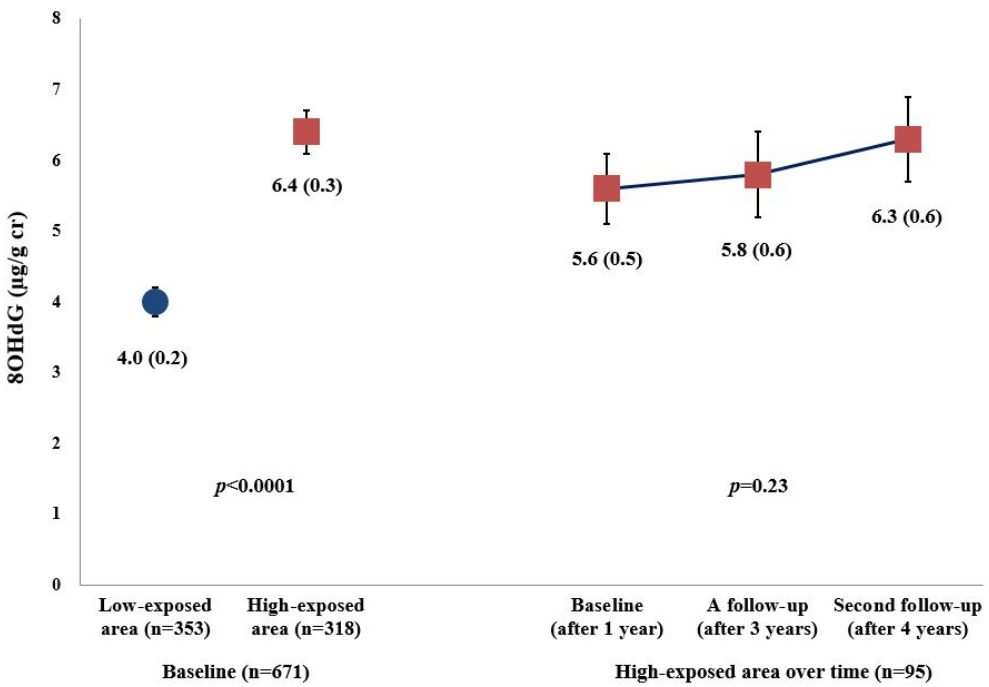


Figure 3. Levels of oxidative stress biomarkers (8-OHdG) 1 year, 3 years, and 4 years after the spill. Values are shown as geometric mean and geometric standard error.
Age on survey, gender, smoking, and education levels were adjusted by multiple linear regression model using PROC GLM procedure of the SAS in baseline analysis and linear mixed model using PROC MIXED procedure in follow-up analysis.

An increase in levels of the oxidative DNA damage was marginally significant during three years follow-up period from one year (baseline) to four years after the spill (β=0.123; p=0.09). In longitudinal analysis using linear mixed models, age, gender, smoking, and education levels were no significant association with the levels of oxidative DNA damage (Table 2).

Table 2. Levels of oxidative DNA damage (8-OHdG) over time (n=95)

	Log 8-OHdG (μg/g cr)	
	β (SE)	p-Value
Age (years)	0.005 (0.005)	0.32
Gender, female	-0.027 (0.088)	0.76
Smoking, yes	-0.112 (0.141)	0.43
Education, <6 years	0.018 (0.111)	0.87
Elapsed time		
Baseline (after 1 year)	Ref.	
A follow-up (after 3 year)	0.047 (0.083)	0.57
Second follow-up (after 4 years)	0.125 (0.073)	0.09

Used linear mixed models with an unstructured covariance matrix.

There were no significant relationship between total duration of clean-up works and levels of oxidative stress biomarker, 8-OHdG, three and four years after spill. There were no significant relationship between a metabolite of oil constituents, log-transformed 1-OHP, and levels of oxidative stress biomarker, log-transformed 8-OHdG, three and four years after spill (Figure 4).

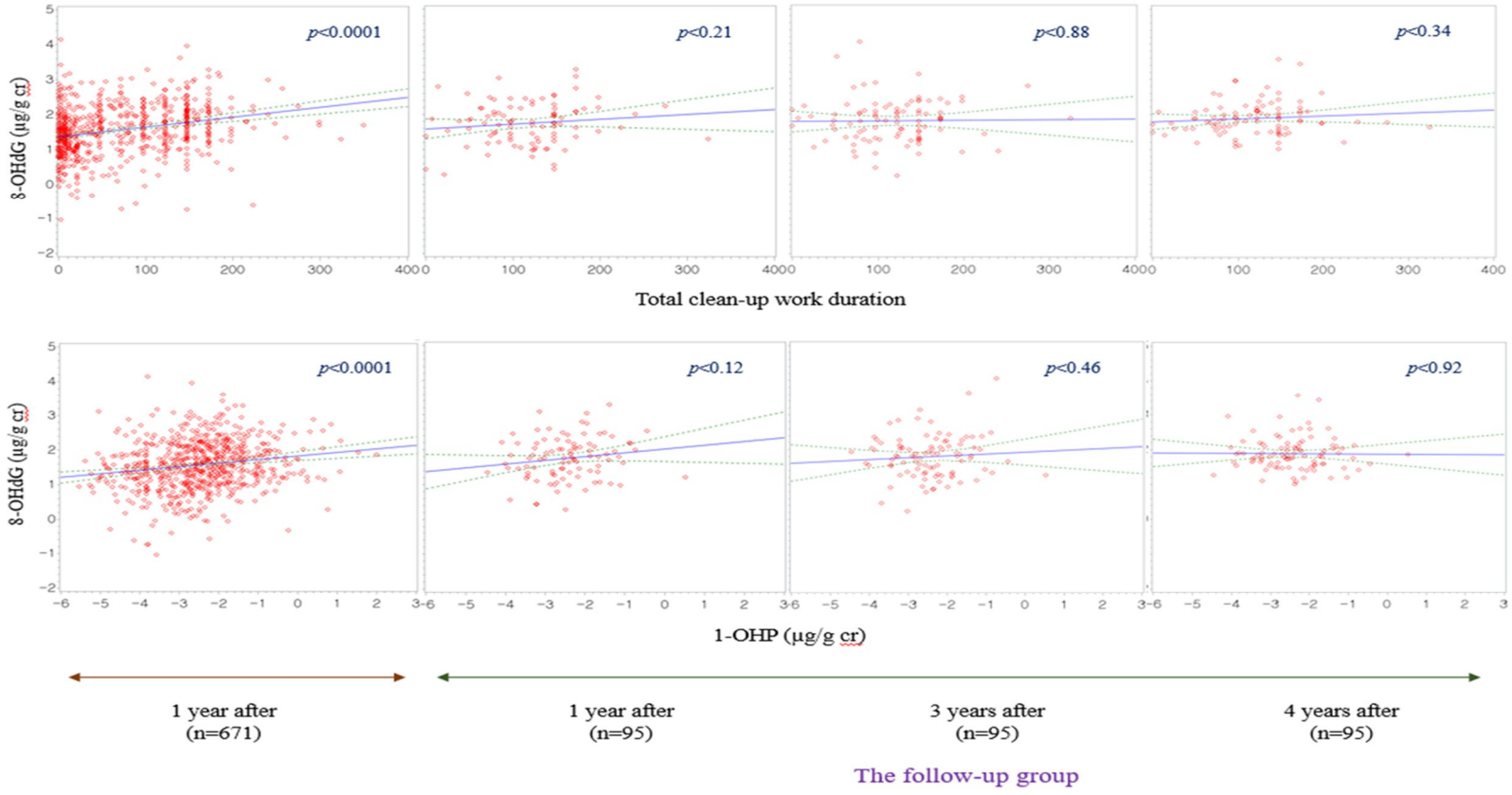


Figure 4. Scatter plots showing relationship between oil exposure and levels of oxidative stress biomarker 3 years and 4 years after the spill. Oil spill exposure was assessed by total clean-up work duration (between Dec. 2007 and Feb. 2009) and by levels of a urinary metabolite of major oil components, 1-hydroxypyrene (1-OHP). Oxidative stress was measured using 8-hydroxydeoxyguanosine (8-OHdG) as indicators of oxidative DNA damage. Levels of log-transformed urinary 1-OHP and log-transformed 8-OHdG were used.

With adjustment for a number of potential confounders including age (<50, 50–64, ≥65), gender (male, female), smoking (no, yes), and education levels (<6, 6–11, ≥12 years), the log-transformed 8-OHdG levels did not increased according to the total duration of clean-up activity both 3 years and 4 years after the spills (Table 3).

Table 3. Relationship between total clean-up work duration and levels of oxidative stress biomarker 1 year, 3 years, and 5 years after the spill

8-OHdG (μg/g cr)	Duration of clean-up work				*p*-Trend
	0–49 days	50–99 days	100–149 days	≥150 days	
	GM (GSE)	GM (GSE)	GM (GSE)	GM (GSE)	
Both high-exposed area and low-exposed area (n=671)					
After 1 year	4.2 (1.1)	5.2 (1.1)	6.1 (1.1)	6.5 (1.1)	<0.0001
Only high-exposed area (n=95)					
After 1 year	4.2 (1.2)	5.4 (1.2)	4.4 (1.2)	6.4 (1.2)	0.23
After 3 years	5.1 (1.3)	5.7 (1.2)	5.5 (1.2)	6.9 (1.2)	0.29
After 4 years	5.1 (1.2)	5.9 (1.1)	6.2 (1.1)	5.9 (1.2)	0.46

High-exposed area: <2 km from early contaminated coastline to residence, Low-exposed area: ≥2 km from early contaminated coastline to residence.

GM: geometric mean, GSE: geometric standard error. 8-OHdG: 8-hydroxydeoxyguanosine.

Adjusted for age (<50, 50–64, ≥65), gender (male, female), smoking (no, yes), and education levels (<6, 6–11, ≥12 years).

p-Trend estimated by a simple linear regression model using the ordinal scale of the total clean-up work duration (coded as 1,2,3,4).

With adjustment for a number of potential confounders including age (<50, 50–64, ≥65), gender (male, female), smoking (no, yes), and education levels (<6, 6–11, ≥12 years), the log-transformed 8-OHdG levels was not associated with the log-transformed 1-OHP levels significantly both 3 years and 4 years after the spills. Beta coefficients between the log-transformed 1-OHP levels and the log-transformed 8-OHdG levels, which are the estimates resulting from the multiple regression analysis, were decreased over time (baseline: 0.12, a follow-up: 0.06, second follow-up: -0.00) (Table 4).

Table 4. Relationship between levels of PAH metabolite and the levels of oxidative DNA damage 1 year, 3 years, and 5 years after the spill

Log 1-OHP (μg/g cr)	Log 8-OHdG (μg/g cr)	
	β (SE)	*p*-Value
Both high-exposed area and low-exposed area (n=671)		
After 1 year	0.09 (0.02)	0.0001
Only high-exposed area (n=95)		
After 1 year	0.12 (0.07)	0.11
After 3 years	0.06 (0.07)	0.45
After 5 years	-0.00 (0.05)	0.97

Low-exposed area: ≥2 km from early contaminated coastline to residence, High-exposed area: <2 km from early contaminated coastline to residence.
1-OHP: 1-hydroxypyrene, 8-OHdG: 8-hydroxydeoxyguanosine.
Adjusted for age (<50, 50–64, ≥65), gender (male, female), smoking (no, yes), and education levels (<6, 6–11, ≥12 years).

Discussion

In this two follow-up studies three years later and four years later among residents living high-exposed area, there was no recovery in 8-OHdG levels as biomarker of oxidative DNA damage. The 8-OHdG levels rather slightly increased over time with marginal significance in three years follow-up period. The geometric means of 8-OHdG over one year, three years, and four years after the spills were 5.7 μg/g cr, 6.1 μg/g cr, and 6.6 μg/g cr after adjusting for well-known risk factors, age on survey, gender, smoking and education levels, respectively. Cross-sectional relationships between the indicators of oil spill, including 1-OHP as the PAH metabolite or the total clean-up work duration, and the levels of 8-OHdG were not found 3 years after and 4 years after the Hebei Spirit oil spill. These no associations are probably that the limited study design, a relatively small and non-tracted non-exposed or low-exposed population.

In my preceding study (Noh et al. 2015), a significant positive exposure-response relationship between oxidative stress biomarkers and oil spill exposure after over one year was found. Levels of the oxidative stress biomarkers, including urinary malondialdehyde (MDA) as indicators lipid peroxidation and 8-OHdG as indicators of oxidative DNA damage, were significantly elevated by increasing number of days of clean-up activity and by increasing 1-OHP levels after adjustment for potential several confounders, such as age, gender, urinary cotinine levels, alcohol consumption, education levels, distance to road, industrial facilities within 2 km, and intake of fishes and shellfishes. The levels of oxidative stress biomarkers, the levels of PAH metabolites, and total duration of clean-up work were slowly decreased by more time elapsed from last clean-up exposure to health

examination (range: 0–20 months). The levels of oil exposure biomarker, 1-OHP, showed a positive relation to the levels of two oxidative stress biomarkers, urinary MDA and 8-OHdG levels. This positive relationship gradually disappeared over time from the last clean-up exposure to the health check-up.

It is not easy to hypothesis a straightforward explanation of these observations that the levels of biomarker of oxidative DNA damage over time. Presumably, numerous compounds of oil spill, including volatile organic compounds (VOCs) and PAHs, are known to cause adverse health consequences. These oil chemicals are classified as potent carcinogens and triggers of immune responses, which form covalent DNA adducts and cause oxidative DNA damage (Singleton et al. 2016; Wilk et al. 2013). One study showed that long-term exposure to low-level VOCs caused oxidative damage in lung and genotoxicity in liver in mice (Wang et al. 2013). This results support oxidative stress having a role in mechanisms of VOC genotoxicity. There were also several studies that environmental level of PAH exposure was associated with DNA damage in sperm (Han et al. 2011), DNA adducts in human cells (Tarantini et al. 2011), or induction of DNA strand breaks 13 years after the Exxon Valdez spill in mussel and clam (Thomas et al. 2007). Excessively produced reactive oxygen species (ROS) can injure various organs in human body such as, lung, liver, kidneys and brain, unless ROS are timely scavenged by endogenous antioxidant defense system. These oxidative stress results in the accumulation of dysfunctional proteins, lipid peroxidation products and damaged nuclear or mitochondrial DNA (Rahman 2003; Wang et al. 2013).

In addition, a possibility is open in the future that parental environmental exposure elicits life-long biological change through like altering methylation

(Buchen 2010). Exposure to antioxidant including VOCs and PAHs can influence the addition or removal of chemical tags on DNA that turn genes on and off (Hughes 2014). Recently, epigenetic transgenerational inheritance of disease and phenotypic variation of environmental toxins (Anway et al. 2005; Skinner et al. 2013) and behaviours (Dias and Ressler 2014) require either a chromosomal or epigenetic alteration in primordial germ cells and subsequent germ line have been shown. Likewise, high levels of chemical exposures in early active period and low levels of chronic exposure during several years from the large oil spills could affect epigenetic transgenerational effects on future children.

In this study, no longer those distinct relationships between the oil exposure and the oxidative stress remain three years and four years after the spill. There might be recovery mechanisms over time. Studies of the Prestige oil spill, the only report of the long-term health risks from the large scale oil spills so far, support the recovery mechanisms. Two years after the Prestige oil exposure, clean-up workers had chromosomal damage (Rodriguez-Trigo et al. 2010). There was no evidence of the persistence of genotoxic damage in individuals exposed to Prestige oil seven years later, when they carried out the follow-up of genotoxic effects seven years after the exposure to determine the possible persistence of genotoxic damage in individuals exposed to Prestige oil (Laffon et al. 2013a). Fishermen, who had participated in clean-up activities of the Prestige oil spill, showed higher levels of respiratory oxidative stress biomarkers two years later (Rodriguez-Trigo et al. 2010), but at the time of six years after the Prestige oil spill, no clear differences were found in levels of respiratory oxidative stress biomarkers and growth factors

(Zock et al. 2014). Given these results, there could be a partial recovery from temporary adverse health effects.

Another explanation is that there might be shown no associations between oil exposure and oxidative stress three years and four years, because this study group has similar characteristics related to oil exposure including residential area and exposed total duration from clean-up activity. All residents, who followed up three years and five years later, have lived <2 km from the oil contaminated coastline (mean: 0.6 km; SD: 0.3 km; range: 0.1–1.3 km) and approximately 90% participated clean-up activity during >50 days (Q1–Q3: 79–148 days), whereas over 50% of the residents of baseline research, which studied both low-exposed and high-exposed area, participated in clean-up work during <50 days.

The main strength of this unique study is the longitudinal design using identical methodology at baseline and follow-up two times that allowed a proper evaluation of levels of oxidative stress over time. Longitudinal studies and repeated surveillance on long-term health effects in cleanup workers of oil spills are important. Very few longitudinal studies have assessed long-term health effects of oil spills. Present study support the study results of longitudinal design of the Prestige oil spill. Another is that urinary 8-OHdG as a marker of oxidative DNA damage was adopted. It is considered to be one of the most promising markers for quantifying in vivo oxidative stress (Wu et al. 2004). Particularly, 8-OHdG is very stable and is excreted in the urine without being metabolized (Valavanidis et al. 2009). Therefore, the biomarkers used in the present study might sufficiently represent the oxidative stress due to the crude oil exposure.

Regardless of the strength, the main limitation of this study was that low-exposed group who were relatively apart from the clean-up work were not followed. In addition, a relatively small high-exposed group rather than baseline survey were followed. In addition, a health-related selection might influence the results of three years and four years later in terms of similar characteristics of the participants including the total duration of clean-up work and distance. These would make unforeseen changes in the levels of oxidative stress biomarker because of not a proper comparison. Besides, oxidative DNA damage can be influenced by genetic and other environmental factors like non-measured or unfamiliar modifying factors. Though limited, age, gender, smoking, and education levels, which are well-known risk factors, were considered in this study.

In conclusion, in this longitudinal study, the levels of oxidative stress biomarker were slightly increased after going over one year, three years, and four years after the Hebei Spirit oil spill. These persistent excess risk of oxidative stress bring about worries concerning current of future long-term health consequences, although cross-sectional relevance between the oil spills and oxidative stress was less apparent over time, which was suggestive of a potential reversibility of the adverse health effects. Continuing surveillance of the high-exposed group for early detection of possible health consequences and additionally including the low-exposed group of baseline for comparison of the health effects related to oil spills would is required in next step.

Ⅲ. LONG-TERM HEALTH EFFECTS OF THE HEBEI SPIRIT OIL SPILL IN CHILDREN

CHAPTER 3.

Asthma symptoms in children: A cross-sectional and longitudinal observational study 1 year, 3 years, and 5 years after the Hebei Spirit oil spill

Introduction

The Hebei Spirit oil spill episode is the one of the largest recent oil spill disasters in the world, second only to the Deepwater Horizon oil spill in the Gulf of Mexico in April 2010. On the morning of December 7, 2007, the Hebei Spirit crude oil carrier collided with a crane barge, off the west coast of Republic of Korea. After the collision, an estimated 12,547 kL of three types of crude oil–UAE Upper Zakum, Kuwait Export, and Iranian heavy–were spilled. Oil is mainly composed of hydrocarbons, and among those compounds are aromatic hydrocarbons, such as volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs). Aromatic hydrocarbons accounted for approximately 30% of the Hebei Spirit oil (Ha et al. 2012) and are relatively well-known for their human health risk (Goldstein et al. 2011).

The population at risk is persons working or living near the affected shoreline. Among those persons, children are more vulnerable to toxic chemicals in that they are still actively developing mature organs and systems, and they proportionally intake or breath more toxicants from food or air (Au 2002). The accident occurred

just 5 miles off the coast that contained densely populated fishing villages, and consequently the spilled crude oil was released into the nearby shore within 14 hours after the spill; the oil slick was 33 km long, 10 m wide, and 10 cm thick after two days (Sim et al. 2010). Moreover, all VOCs appeared to completely evaporate within a few days after the accident–approximately 10 hours for benzene, 48 hours for toluene, ethylbenzene and xylene; and 4 days for the other VOCs (Kim et al. 2012). Therefore, children who were living near the site of the spill were inevitably exposed to extremely high ambient levels of benzene, toluene, ethylbenzene, and xylene (BTEX) during the initial period and several carcinogenic compounds, such as PAHs, which can be much more persistent in various environmental media and can bioaccumulate during the long-term period (Lee et al. 2013).

According to previous epidemiological or toxicological studies related to large oil spills, these crude oil components affect the hematological profile and liver function (D'Andrea and Reddy 2013, 2014b), pose genotoxic and endocrine alterations (Ji et al. 2011; Laffon et al. 2006; Perez-Cadahia et al. 2008a), and lead to functional and biological respiratory health effects (Zock et al. 2007; Zock et al. 2012). These health effects may appear in children who were living in oil-exposed area, however, little evidence is present regarding health effects of oil spills in children compared to adults. Only two epidemiological studies have reported children's health from oil exposure so far (Crum 1993; Jung et al. 2013), and both these studies showed respiratory health effects of oil spills on children. Several oil spill studies in adults also indicate persistent, long-term respiratory health effects (Zock et al. 2012).

Exposure to VOCs (Rumchev et al. 2004) and PAHs (Gale et al. 2012) has been associated with asthma. Asthma has strong environmental influence, especially in early life (Platts-Mills et al. 2000; Toskala and Kennedy 2015). Childhood is the most important age for asthma development (Arruda et al. 2005; Bjerg and Ronmark 2008), and lifetime asthma prevalence generally decreases with age (Ryan-Ibarra et al. 2016). Meanwhile, there are very few longitudinal studies that have evaluated long-term health consequences of exposure to large oil spills (Zock et al. 2014). Therefore, this study was to investigate long-term health consequences in asthma symptoms of children during the 5 years after the Hebei Spirit oil spill. Asthma symptoms were measured a total of three times–1 year, 3 years, and 5 years after the spill–from 2009 to 2013. These symptoms were then interpreted in light of the location of the child's home and/or school, determined by distance from both the site of the spill and the affected shoreline, and the cumulative BTEX exposure of the child during initial 4 days of the oil spill.

Materials and Methods

Study population

The study area included four townships that were composed of 83 small villages. These townships were located closest to the site of the spill along the heavily contaminated coastline (Figure 1). The heavily contaminated coastline was defined as approximately 35 km of seashore, which was quickly contaminated with a thick layer of spilled oil beginning 4 days after the accident. The area was typical of rural villages and was relatively clean and lacked environmental pollution before the spill. At the time of the accident, school students accounted for 5.2% (1,020 people) of the total population of the study area (19,690 people) (Taean-gun 2007). There were 13 elementary and 4 middle schools. Among these schools, an elementary school adjacent the coastline was closed in 2010 because of a small number of students. Surveys were administered a total of three times biennially to elementary and middle school students. The surveys were conducted between June and November 2009 (first survey, 1 year after the spill), March to April 2011 (second survey, 3 years after the spill), and October to November 2013 (third survey, 5 years after the spill). A total of 828 students (participation rate, 87.7%) participated in the baseline survey, 760 students (90.4%) completed the second survey, and 783 students (95.6%) completed the third survey compared to the population census (Taean-gun 2009, 2011, 2013). Subjects for this study were limited to children (age 0−12 years at the time of the accident). Data from 655 students in the baseline survey, 664 students in the second survey, and 611 students in the third survey were used, after excluding students who transferred from other regions, were over

13 years old at the time of accident, were current smokers, and had missing information about asthma symptoms (Figure 2).

Each study was approved by the Institutional Review Board of Dankook University Hospital, Cheonan, Republic of Korea (DKUH IRB 2009-04-027, DKUH IRB 2012-02-062, DKUH IRB 2014-06-013). Each time, written informed consent of the participants was obtained from them or their parents with their assent.

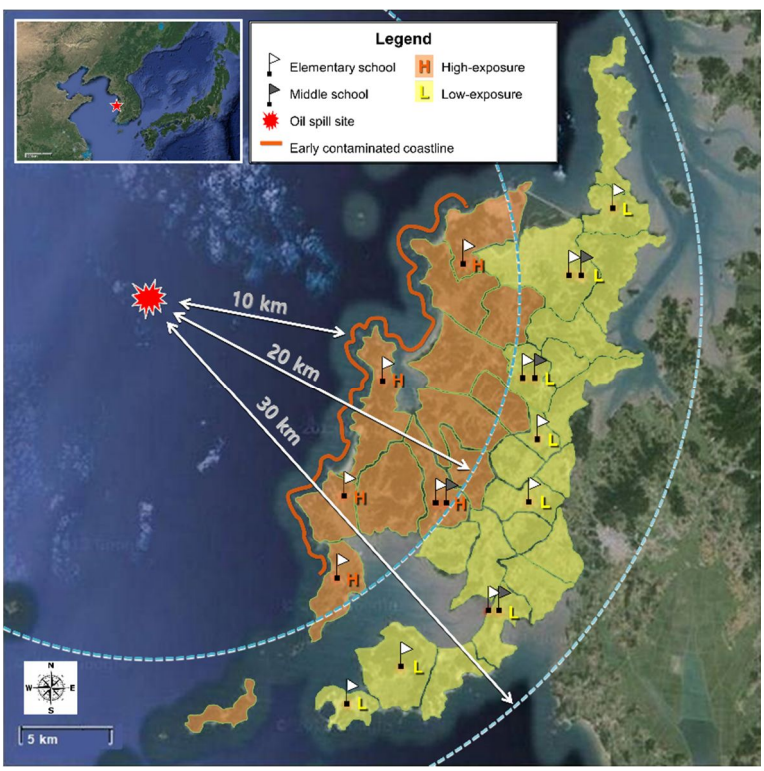


Figure 1. Geographic map of study area including school location and distance from oil spill. Area was divided into low- and high-exposure after considering distance from both the affected shoreline and the spill site.

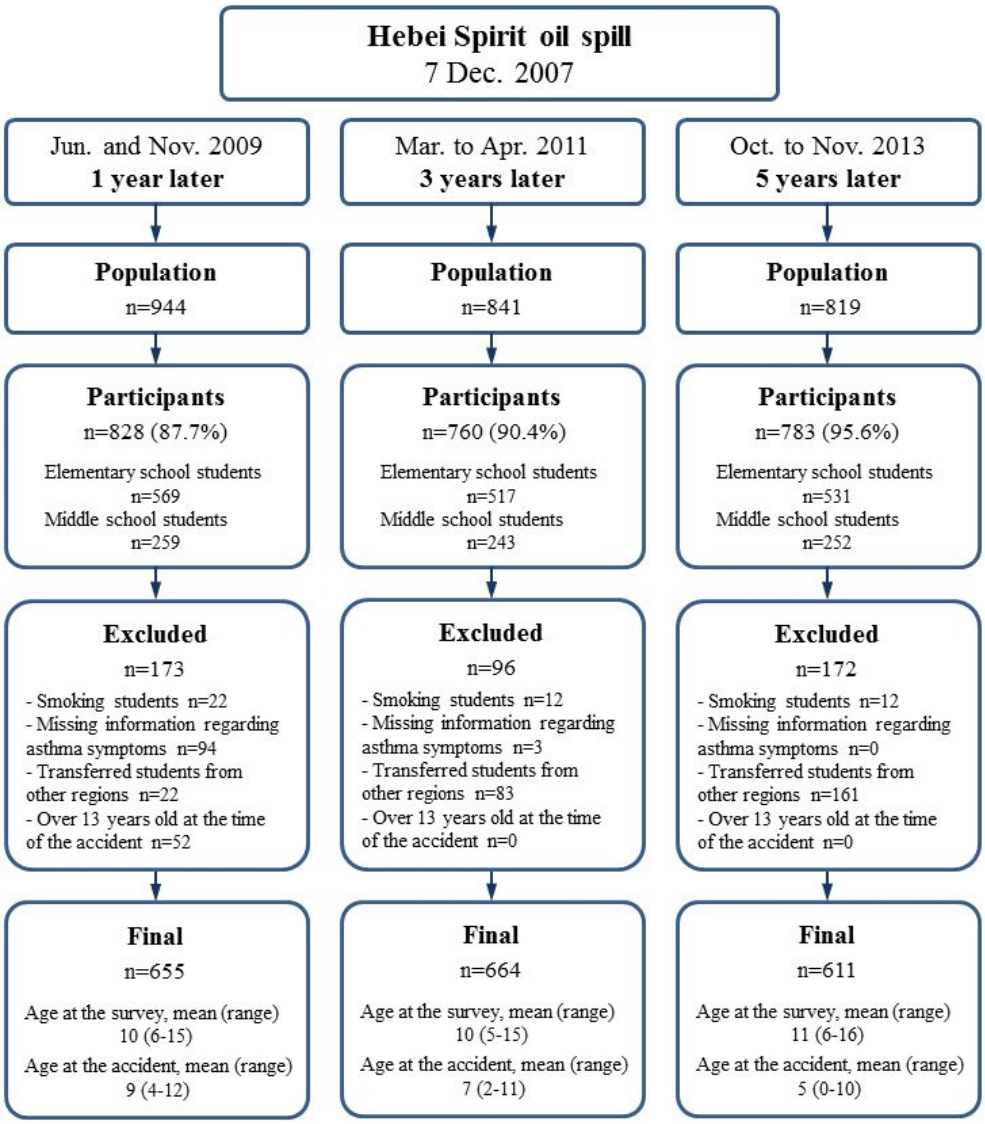


Figure 2. Flow diagram of student enrollment. Population was based on resident registration data.

Oil exposure assessment

Participation of clean-up work was excluded as an oil exposure indicator because less than 1% of the study subjects were involved in clean-up activity. Therefore, distance of their home and school from the spill site and ambient cumulative levels of BTEX were used as the oil exposure indicators in this study.

First, in case of the distance from the spill, the home and school address of each student was geocoded with the ArcGIS Desktop ver.9.3 geographic information system (Esri, Redlands, CA, USA), and distance from the heavily contaminated coastline or the oil spill point to the home or the school was calculated in each study subject. In preschool children at the time of the accident, only the home location was considered, and in school children at the time of the accident, location of both the home and the school were considered. The study area was dichotomously divided into high-exposed area and low-exposed area according to whether the home and/or the school was located within 2 km of the heavily contaminated coastline and 20 km from the oil spill point.

Second, in case of the ambient cumulative levels of BTEX, a modeling technique integrating a weathering algorithm and California Puff (CALPUFF) air dispersion model was applied to simulate the air dispersion of oil components, which has been previously described (Kim et al. 2012). The hourly concentration of the each VOC component, such as benzene, toluene, ethylbenzene, and xylene, was calculated for each small village (n=83). All VOC components completely evaporated within four days, so cumulative levels of BTEX were estimated from adding all hourly benzene, toluene, ethylbenzene, and xylene concentrations during initial four days after the spill. In preschool children at the time of the accident,

only the levels of the home area (24 hours per day) were considered, and in school children at the time of the accident, the levels of both the home (16 hours per day) and the school area (8 hours per day) were considered when calculating the cumulative BTEX levels of the each participant.

Prevalence of asthma symptoms

The modified International Study of Asthma and Allergies in Childhood questionnaire (Korean version) was used to evaluate characteristics related to asthma (Hong et al. 2003). A questionnaire was distributed in advance of the survey through the schools. It was completed by their parents in the elementary school students and self-administered in the middle school students, and was collected on the day of the survey. The questionnaires were inspected on the survey site and missing information was completed before a health check-up; still remaining missing information was accomplished during a telephone interview.

Current asthma symptoms were dichotomously determined according to the question, “Has your child had wheezing or whistling in the chest in the last 12 months?” in elementary school students and “Have you had wheezing and whistling in your chest in the last 12 months?” in middle school students.

The parental asthma history, which was considered one of the confounding factors, was defined as a reported history of physician-diagnosed asthma in one or more parents.

Statistical analyses

SAS version 9.4 was used for the statistical analysis (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were evaluated by the PROC FREQ and PROC MEANS procedureS. Cross-sectional associations between the oil exposure levels, such as the area and the cumulative BTEX levels, and the prevalence of current asthma symptoms were evaluated using logistic regression analysis (the PROC LOGISTIC procedure) after adjusting for age, gender, and parental history of asthma. The odds ratios (OR) and 95% confidence interval (95% CI) were automatically calculated by the PROC LOGISTIC procedure. Longitudinal associations were assessed by generalized estimating equation (GEE) models (the PROC GENMOD procedure), which can consider repeated measures within subjects, assuming an autoregressive working correlation structure.

Results

The number of participants was 655 in the first survey (1 year after the accident), 664 in the second survey (3 years after the accident), and 611 in the third survey (5 years after the accident). The mean age at the time of the accident was 9 years, 7 years, and 5 years in the first, second, and third survey, respectively. The mean age at the time of the first, second, and third survey was 10 years, 10 years, and 11 years, respectively, and approximately 30% were elementary students and 70% were middle school students. Approximately 5% of the children had parents with a history of asthma. Very few children (less than 1%) had participated in clean-up activity over a week (Table 1).

Table 1. Demographic and oil exposure characteristics of the study participants at the three surveys after the Hebei Spirit oil spill

	First survey	Second survey	Third survey
Year	2009	2011	2013
Participants (n)	655 (100.0)	664 (100.0)	611 (100.0)
Participation in clean-up work[ζ]	4 (0.6)	NS	2 (0.3)
Demographic aspect			
Age at the survey			
Mean (SD)	10.4 (2.4)	10.3 (2.6)	11.0 (2.6)
Elementary school students	463 (70.7)	445 (67.0)	409 (66.9)
Middle school students	192 (29.3)	219 (33.0)	202 (33.1)
Age at the time of the accident			
Mean (SD)	8.7 (2.3)	6.9 (2.5)	5.0 (2.6)
0–6 years	138 (21.1)	294 (44.3)	413 (67.6)
7–12 years	517 (78.9)	370 (55.7)	198 (32.4)
Girls	361 (55.1)	342 (51.5)	310 (50.7)
Parental asthma history	26 (4.0)	40 (6.0)	21 (3.4)
Exposure assessment			
Area[†]			
High-exposure	208 (31.8)	190 (28.6)	157 (25.7)
Low-exposure	447 (68.2)	474 (71.4)	454 (74.3)
BTEX exposure[‡]			
≥20 mg/m^3·4 d	348 (53.1)	346 (52.1)	302 (49.4)
<20 mg/m^3·4 d	307 (46.9)	318 (47.9)	309 (50.6)

Data are presented as n (%), unless otherwise indicated.

NS, not surveyed

[ζ]More than a week

[†]Area was divided into home and school located within 2 km from the early contaminated coastline and 20 km from the oil spill point among school children; only home location was considered among preschool children at the time of the accident.

[‡]Cumulative concentrations of benzene, toluene, ethylbenzene, and xylene until 4 days after the accident from the modeling technique (Kim et al. 2012)

A total of 1,123 students participated in the surveys including 655 students in the first, 664 students in the second, and 611 students in the third survey. Among these students, 391 students participated both the first and the second survey, and 195 students participated all three surveys (Table 2).

Table 2. Status of the participation at the three survey 1, 3, and 5 years after the Hebei Spirit oil spill accident (total n=1,123)

	n
One-time participation	
In 2009 (1 year later)	248
In 2011 (3 years later)	68
In 2013 (5 years later)	195
Two-times participation	
In 2009 and 2011	196
In 2011 and 2013	205
In 2009 and 2013	16
Three-times participation	
In 2009, 2011, and 2013	195

This study was proceeded by school-based surveys in the exposed area.

To analyze a cross-sectional relationship between oil exposure and asthma symptoms, Table 3 included subjects who participated in the first (n=655), second (n=664), and/or third survey (n=611). Prevalence rates of the asthma symptoms were significantly higher in children who resided in the high-exposure area compared to those in the low-exposure area 1 year and 3 years after the spill. There was no longer a statistically significant association 5 years after the spill. When stratified into two age groups (0–6 years and 7–12 years at the time of the accident), a difference in effect size assessed by OR between oil exposure and asthma symptoms was higher in the 0–6 age group compared to the 7–12 age group. In the case of 0–6 year olds (at the time of the spill), the prevalence rates of asthma symptoms were approximately three times higher in children lived in the high-exposure area than those who lived in the low-exposure area at the time of the spill: 1 year later (OR: 2.7, 95% CI: 1.0–7.0) and 3 years later (2.8, 1.3–5.9). When using BTEX levels as the oil exposure index, a similar trend was shown as using the area index. In addition, there were significant differences between the BTEX levels and the asthma symptoms 5 years after the spill (1.8, 1.1–2.8). When stratified by age, among the 0–6 age group, there was a significant association between asthma symptoms and BTEX levels 1 year (2.5, 1.0–6.4) and 5 years after the spill (1.7, 1.0–3.0).

Table 3. Cross-sectional associations between oil exposure and current asthma symptoms

	1 year later, 2009 (n=655)			3 year later, 2011 (n=664)			5 year later, 2013 (n=611)		
	Oil exposure		OR (95% CI)	Oil exposure		OR (95% CI)	Oil exposure		OR (95% CI)
Area[†]	High-exposure	Low-exposure		High-exposure	Low-exposure		High-exposure	Low-exposure	
All	14.4%	8.7%	1.9 (1.1–3.1)	13.7%	8.7%	1.9 (1.1–3.2)	14.0%	13.4%	1.2 (0.7–1.9)
Age 0 to 6[ζ]	25.7%	12.6%	2.7 (1.0–7.0)	21.7%	9.3%	2.8 (1.3–5.9)	16.7%	14.5%	1.3 (0.7–2.3)
Age 7 to 12[ζ]	12.1%	7.6%	1.7 (0.9–3.2)	9.1%	8.0%	1.2 (0.6–2.7)	9.8%	11.0%	0.9 (0.3–2.4)
BTEX levels[‡]	≥20 $mg/m^3 \cdot 4$ d	<20 $mg/m^3 \cdot 4$ d		≥20 $mg/m^3 \cdot 4$ d	<20 $mg/m^3 \cdot 4$ d		≥20 $mg/m^3 \cdot 4$ d	<20 $mg/m^3 \cdot 4$ d	
All	11.8%	9.1%	1.6 (0.9–2.7)	11.3%	8.8%	1.4 (0.8–2.3)	16.6%	10.7%	1.8 (1.1–2.8)
Age 0 to 6[ζ]	24.0%	14.2%	2.5 (1.0–6.4)	15.3%	9.6%	1.7 (0.8–3.5)	18.9%	11.7%	1.7 (1.0–3.0)
Age 7 to 12[ζ]	9.8%	8.2%	1.3 (0.7–2.4)	8.6%	8.1%	1.1 (0.5–2.3)	12.6%	8.1%	1.5 (0.6–4.1)

[ζ]At the time of the accident

[†]Area was divided in both home and school located within 2 km from the early contaminated coastline and 20 km from the oil spill point among school children; only home location was considered among preschool children at the time of the accident.

[‡]Ambient cumulative concentrations of benzene, toluene, ethylbenzene, and xylene until four days after the accident from modelling (Kim et al. 2012)

Asthma symptoms were determined according to the question, "Has your child had wheezing or whistling in the last 12 months?"

Values are presented by prevalence (%), odds ratio (OR), and 95% confidence interval (CI). ORs (95% CIs) were adjusted for age, gender, and parental asthma history.

To observe persistent asthma symptoms due to the oil spill (Table 4), children who participated in both the first and second survey (n=391) were assessed. Persistent rates of the asthma symptoms were significantly higher in children who resided in the high-exposure area than those in the low-exposure area among the 0−6 age group (16.7% vs 1.4%; OR (95% CI): 14.3 (1.6−130.7)).

Table 4. Persistence rate of the asthma symptoms during a 2-year follow-up from 2009 to 2011 (n=391)

Persistent asthma symptoms[§]	Follow-up (2009−2011)			
Area[†]	High-exposure	Low-exposure	OR (95% CI)	*p*-Value
All	4.4%	2.3%	2.1 (0.6−6.6)	0.23
Age 0 to 6[ζ]	16.7%	1.4%	14.3 (1.6−130.7)	0.02
Age 7 to 12[ζ]	1.0%	2.7%	0.5 (0.1−4.3)	0.50
BTEX levels[‡]	≥20 mg/m^3·4 d	<20 mg/m^3·4 d	OR (95% CI)	*p*-Value
All	4.0%	2.1%	2.3 (0.7−8.1)	0.19
Age 0 to 6[ζ]	11.9%	1.7%	8.3 (0.9−74.0)	0.06
Age 7 to 12[ζ]	1.9%	2.3%	1.0 (0.2−6.2)	0.96

[§]When having asthma symptoms both at baseline (2009) and at follow-up (2011)

[ζ] At the time of the accident

[†]Area was divided into home and school located within 2 km from the early contaminated coastline and 20 km from the oil spill point among school children; only home location was considered among preschool children at the time of the accident.

[‡]Cumulative concentrations of benzene, toluene, ethylbenzene, and xylene until 4 days after the accident from modelling (Kim et al. 2012)

Asthma symptoms were determined according to the question, "Has your child had wheezing or whistling in the last 12 months?"

Values are presented by prevalence (%), odds ratio (OR), and 95% confidence interval (CI). ORs (95% CIs) and *p*-values were adjusted for age, gender, and parental asthma history.

When a longitudinal relationship between oil exposure and asthma symptoms was assessed (Table 5), there were significant associations between the oil exposure surrogates, both the area and the BTEX levels, and the asthma symptoms. These associations were observed for up to five years after the oil spill when including all participants (n=1,123). However, there were no associations in the 7–12 age group when stratified into two age groups.

Table 5. Longitudinal associations between oil spill exposure levels and asthma symptoms up to 5 years after the Hebei Spirit oil spill (total n=1,123)

	Asthma symptoms	
	OR (95% CI)	*p*-Value
Area[†], high-exposed		
All	1.58 (1.15−2.16)	0.004
Age 0 to 6	1.86 (1.19−2.90)	0.006
Age 7 to 12	1.34 (0.87−2.08)	0.19
BTEX exposure[‡], ≥20 mg/m^3·4 d		
All	1.55 (1.13−2.13)	0.006
Age 0 to 6	1.81 (1.18−2.79)	0.007
Age 7 to 12	1.28 (0.81−2.02)	0.29

[ζ] At the time of the accident

[†]Area was divided into home and school located within 2 km from the early contaminated coastline and 20 km from the oil spill point among school children; only home location was considered among preschool children at the time of the accident.

[‡]Cumulative concentrations of benzene, toluene, ethylbenzene, and xylene until 4 days after the accident from modelling (Kim et al. 2012)

Generalized estimating equation (GEE) models assuming an autoregressive working correlation structure were used for this longitudinal analysis, and were adjusted for age (continuous), gender (male, female), parental asthma history (no, yes), and elapsed time (ordinal scale; coded as 1 (first survey), 2 (second survey), or 3 (third survey)).

To more precisely analyze the age effects on the relationship between oil exposure and asthma symptoms, data from participants from all three surveys (n=195) were stratified into 1-year age groups (Figure 3). The risk differences in the asthma symptoms between the low-exposed and the high-exposed were larger in the younger age group. Such age-dependent associations were apparent in the area index as one of the oil exposure surrogates rather than in the BTEX levels.

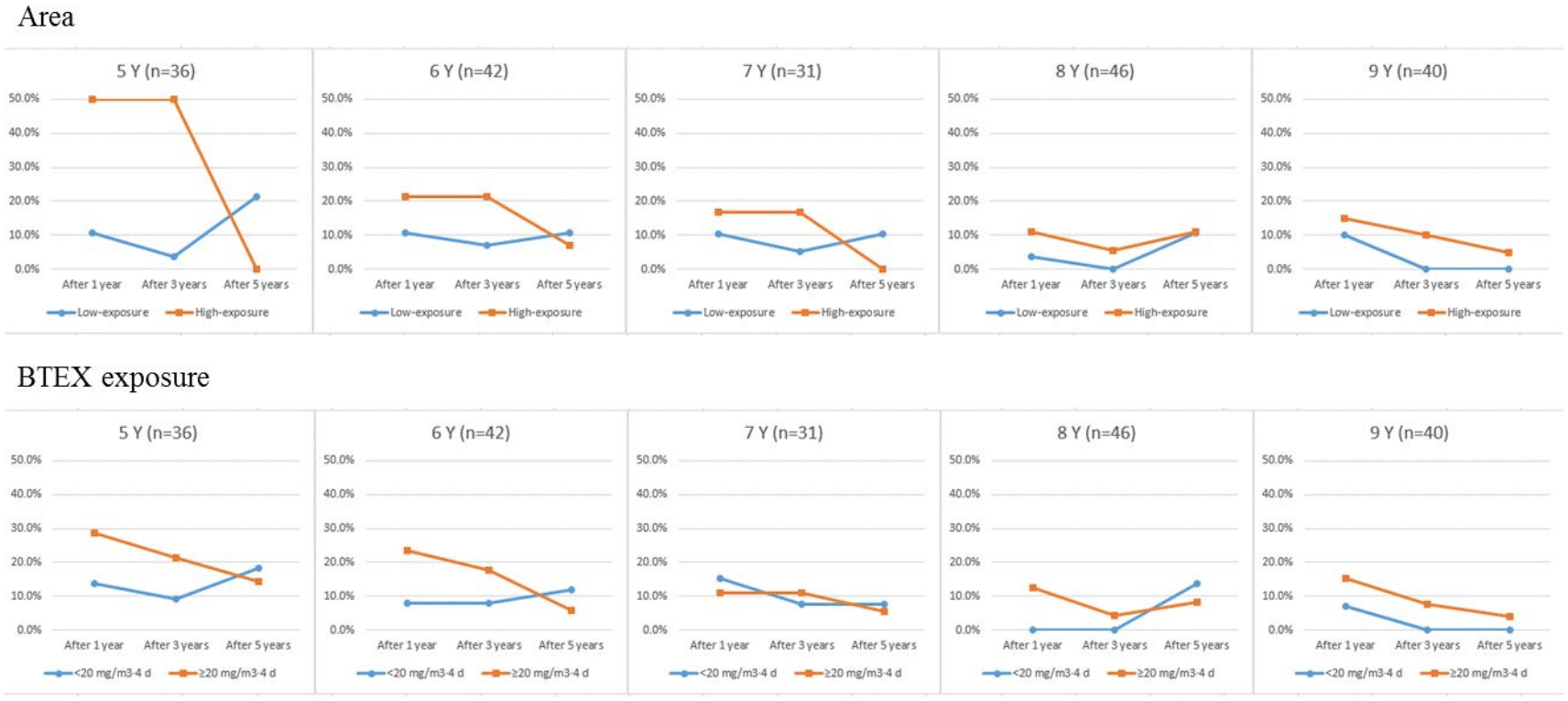


Figure 3. Age effects on the relationship between oil exposure and asthma symptoms.
Data was used from children who participated in all three surveys (n=195) and was stratified into 1-year age groups.

Discussion

In this biennial longitudinal study five years after the Hebei Spirit oil spill, oil exposure was significantly associated with asthma symptoms in children. These associations considerably persisted up to five years; however, the strength of the associations was slightly weakened over time. A younger age at the time of the accident had a greater influence on the association between asthma symptoms and oil exposure. My findings suggest that young children living in highly oil-exposed area even without participation in clean-up work of oil spills, which is a well-known direct oil exposure route (Zock et al. 2012), may result in persistent asthma symptoms.

As far as I know, this is the first longitudinal study on the health effects of a major oil spills in children. Indeed, in children, evidence is lacking regarding short-term and long-term health effects of oil spills (Goldstein et al. 2011). Even in adults, there have been very few longitudinal studies assessing long-term health effects of oil spills (Zock et al. 2014). Nine major oil spills have been evaluated through epidemiological studies on residents and/or clean-up workers since the late 1980s (Goldstein et al. 2011). However, until the late 2000s, those studies only assessed acute health effects from oil spills, which were typically performed immediately or less than a year after the accident; they were also based on relatively small study populations. A common finding was an increased prevalence of acute respiratory symptoms. Since 2007, the Prestige oil spill has suggested that clean-up workers may experience long-term respiratory effects (Rodriguez-Trigo et al. 2010; Zock et al. 2007), and may result in persistent respiratory symptoms (Zock et al. 2012). When respiratory symptoms in local fishermen were observed 1

year after having participated in clean-up work, lower respiratory tract symptoms were more prevalent in clean-up workers (Zock et al. 2007). The risk was increased with the duration of the clean-up work and the number of performed tasks, and decreased with elapsed time since the last exposure. When Zock et al. followed up on the respiratory symptoms of the same population 5 years after the Prestige oil spill, the clean-up workers still had an increased prevalence of respiratory symptoms compared with non-exposed controls (Zock et al. 2012). The risk of having persistent respiratory symptoms increased with the degree of exposure compared with those without any symptoms. In these Prestige results of long-term respiratory health, although adults were studied and followed, findings were in line with this study.

Notable observations of the present study is that children who were younger at the time of the accident suffered larger health effects, such as having a higher prevalence and a higher persistent rate of asthma symptoms than the children who were relatively older at the time of the accident. Given these observations, exposure during the early years of life is likely to play a crucial role regarding asthma etiology (Platts-Mills et al. 2000; Raedler and Schaub 2014). It is generally well-known that children might be more susceptible to adverse effects from oil exposure than adults, because there immune system and lungs are still developing during early childhood (Schwartz 2004). In addition, children detoxify environmental chemicals less efficiently and their bodies breathe more air per kilogram of body weight than adults. By extension, given the results of this study, the younger the child when oil spill occurred, the greater the chance asthma symptoms will appear and persist. Early environmental exposure, when the child is

in the process of growth and development, could be a critical time, which could cause future asthma symptoms of the child and lead to lasting effects (Michel et al. 2013).

In addition, these age effects might be explained by the characteristics of the disease in that asthma reverses spontaneously as child grows. Childhood asthma generally manifests during the first 5 years of life from environment-driven exacerbation (Thomas et al. 2008), and typically tends to remit with age (Bjerg and Ronmark 2008; Yao et al. 2011). In addition, chronic symptoms, such as higher rates of episodic wheezing and bronchial hyper-responsiveness, are detected more frequently in early life than in young adults (Martinez and Vercelli 2013). Likewise, minor impacts from the oil spill among the older children group (age 7−12 years) could be related to lower asthma prevalence and better resistance of the group than the younger children group (age 0−6 years). Results from one of the World Trade Center disaster studies support this hypothesis. Among children <5 years of age, an increase in the prevalence of asthma diagnosis was observed even among those not directly exposed to the dust cloud after 2 years (Thomas et al. 2008). When followed up after 6 years, respiratory symptoms were still associated with 9/11 exposure in younger children (Stellman et al. 2013). Likewise, childhood, particularly young childhood, seems to be a vulnerable time for environmental disaster to cause asthma symptoms.

The children in this study appear to have been directly and indirectly exposed to numerous oil toxicants. Participation in clean-up activities of the oil spill are known as a major risk factor inducing longer-term adverse health effects among adults (Rodriguez-Trigo et al. 2010), but nevertheless nearly all children (>99%)

had not worked in clean-up efforts after the spill, and even those who participated in the clean-up activity worked for 1 day or less than 1 week. One could cautiously speculate that children are affected by different sources, pathways, and routes of exposure than adults through intake of air, food, soil, and dust per unit body weight and surface area (Sexton et al. 2005). The predicted major routes of long-term exposure to chemicals from the oil spill, such as PAHs and alkylated PAHs, can persist in the environment, accumulate, and be toxic to humans (Goldstein et al. 2011; Solomon and Janssen 2010). These compounds are inhaled while residing at home or attending school near the affected shoreline as well as via dermal contact, ingestion of food and water, and contact with beach sand or oil sediment, especially by children. Another possible route of oil exposure is inhalation and partially dermal contact of ambient BTEX components, which are carcinogens in crude oil, at the initial time after the spill (<4 days). The oil spill accident occurred only 5 miles off the coast, and released oil onto the shore of the study area within 14 hours after the spill. All benzene (<10 hours) and other volatile components of crude oil components (<4 days) evaporated very quickly right after the spill, and the atmospheric BTEX concentrations were abnormally high in the study area, as determined by modelling (Kim et al. 2012). Atmospheric photochemical activity converts volatile hydrocarbons into reactive aldehydes, and leads to ozone formation. Both of these activities can synergistically enhance acute and chronic IgE-mediated inflammation including asthma attack (Eggleston 2007; Goldstein et al. 2011). In addition, BTEX (Arif and Shah 2007; Rumchev et al. 2004) and PAH (Miller et al. 2010) components of the crude oil are well known to increase the prevalence of asthma among adults and children.

Mechanisms of these prolonged asthma symptoms from the oil spill remain largely unknown. The main indications of this study are that the persistent rate of the asthma symptoms was more pronounced among those with asthma symptoms at the first survey during a 2-year follow-up, and that there were more prevalent asthma symptoms in younger children than older children. Possible mechanisms underlying these observations are increased bronchial responsiveness, induced airway sensitization, increased oxidative stress, and/or elevated growth factor activity suggestive of a lasting airway remodelling process related to inhalation of high levels of various irritants (Maestrelli et al. 2009; Zock et al. 2012). Also, the oil irritants could induce a disruption or injury of the bronchial epithelial structure that bring increased inflammatory reactions mediated by cytokines and leukotrienes, increased secretion of growth factors, and eventually lasting airway inflammation and bronchial hyper-responsiveness. Furthermore, all these processes described above could be involved in epigenetic mechanisms, such as DNA methylation, histone modification, and microRNA transcript silencing (DeVries and Vercelli 2016; Klingbeil et al. 2014). A recent RNA-seq study showed that crude oil treatment of human airway epithelial cells had strong effects on several genes that were differentially expressed compared to controls (Liu et al. 2016). Maternal exposure to PAHs altered DNA methylation (Herbstman et al. 2012; Tang et al. 2012), and DNA methylation of 5'-CpG islands was associated with asthma symptoms in young children (Perera et al. 2009). Likewise, exposure to oil components could promote cytosine methylation-mediated gene silencing that might cause persistent and long-lasting asthma symptoms in childhood (Hew et al. 2015; Ho 2010).

The main strength of this study is the longitudinal design including all school-aged students of the most affected area every 2 years over 5 years after the spill (a total of three surveys; after 1 year, 3 years, and 5 years). Another strength is that this study inspected the entire school-based population of the study area. Participation rate (%) was very high for all 3 surveys, in the first (88%), the second (90%), and the third (96%) based on population. By observing all of the students in the affected area, the potential for selection bias hardly exists, as it might be impossible that the children having new-onset respiratory symptoms of high-exposure area may be more likely to participate in this study compared to those of low-exposure area. In addition, the methodology, the asthma symptom measurements, and the interpretation of the responses were identical in all three surveys. Nevertheless, there are some limitations. No information on asthma symptoms was available before the oil spills. However, when compared to the low-exposure area, the high-exposure area is a more rural area, where asthma symptoms of children is often as low as in more urban areas. Another limitation is that there might be reporting bias by using parents as proxies for the children, or by changing the respondent of asthma symptoms from the child to the adult as the child graduated from elementary school to middle school during the follow-up period.

In conclusion, oil exposure was associated with aggravation and persistence of asthma symptoms from cross-sectional and longitudinal observations up to five years after the spill. In a cross-sectional design, an increase in the prevalence of asthma symptoms was found at 1 year, 3 years, and 5 years after the Hebei Spirit oil spill. Although slowly, the prevalence of asthma symptoms decreased over time, and excess risk of the asthma symptoms was less apparent. This finding may

suggest lasting and perhaps irreversible airway damage 5 years later; there may also be reversibility of the adverse effects over time, although it is slow. Children are susceptible subpopulations among oil-exposed residents. Continued, long-term medical monitoring of children is required to track persistence of these asthma symptoms. Guidelines of preventive and therapeutic interventions are essential. Lessons learned should guide future responses to oil spill disasters.

CHAPTER 4.

Exposure to volatile organic compounds and loss of lung function in children: 1 year, 3 years, and 5 years after the Hebei Spirit oil spill

Introduction

The crash of the oil tanker Hebei Spirit resulted in a spillage of 12,547 kL of crude oil only 5 miles off the west shore of the Republic of Korea on December 7, 2007. Volatile organic compounds (VOCs), which occupied a large portion of the spilled crude oil (Ha et al. 2012), were estimated to completely evaporate within four days of the accident (Kim et al. 2012). Among the VOCs, benzene was completely evaporated within 10 hours, and toluene, ethylbenzene, and xylene were completely evaporated within 48 hours. The other VOCs were totally evaporated within 4 days after the spill. In addition, VOCs rapidly moved toward land with a southeast wind until December 10, 2007, and brought abnormally high concentrations of VOCs into several villages during the initial period. The concentrations of VOCs were 100-fold to 10,000-fold higher than non-exposed surrounding villages (Kim et al. 2012).

Among all crude oil compounds, VOCs are particularly toxic and raise a potential human health risk (Perez-Cadahia et al. 2007). One of the VOCs, benzene, has been classified by the International Agency for Research on Cancer (IARC) as

a human carcinogen (group 1). Toluene and ethylbenzene have been classified by the IARC as possible human carcinogens (group 2B). These VOCs have been shown to cause respiratory, hepatic, renal, neurologic, hematologic, reproductive, and developmental toxicity (Bahadar et al. 2014; Donald et al. 1991; Lan et al. 2004; Rajan and Malathi 2014). Among these health risks, there are some reports that even low levels of VOCs can lead to loss of lung function in the general population (Elliott et al. 2006; Yoon et al. 2010).

With regard to people who were exposed to crude oil components from the Hebei Spirit oil spill, some studies reported not only acute, but also longer-term respiratory health risks. Within one month after the spill, volunteers of the clean-up effort presented physical respiratory symptoms, such as bronchial irritation as an acute phase response (Ha et al. 2012). These respiratory symptoms increased according to duration of clean-up participation (Cheong et al. 2011) and had persisted until one year after the spill (Na et al. 2012). I also observed that children who lived close to the oil spill area had a significant loss of lung function 1 year after the spill compared to those who lived far from the oil spill area (Jung et al. 2013). In addition, the Prestige oil spill of Spain in 2002 reported respiratory health consequences after 1 year (Zock et al. 2007) and after 2 years (Rodriguez-Trigo et al. 2010), and these risks persisted 5 years after the spill (Zock et al. 2012).

Still, while there are many concerns about human health effects of a large oil spill disaster, there have been very few studies assessing the long-term health consequences (Goldstein et al. 2011). In addition, study participants of nearly all published research related to major oil spills included residents, clean-up workers, or both, but did not include children. Children are particularly vulnerable to health

risks from exposure to excessive VOCs compared to adults because they breathe in more air per unit of body mass, and their bodies detoxify many chemicals less efficiently. Therefore, I investigated long-term health consequences of exposure to VOCs in lung function in children, as assessed by a cross-sectional and longitudinal relationship between each subject's estimated exposure to VOCs during the initial period (≤4 d) and repeated measures of their lung function at 1 year, 3 years, and 5 years after the Hebei Spirit oil spill.

Materials and Methods

Study population

The study area was 83 small villages of 4 townships located closest to the oil spill (Figure 1). The study area had a small population of children, which is a typical feature of rural villages, and that number declined in the aftermath of the oil spill. I administered school-based health surveys in all elementary and middle school students of the study area 1 year (Jun–Nov 2009), 3 years (Mar–Apr 2011), and 5 years (Oct–Nov 2013) after the spill. Participation rates of the baseline survey (after 1 year), the first follow-up survey (after 3 years), and the second follow-up survey (after 5 years) were 87.7% (n=828), 90.4% (n=760), and 95.6% (n=783), respectively, compared to the population census. My study subjects were 224 children exposed to oil-induced VOCs at the initial period of the spill ($\leq$4 d) and who reported their lung function in all of the surveys for a total of three times, after 1 year, 3 years, and 5 years.

Each study was approved by the Institutional Review Board of Dankook University Hospital, Cheonan, Republic of Korea (DKUH IRB 2009-04-027, DKUH IRB 2012-02-062, DKUH IRB 2014-06-013). Each time, written informed consent of the participants was obtained from them or their parents with their assent.

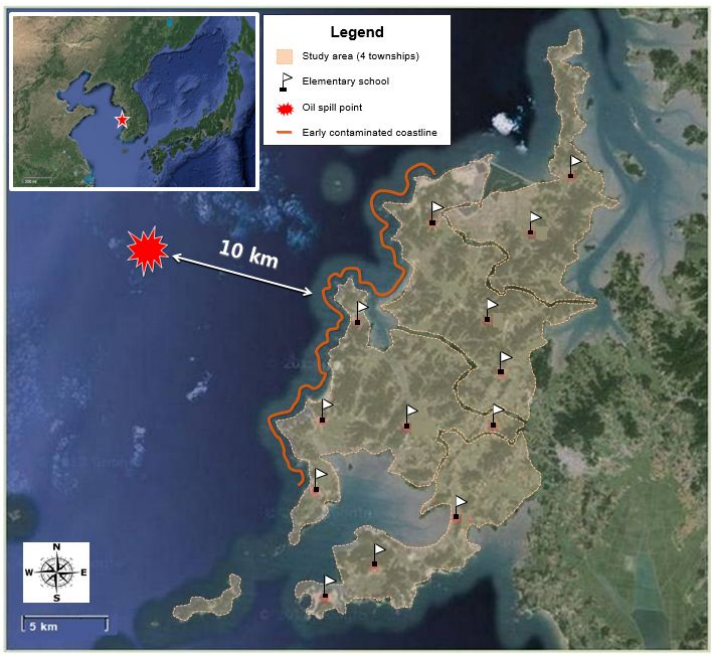


Figure 1. Map showing the geographic location of the study area associated with the Hebei Spirit oil spill

Estimation of exposure to VOCs

Exposure to oil-induced VOCs of the study subjects was estimated with a modeling technique integrating the weathering model and the California Puff (CALPUFF) air dispersion model, and detailed methods were described in my colleague's previous study (Kim et al. 2012). From these modeling processes, ambient 1-hour concentrations ($\mu g/m^3$) of each village (total villages of the study area, n=83) were calculated from the time of the accident. After that, all hourly concentrations in the village from December 7 to December 10, 2007 ($mg/m^3 \cdot 4$ d) were added to predict the total exposure levels of VOCs. Consequently, the cumulative ambient concentrations of each home and school location of each study subject were calculated. For preschool children at the time of the accident, only the concentrations of VOCs at home were considered. For school, both the concentrations at home and school were considered, assuming that they spent 16 hours a day at home and 8 hours a day at school.

Benzene, toluene, ethylbenzene, xylene, and total VOCs (TVOCs) were used as oil-induced components among the group of VOCs. TVOCs included benzene, toluene, ethylbenzene, xylene, paraffin (C6–C12), cycloparaffin (C6–C12), and aromatic mono- and dicyclic components (C6–C11), as described in my colleague's previous study (Kim et al. 2012).

Measurement of lung function

Spirometric measurements were performed according to American Thoracic Society (ATS) guideline (Miller et al. 2005). A Microspiro HI-298 (Chest Corporation, Tokyo, Japan) was used at the baseline survey and a Spirovit SP-1

(Schiller AG, Baar, Switzerland) was used at the first and second follow-up surveys to measure forced expiratory volume in one second (FEV_1). I calculated each participant's percent of predicted FEV_1 on the basis of their age, height, and sex using the global lung function 2012 equations, which are endorsed by the ATS and the European Respiratory Society (ERS) (Quanjer et al. 2012).

Other measures

Information concerning clean-up activity participation and current smoking of the study subject was obtained from the survey.

Statistical analyses

Statistical analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). General characteristics were analyzed using the PROC MEANS and PROC FREQ procedures. A cross-sectional relationship between exposure to VOCs and loss of lung function was analyzed by multiple linear regression models (the PROC GLM procedure). A longitudinal relationship between exposure to VOCs and loss of lung function was analyzed by a mixed linear regression model (PROC MIXED procedure). An unstructured covariance matrix, which allows unequal variances over time and unequal covariances for each time combination, was assumed based on the smallest AIC value. The concentrations of VOCs were used as both a continuous variable and a dichotomous variable divided by the median value. All analyses were adjusted for current smoking.

Results

Study subjects (n=224) were followed for a mean period of 4.4 years from the baseline survey to the second follow-up survey (Table 1). The age range of the 224 children was from 4 to 9 years of age on the day of the accident, from 6 to 11 years of age for the baseline survey, from 8 to 13 years of age for the first follow-up survey, and from 10 to 15 years for the second follow-up survey. Among the 224 children, one subject (0.4%) participated in clean-up activity over one week in the aftermath of the accident. About half of the children (54.5%) lived within 2 km of the heavily crude oil-contaminated coastline at the time of the accident. Girls comprised 54.0% of study subjects. A few children were current smokers: 0.9% of the study subjects on the baseline survey, 0.5% on the first follow-up survey, and 3.6% on the second follow-up survey.

Table 1. Characteristics of the participants including lung function

Characteristic	Total (n=224)
Girls	121 (54.0%)
Clean-up work†	1 (0.4%)
<2 km area‡	122 (54.5%)
Age (years)	
At accident, 2007	7.1 (range 4−9)
Baseline survey, 2009	8.7 (range 6−11)
First follow-up, 2011	10.5 (range 8−13)
Second follow-up, 2013	13.0 (range 10−15)
Current smokers	
Baseline survey, 2009	2 (0.9%)
First follow-up, 2011	1 (0.5%)
Second follow-up, 2013	8 (3.6%)
Height (cm)	
Baseline survey, 2009	134.3 ± 9.4
First follow-up, 2011	145.2 ± 9.9
Second follow-up, 2013	159.1 ± 8.2
Weight (kg)	
Baseline survey, 2009	32.4 ± 9.7
First follow-up, 2011	40.3 ± 12.4
Second follow-up, 2013	53.7 ± 14.3
FEV_1 (L)	
Baseline survey, 2009	1.69 ± 0.36
First follow-up, 2011	2.01 ± 0.50
Second follow-up, 2013	2.65 ± 0.62
FEV_1 (% predicted)	
Baseline survey, 2009	96.5 ± 10.6
First follow-up, 2011	92.0 ± 14.5
Second follow-up, 2013	90.6 ± 12.9

†>7 days

‡Distance from the early contaminated coastline to their residence

The height, weight, and FEV_1 gradually increased during the follow-up period (Table 1). The mean FEV_1 on the baseline survey, the first-follow up survey, and the second-follow up survey was 1.7 L, 2.0 L, 2.7 L, respectively. However, the percent of the predicted FEV_1 was significantly reduced over time (Table 1 and Figure 2). The mean the percent of the predicted FEV_1 on the baseline survey, the first-follow up survey, and the second-follow up survey, after adjusting for smoking, was 100.7%, 96.3%, 94.6%, respectively (p=0.0001) (Figure 2).

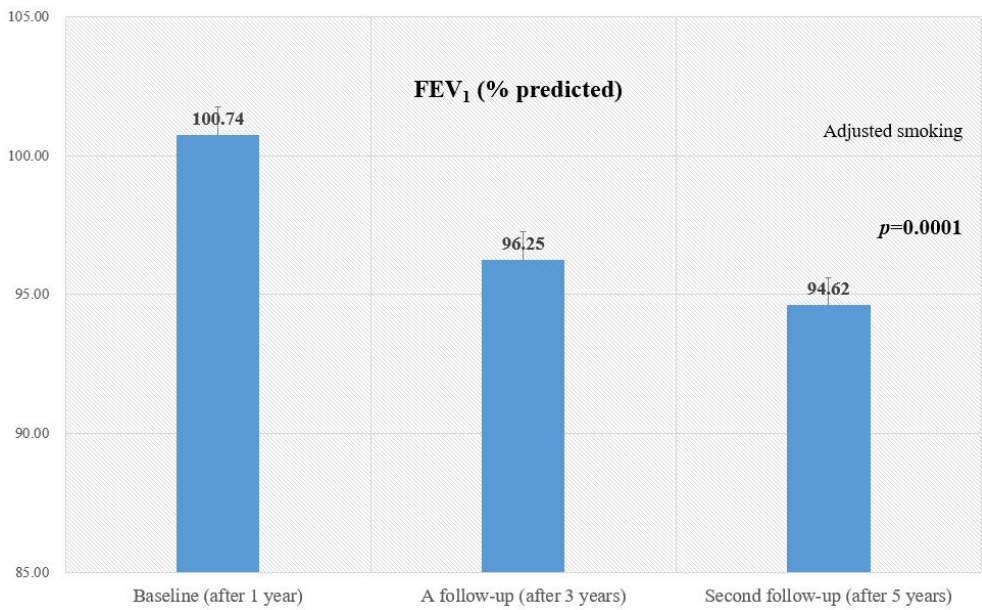


Figure 2. Lung function in children over time (n=224)

We used linear mixed models after adjusting for smoking (no, yes) and visit time (baseline, first follow-up, second follow-up) with an unstructured covariance matrix.

The mean of the cumulative concentrations of the atmospheric benzene, toluene, ethylbenzene, xylene, and TVOCs for four days after the oil spill was 2.3 $mg/m^3 \cdot 4$ d, 10.1 $mg/m^3 \cdot 4$ d, 6.2 $mg/m^3 \cdot 4$ d, 11.2 $mg/m^3 \cdot 4$ d, and 1,561.6 $mg/m^3 \cdot 4$ d, respectively (Table 2). Consequently, total ambient VOC exposure of the 224 children in order of volume was xylene, toluene, ethylbenzene, and benzene.

Table 2. Cumulative concentration of atmospheric benzene, toluene, ethylbenzene, xylene, and total volatile organic compounds (TVOCs) for 4 days after the oil spill

Cumulative concentration ($mg/m^3 \cdot 4$ d)[†]	Median	Mean ± SD	Min − Max
Benzene	1.8	2.3 ± 1.4	0.9 – 9.5
Toluene	6.9	10.1 ± 6.1	3.0 – 23.0
Ethylbenzene	4.6	6.2 ± 3.7	1.8 – 13.6
Xylene	8.2	11.2 ± 6.7	3.3 – 24.8
TVOCs[‡]	1157.0	1,561.6 ± 933.1	466.7 – 3,446.3

[†]We used cumulative concentrations for the four days after the oil spill.
[‡]TVOCs: total volatile organic compounds including benzene, toluene, ethylbenzene, xylene, paraffin (C6−C12), cycloparaffin (C6−C12), and aromatic mono- and dicyclic components (C6−C11)

In a cross-sectional design to assess associations between exposure to VOCs and loss of lung function in children (Table 3 and Figure 3), the cumulative concentrations of toluene, ethylbenzene, xylene, and TVOCs were significantly associated with the percent of the predicted FEV_1 after 1 year and after 3 years both in crude models (Figure 3) and in smoking-adjusted models (Table 3). The cumulative concentrations of ethylbenzene, xylene, and TVOCs were marginally associated with the percent of the predicted FEV_1 after 5 years in the smoking-adjusted models.

Table 3. Cross-sectional associations between exposure to VOCs and loss of lung function 1, 3, and 5 years after the Hebei Spirit oil spill (n=224)

	FEV_1 (% predicted)					
	Baseline (after 1 year)		First follow-up (after 3 years)		Second follow-up (after 5 years)	
Cumulative concentration ($mg/m^3 \cdot 4$ d)[†]	β	*p*-Value	β	*p*-Value	β	*p*-Value
Benzene	-0.670	0.18	-0.699	0.31	-0.435	0.48
Toluene	-0.353	0.003	-0.481	0.003	-0.209	0.14
Ethylbenzene	-0.656	0.0005	-0.884	0.0006	-0.397	0.09
Xylene	-0.353	0.0007	-0.477	0.0009	-0.213	0.10
TVOCs[‡]	-0.003	0.0007	-0.004	0.0007	-0.002	0.10

Estimates and *P* values were from generalized linear regression analysis adjusted for current smoking.

[†]We used cumulative concentrations for the four days after the oil spill.

[‡]TVOCs: total volatile organic compounds including benzene, toluene, ethylbenzene, xylene, paraffin (C6−C12), cycloparaffin (C6−C12), and aromatic mono- and dicyclic components (C6−C11)

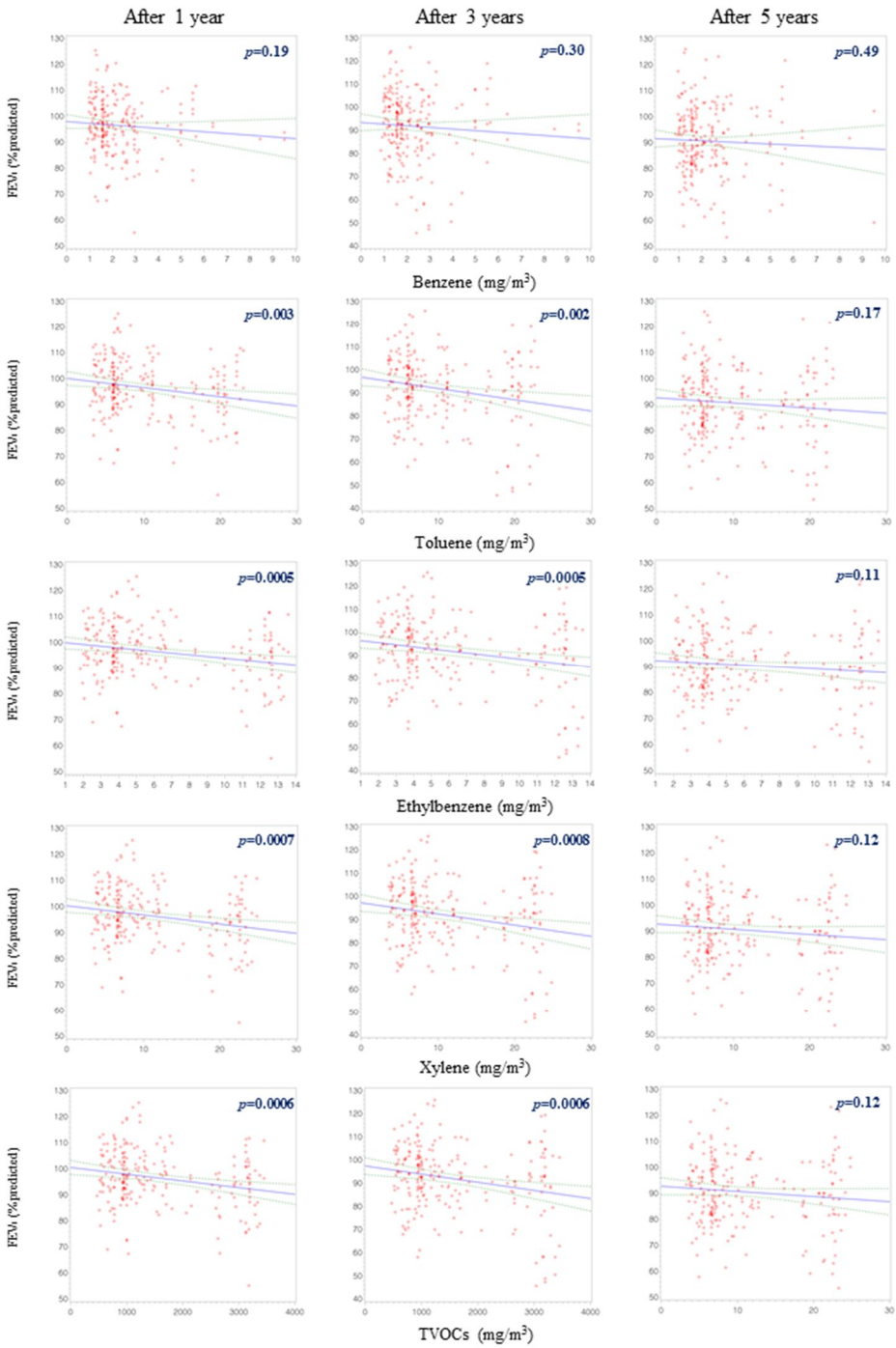


Figure 3. Cross-sectional relationship between cumulative ambient concentrations of VOCs of each child and loss of lung function 1 year, 3 years, and 5 years after the Hebei Spirit oil spill

P values were from a simple linear regression analysis.

Growth of lung function of study subjects during the first follow-up period, measured by an increase in FEV1 between the surveys, had a marginally significant negative association with the exposure to ethylbenzene, xylene, and total VOCs after adjusting for age, sex, height, FEV_1, and smoking at baseline (Table 4).

Table 4. Effects of the VOC exposure levels on the differences in lung function between surveys of each participant

	Differences in FEV_1 (L)			
	First follow-up - baseline		Second follow-up - baseline	
VOC variable[†] ($mg/m^3 \cdot 4$ d)	Coefficient	*P* value	Coefficient	*P* value
Benzene	0.003	0.83	0.002	0.85
Toluene	-0.005	0.12	0.001	0.80
Ethylbenzene	-0.010	0.06	0.039	0.84
Xylene	-0.005	0.07	0.023	0.83
TVOCs[‡]	-0.000	0.06	0.000	0.83

P values are from generalized linear regression analysis adjusted for age, sex, height, FEV_1 (L), and current smoking.

[†]We used cumulative concentrations of each VOC variable for four days right after the oil spills

[‡]Including benzene, toluene, ethylbenzene, xylene, paraffin (C6−C12), cycloparaffin (C6−C12), and aromatic mono- and dicyclic components (C6−C11)

In a longitudinal design to assess associations between exposure to VOCs and loss of lung function up to 5 years after the accident (Table 5 and Figure 4), the cumulative concentrations of toluene, ethylbenzene, xylene, and TVOCs, in the form of continuous variables, were significantly associated with the percent of the predicted FEV_1 after adjusting for smoking (Table 5). Exposure to benzene, toluene, xylene, and TVOCs, which was dichotomized by median value, was significantly associated with the percent of the predicted FEV_1 (Figure 4).

Table 5. Longitudinal associations between exposure to VOCs and loss of lung function up to 5 years after the Hebei Spirit oil spill (n=224)

	FEV_1 (% predicted)	
Cumulative concentration ($mg/m^3 \cdot 4$ d)[†]	β	*p*-Value
Benzene	-0.612	0.18
Toluene	-0.339	0.002
Ethylbenzene	-0.633	0.0003
Xylene	-0.340	0.0004
TVOCs[‡]	-0.002	0.0003

We used linear mixed models after adjusting for smoking (no, yes) and time (baseline, first follow-up, second follow-up) with an unstructured covariance matrix.

[†]We used cumulative concentrations for the four days after the oil spill.

[‡]TVOCs: total volatile organic compounds including benzene, toluene, ethylbenzene, xylene, paraffin (C6−C12), cycloparaffin (C6−C12), and aromatic mono- and dicyclic components (C6−C11)

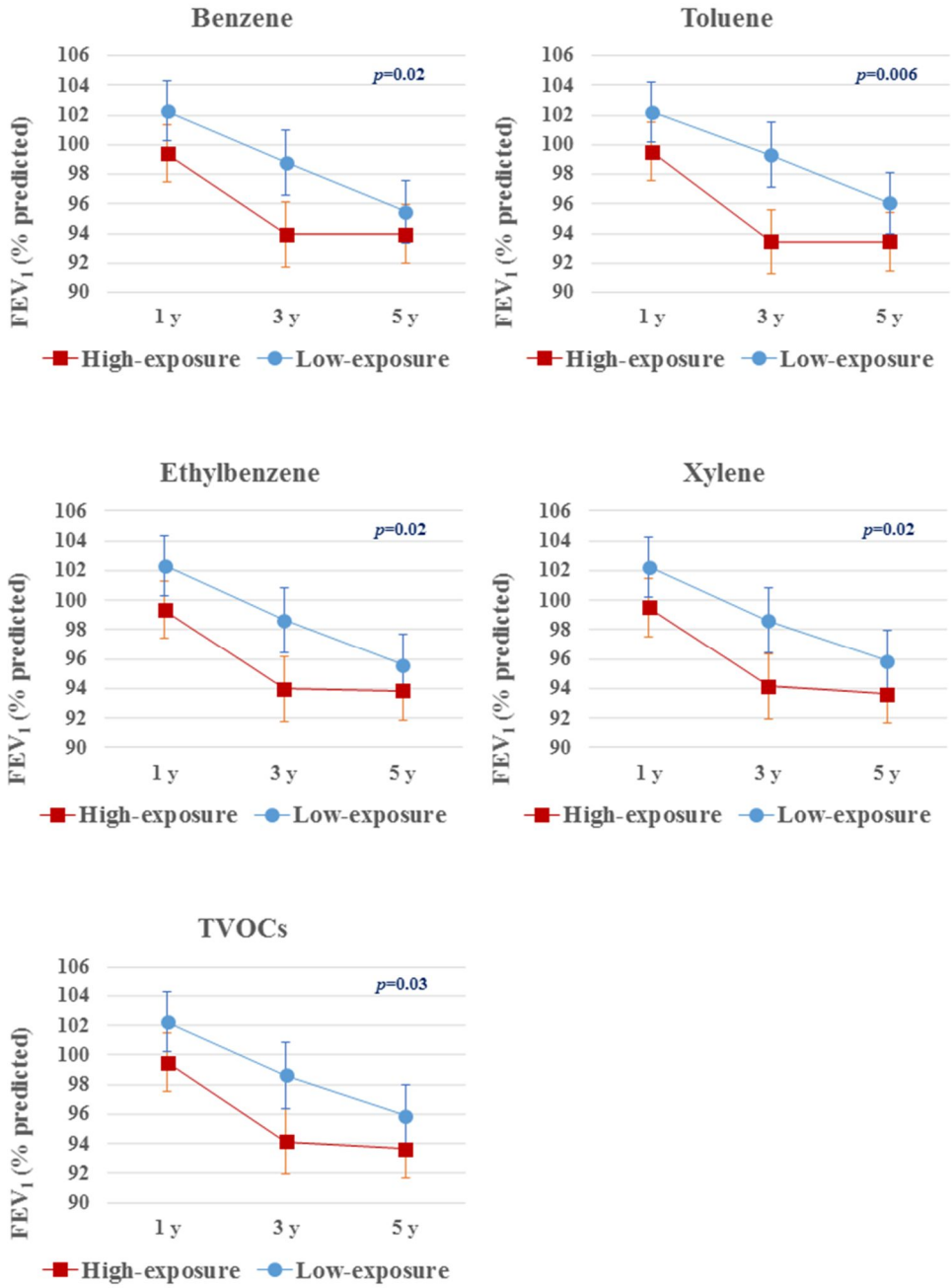


Figure 4. Longitudinal relationship between dichotomized cumulative ambient exposure to VOCs and loss of lung function from 1 year to 5 years after the Hebei Spirit oil spill

We used linear mixed models after adjusting for smoking (no, yes), visit time (baseline, first follow-up, second follow-up), and an interaction term between VOCs and visit time with an unstructured covariance matrix.

Discussion

The exposure to oil-induced VOCs caused loss of lung function in children up to 5 years after the spill. The lung function of the children got worse without recovery during the 4-year follow-up period. Cross-sectional associations between the exposure to VOCs, such as toluene, ethylbenzene, xylene, and TVOCs, and the loss of lung function were significant at 1 year and at 3 years after the spill and were marginally significant at 5 years after the spill. Longitudinal associations between the exposure to VOCs, such as benzene, toluene, ethylbenzene, xylene, and TVOCs, and the loss of lung function were statistically significant in the children.

As far as I am aware, this is the first study to observe long-term lung health consequences of exposure to oil-induced VOCs in children. There have been few studies on long-term health consequences of oil spills, despite growing concern after the 2010 Deepwater Horizon oil spill in the Gulf of Mexico in the USA (Goldstein et al. 2011). Even among the studies that have been performed, there have been very few longitudinal studies (Zock et al. 2014). Furthermore, hardly any of the studies assessed the health effects of the oil spill in children (Goldstein et al. 2011). Children may suffer a greater health risk than adults assuming an identical amount of exposure to VOCs because they breathe in more air per unit of body mass, and their bodies detoxify VOCs less efficiently. My study results came from the total of three time points of repeated surveillance to assess long-term lung health in children, despite the difficulty of enrollment and tracking of a cohort for an environmental epidemiology study (Reardon 2011).

VOCs, including benzene, toluene, and xylene, are the main components of crude oil and have potential long-term health risks (Solomon and Janssen 2010). VOCs evaporate within hours after the oil reaches the surface (Solomon and Janssen 2010) and therefore are emitted only from a narrow area (de Gouw et al. 2011). It is estimated that all VOCs from the Hebei Spirit oil spill polluted several villages within a few days (Kim et al. 2012). In the case of the Deepwater Horizon oil spill, all VOCs appeared to evaporate before reaching the shore (Goldstein et al. 2011). This difference is due to the proximity of the residential areas from the accident point. The location of the Hebei Spirit oil spill was just 5 miles off the coast, while the location of the Deepwater Horizon oil spill was 50 miles off the coast (D'Andrea and Reddy 2014a). Consequently, it was estimated that many children were exposed to a large volume of VOCs within a few days of the Hebei Spirit oil spill (Kim et al. 2012).

Exposed total volume and a toxicity component appeared important to reduce lung function. The present study shows that benzene among the other VOCs, such as toluene, ethylbenzene, xylene, had relatively less of an effect on the loss of lung function in children. This is probably due to the total exposed amount of benzene. Children were exposed to the least amount of benzene at home and at school during the initial oil-spill period compared to the other VOCs, because benzene completely evaporated within 10 hours, while the oil and volatiles did not reach the shore of the residential area until 14 hours after the spill. Consequently, the total amount of benzene exposure in children (mean: 2.3 $mg/m^3 \cdot 4$ d) was one-fifth that of xylene (11.2 $mg/m^3 \cdot 4$ d).

The time since exposure to VOCs also seems to be important in the association with the loss of lung function. From the cross-sectional analysis, the associations between the exposure to VOCs and the loss of lung functions were statistically significant at 1 year and at 3 years after the spill, but were only marginally statistically significant at 5 years after the spill. Also, the number of the VOC variables, which had an association with the loss of lung function, was reduced at 5 years after the spill. Nevertheless, lung function was not recovered even 5 years after the spill in children. The percent of the predicted FEV_1 after adjusting for smoking after 1 year, 3 years, and 5 years in children was 100.7%, 96.3%, 94.6%, respectively (p=0.0001). Given the observed trends, there could be partial recovery from reversible effects of the VOCs on lung function, though the recovery over time occurred slowly.

There were no significant interactions between the exposure to VOCs and the loss of lung function over time. I was curious whether loss of lung function in the higher VOC exposed group had a greater decrease than that in the lower VOC exposed groups. This tendancy was observed between the baseline survey (after 1 year) and the first follow-up survey (after 3 years) with a marginal significance or no significance in some components of the VOCs (results not presented). In addition, the initial exposure to VOCs disrupted growth of lung function between 1 year and 3 years after the spill after adjusting for covariates (marginal significances in ethylbenzene, xylene, and TVOCs; see Table 4).

Overall, exposure to completely evaporated VOCs in the initial period of the oil spill seems to be an important risk factor in the lung health effects in children. Participation of clean-up work due to oil spills is a well-known major risk factor in

adults. However, the children in this survey hardly participated in clean-up work (0.4%), whereas most adults participated in clean-up work for a prolonged period among residents living in heavily oil-exposed areas (Noh et al. 2015). There are various exposure routes, such as direct participation in clean-up activity and indirect exposure from multiple media (i.e., air, water, and soil) via multiple routes (i.e., inhalation, ingestion, and dermal) resulting from living close to the oil spill site. Among these exposure routes, exposure to VOCs thus seems to appropriate in loss of lung function in children. Several studies that report a relationship between VOC exposure and a decline in lung function support my results. In a longitudinal panel study of 154 elderly people, metabolites of toluene and xylene were significantly associated with a reduction of FEV_1 (Yoon et al. 2010). Also, in the U.S. population, blood concentrations of 1,4-dichlorobenzene were significantly associated with reduced FEV_1 (Elliott et al. 2006).

A better understanding of the lung health consequences of the Hebei Spirit oil spill among children could be achieved when bringing together studies from previous disasters. My colleague's previous study, which found lower lung function in children who lived <2 km of the oil-contaminated coastline compared to those who lived ≥2 km of the oil-contaminated coastline 1 year after the Hebei Spirit oil spill, were in line with the present study. In adults, persistent respiratory health effects were reported at 5 years after the Prestige oil spill (Zock et al. 2012); these effects disappeared at 6 years after the spill (Zock et al. 2014). One notable disaster, terrorist attacks on the World Trade Center (WTC) on September 11, 2001, USA, also showed a large decline in lung function in rescue workers at the WTC

during the first year (Aldrich et al. 2010). These declines were persistent without recovery over the next 6 years.

A particular strength of this study was the longitudinal panel design of the children over a four-year period. To the best of my knowledge, this is the first long-term follow-up study assessing lung function after an oil spill in children. Nevertheless, there are limitations. The exposed levels of VOCs for the 4 days right after the spill were estimated from a modeling technique, because actual amibient exposure assessments were not established at the initial period. In addition, lung function data before the oil spill or at right after the oil spill were not available.

In conclusion, completely evaporated VOCs in the initial period of oil spills could reduce lung function in children for at least 5 years. The airborne exposure levels of VOCs for a few days right after an oil spill are generally abnormally high. Therefore, children, a group vulnerable to environmental toxicant exposure, could endure adverse lung health impacts of oil spills, even without direct contact with oil chemicals, such as from clean-up activity, and these effects could persist over the next several years. Guidelines for preventive management and the continued surveillance of lung function in children are required for this spill, past oil spills, and future oil spills.

Ⅳ. CONCLUSIONS AND

IMPLICATIONS

Conclusions

Exposure to the Hebei Spirit oil spill was associated with long-term health consequences up to 5 years after the disaster.

In chapter 1 (adults), levels of oxidative stress biomarkers were significantly increased with the total duration of clean-up efforts and a metabolite of polycyclic aromatic hydrocarbons (PAHs) 1 year after the disaster.

In chapter 2 (adults), levels of oxidative stress biomarkers in high-exposure group up to 4 years after the disaster slightly increased over time.

In chapter 3 (children), asthma symptoms were significantly associated with oil exposure up to 5 years after the disaster.

In cahpter 4 (children), loss of lung function was significantly associated with exposure to volatile organic compounds (VOCs) up to 5 years after the disaster.

Implications

A comprehensive study of long-term health effects are required for this oil spill disaster and future oil spill disasters.

For the Hebei Spirit oil spill disaster, a continued long-term medical monitoring is recommended. In addition, an epigenetic study and a cancer study are suggested as a next step because oil exposure may cause late-onset diseases.

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국문초록

허베이스피리트호 유류유출사고로 인한 중장기 건강영향

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노 수 련

연구배경: 허베이스피리트호 유류유출사고는 2007 년 12 월 7 일 오전 7 시 경에 삼성중공업 해상크레인과 허베이스피리트호의 충돌로 인하여 충남 태안군 만리포 북서방 5 마일 해상에서 원유 12,547 kL 가 해상에 유출된 사고이다. 약 1,052 km 의 서해안을 오염시켰고, 현재까지 국내에서 발생한 가장 큰 규모의 해양오염 사고로 기록되고 있다. 유출된 원유에는 수백만 가지의 유해물질이 포함되어 있으나 독성과 건강영향이 알려진 물질은 극히 일부에 불과하다. 그 중에서 주목해 보아야 할 화학물질은 휘발성유기화합물질 (VOCs), 다환방향족탄화수소 (PAHs), 중금속 등이 있다. 현재까지 유류유출사고로 인한 중장기적 건강영향 연구는 전 세계적으로 2002 년 스페인 프레스티지 사고가

유일하며, 2010 년 미국 걸프만 유류유출사고에 대한 현재 중장기적 건강영향조사를 진행 중에 있다.

연구목적: 이 논문의 목적은 허베이스피리트호 유류유출사고로 인한 중장기적 건강영향을 살펴보고자 함에 있다. 먼저 유류유출사고로 인한 건강영향들을 알기 위해, 중장기적 건강영향 (long-term health effects) 뿐만 아니라 급성 건강영향 (acute health effects)까지 포함한 전세계의 모든 연구들에 대한 문헌고찰을 실시하였다.

다음으로 5 년이 경과한 시점까지의 허베이스피리트호 유류유출 관련 중장기 건강영향조사 데이터를 분석하였고, 네 편의 소 연구의 목적은 다음과 같다.

연구 1. 성인의 산화손상지표 농도를 사고 1 년 후 측정하고, 유류유출사고와 연관성이 있는지 살펴 보았다.

연구 2. 고노출지역 성인의 산화손상지표 농도를 사고 3 년 후와 4 년 후에 추적 조사하고, 농도변화 추이를 관찰하였으며, 유류유출사고와의 연관성 여부를 확인하였다.

연구 3. 어린이의 천식증상을 사고 1 년 후, 3 년 후, 5 년 후에 측정하고, 유류유출사고와 연관성이 있는지 알아 보았다.

연구 4. 어린이의 폐기능을 사고 1 년 후, 3 년 후, 5 년 후에 측정하고, 사고초기 고농도의 VOCs 노출과 관련성이 있는지 관찰 하였다.

연구방법: 성인의 경우, 사고 1 년 후인 2009 년에 9,246 명의 1 차 중장기 건강영향조사를 시작으로, 사고 3 년 후인 2010 년에 1,257 명을 조사하였고, 사고 4 년 후인 2012 년에 1,158 명을 조사하였다. 조사지역은 1 차조사의 경우 고노출지역과 저노출지역을 모두 포함하였지만, 2, 3 차의 추적조사의 경우 고노출지역에서만 시행되었다.

어린이의 경우, 사고 1 년 후인 2009 년에 828 명의 학생들을 조사한 것을 시작으로, 사고 3 년 후인 2010 년에 760 명, 사고 5 년 후인 2012 년에 783 명의 학생들을 조사하였다. 조사지역은 1 차, 2 차, 3 차 모두 동일하게 사고지점에서 가장 가까이에 위치한 4 개면의 모든 학교 모든 학생들이었으며, 조사 참여률은 각각 90% 이상이었다.

허베이스피리트호 유류유출사고로 인한 중장기적 건강영향을 살펴볼 때, 다음과 같은 점을 주목하고 연구방향을 잡았다.

피해지역 성인의 경우, 유출된 유류성분의 노출경로는 기존 논문고찰에서도 가장 중요하다고 보고되는 방제작업 참여를 통한 지속적 노출에 주목하고, 총 방제작업 기간과 PAHs 대사체인 1-hydroxypyrene (1-OHP)와 2-naphthol (2-NAPH) 농도를 노출지표로 정하였다. 건강영향은 유류 속 수많은 물질의 혼합물 형태, 노출경로의

복잡성을 고려할 때, 질병의 형태 및 발생시기와 정도 및 회복가능성이 사람마다 다양하게 나타날 수 있기 때문에, 여러 질병 이완의 전 단계로써 세포의 산화손상 정도를 측정하여, 건강영향지표로 보았다. 산화적 지질과산화의 지표인 malondialdehyde (MDA)와 산화적 DNA 손상 지표인 8-hydroxydeoxyguanosine (8-OHdG)를 측정하였다.

피해지역 어린이의 경우, 설문을 통해 방제작업에 참여하였는지 조사하였는데, 대부분이 참여하지 않았던 것으로 확인되었다. 따라서 유출된 유류성분의 노출경로로 어린이에 있어서는, 사고 며칠 동안에 모두 증발한 VOCs 에의 초기 공기 중 노출을 가장 큰 노출원으로 고려하였다. 지표산출방법은 모델링 결과, 사고 직후 벤젠 (benzene)은 10 시간 이내에, 톨루엔 (toluene), 에틸벤젠 (ethylbenzene), 자일렌 (xylene)의 경우도 48 시간 이내에, 그 외 VOCs 계열의 경우에도 4 일 이내에는 모두 증발한 것으로 사료되어, 사고 직후 4 일 동안 대기로부터 노출된 개인별 누적 VOCs 총량을 계산하였다. 다음으로 학교와 집의 위치가 사고지점에서 얼마나 가까운지 조사하여, 위치지표로 사용하고 직·간접적 노출크기를 대리하였다. 건강영향의 경우 여러 건강영향 가능성들을 살펴본 결과, 어린이에 있어서는 호흡기 관련 건강영향이 주되게 관찰되었다. 따라서 설문조사를 통해 최근 1 년 이내에 천식증상이 있었는지 알아보았고, 폐기능 검사를 이용하여 일초간 노력성 호기량 예측치 (FEV1 % pred)를 산출하였다.

연구결과: 연구 1. 사고발생 1 년후, 총 방제작업 기간에 따라 8-OHdG 와 MDA 의 농도가 유의하게 증가하였다 (둘 다 p-trend < 0.0001). 총 방제작업 기간과 1-OHP 농도는 통계적으로 유의한 양의 상관관계가 있었고, 방제작업에 가장 오랫동안 참여한 그룹 (100 일 이상) 에서 농도가 가장 높았다. 여러 관련 변수들을 보정하고 살펴보았을 때, 1-OHP 농도가 증가할수록, MDA 와 8-OHdG 농도도 유의하게 증가하였다.

연구 2. 사고발생 3 년후와 4 년후에 고노출지역 주민의 8-OHdG 농도는 조금 더 증가하였다. 연령, 성별, 흡연, 교육수준을 보정하고, 1 년 후의 평균농도는 5.6 ㎍/g cr, 3 년 후의 평균농도는 5.8 ㎍/g cr, 5 년 후의 평균농도는 6.3 ㎍/g cr 이었다. 총 방제작업 참여기간과 1 차 조사 시점의 1-OHP 농도는 사고 3 년 후와 4 년 후의 8-OhdG 농도와 통계적으로 유의한 수준의 관련성을 보이지 않았다.

연구 3. 횡적으로 (cross-sectional design) 사고 1 년 후, 3 년 후, 5 년 후 시점 천식증상의 나타남과 집과 학교의 위치를 고려한 거리지표와 사고직후 4 일간의 BTEX 누적 노출량은 통계적으로 유의한 연관성을 나타냈다. 이러한 유류유출과의 천식증상 연관성은 사고가 났을 당시 나이가 더 어린 미취학 아동 그룹에서 더욱더 뚜렷하게 나타났으며, 사고시점으로부터 시간이 더 경과할수록 연관성의 강도는 줄어들었다. 종적으로 (longitudinal design)에서도 유류노출과 천식증상의 관련성을 확인할 수 있었다.

연구 4. 어린이의 VOCs 누적 노출량을 크기 순으로 정렬하면, 자일렌 (평균: 11.2 mg/m3·4 일), 톨루엔 (10.1), 에틸벤젠 (6.2), 벤젠 (2.3) 순이었다. 시간이 갈수록 어린이의 FEV1 예측치는 줄어들었는데, 1 년 후 100.7%, 3 년 후 96.3%, 5 년 후 94.6%를 나타냈다. 횡적으로 (cross-sectional design) 분석한 VOCs 누적 노출량과 폐기능 저하와의 연관성은, 사고 1 년 후와 3 년 후에는 통계적으로 유의였고 5 년 후에는 통계적으로 제한적 (marginal)이었다. 종적으로 (longitudinal design) 관찰하였을 때에도 통계적으로 유의한 연관성을 나타냈다.

연구결론: 허베이스피리트호 유류유출사고는 성인과 어린아이 모두 중장기적인 건강영향을 초래한 것으로 판단된다.

성인의 경우, 오랜 기간 지속된 방제작업이 산화손상을 초래했을 것으로 사료되며, 많이 노출되었을 경우 특히 DNA 산화손상을 가져왔을 경우, 회복이 더딘 것으로 보인다. 어린이의 경우, 사고 초기에 대기 중으로 모두 휘발된 다량의 VOCs 노출이 폐기능 저하를 초래하는 주된 원인으로 보여지며, 어린이 천식의 경우 사고지점으로부터 학교와 집까지의 근접성에 따라 추정되는 잔류유류물질의 지속적 노출 역시 중요해 보인다. 또한 독성물질 노출시점의 나이는 이후 천식증상 지속과 발현에 중요하게 작용하였다. 유류유출로 인한 중장기적 건강영향은 성인과 어린이 모두에서 시간의 흐름에 따라 관련성이 흐릿해졌다.

결론적으로, 허베이스피리트호 유류유출은 성인과 어린이를 포함한 지역주민의 중장기적인 건강에 영향을 미쳤다. 어린이와 성인 모두에서 시간이 지나면서 관련성이 희미해지는 것을 보아, 회복기전이 작용한 것으로 사료된다. 어린이의 경우 나이가 어릴수록 영향이 더 크고 지속되는 것으로 사료된다. 이 논문은 허베이스피리트호 유류유출로 인한 중장기적 건강영향을 이해하고, 혹시 모를 앞으로의 사고를 대비하는데 중요한 자료가 될 것이다.

주요어: 방제작업, 산화적 DNA 손상, 산화적 지질과산화, 유류유출, 종단연구, 중장기 건강, 천식증상, 폐기능, 호흡기 건강, 환경적 재난

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