Exposure and Health (2020) 12:617–628
https://doi.org/10.1007/s12403-019-00320-0

ORIGINAL PAPER

Exposure Assessment of Rayong Oil Spill Cleanup Workers

Thammasin Ingviya[1,2,3] · Chanthip Intawong[4] · Salahaddhin Abubaker[1] · Paul T. Strickland[1]

Received: 23 May 2019 / Revised: 19 August 2019 / Accepted: 27 August 2019 / Published online: 5 September 2019

Abstract

In July of 2013, a pipeline connecting an offshore oil platform to a tanker caused crude oil to spill into the Sea of Rayong off the coast of Thailand. The resulting oil slick, estimated to be between 50 and 190 m^3 (336–1200 barrels), washed ashore 1 day later on the island of Samet. We conducted a study to quantify internal dose of polycyclic aromatic hydrocarbons (PAHs) and benzene in 1262 oil spill cleanup workers, and to examine factors related to their dose. Frozen stored urine samples ($n = 1343$) collected from the workers during the 1 month cleanup period were used to measure the concentration of 1-hydroxypyrene-glucuronide (1-OHPG), cotinine and creatinine. Data from questionnaires and urinary *trans,trans*-muconic acid (*t,t*-MA), a benzene metabolite, measured previously as part of a worker health surveillance plan, were linked with the laboratory data. The internal dose of urinary 1-OHPG was highest in individuals who worked during the first 3 days of cleanup work (median 0.97 pmol/ml) and was 66.7% lower (median 0.32 pmol/ml) among individuals who worked in the final week of the study (days 21–28). After adjusting for age, cotinine and creatinine by regression analysis, the decline in urinary 1-OHPG concentration with days of cleanup remained significant (P-trend < 0.001). A decreasing trend by days of cleanup was also observed for detectable urinary *t,t*-MA percentage (P-trend < 0.001). Rayong oil spill cleanup workers exhibited evidence of elevated levels of PAH and benzene exposure during the early weeks of cleanup, compared to near background levels 4 weeks after cleanup began. Long-term health monitoring of oil spill cleanup workers is advised.

Keywords Oil spill · Cleanup · Exposure · PAHs · Benzene · Biomarkers

Introduction

The frequency and size of offshore oil spills has increased dramatically in the last 50 years (Murphy et al. 2016). More than 11,000 oil spill-related publications have been published since 1968 (Murphy et al. 2016). Spilled crude oil can affect the environment, local economics and the health of local communities (Aguilera et al. 2010; Laffon et al. 2016). A recent medium tier oil spill in the Sea of Rayong resulted in a month-long cleanup effort.

On 27 July, 2013, a pipeline connecting an offshore oil platform to a tanker, operated by PTT Global Chemical (PTTGC), a corporation owned by the government of Thailand, leaked and caused crude oil to spill into the Sea of Rayong off the coast of Thailand (PTT Global Chemical 2013). The crude oil covered an area of approximately 20 km^2 and washed ashore on the island of Samet in an area called "Ao Prao" on 28 July, 2013 (Laemun et al. 2014). The estimated amount of oil spilled was between 50 and 190 m^3 or 336–1200 barrels (PTT Global Chemical 2013). On-land cleanup lasted about a month and was performed by a combination of territorial defense volunteers, citizen volunteers, Thai military personnel and PTTGC employees. Cleanup procedures included oil containment, skimming and dispersal, absorbance, high-pressure water spraying and removal and disposal of contaminated soil, sand and rocks (Laemun et al. 2014).

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12403-019-00320-0) contains supplementary material, which is available to authorized users.

✉ Paul T. Strickland
pstrick1@jhu.edu

[1] Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

[2] Department of Family Medicine and Preventive Medicine, Prince of Songkla University, Songkhla 90110, Thailand

[3] Air Pollution and Health Effect Research Center, Prince of Songkla University, Songkhla 90110, Thailand

[4] Occupational Medicine Department, Rayong Hospital, Rayong 21000, Thailand

Crude oil is a complex mixture of hydrocarbons, including volatile organic compounds (VOCs), such as benzene, and polycyclic aromatic hydrocarbons (PAHs), such as pyrene and benzo[a]pyrene (OSHA 1999; ATSDR 1995; IARC 1983). Several studies from previous oil spill incidents have reported elevated levels of metabolites of PAHs and VOCs in the urine of cleanup workers (Ha et al. 2012; Cheong et al. 2011; D'Andrea and Reddy 2014). During and after the Rayong oil spill cleanup, the Rayong Provincial Public Health Office and Rayong Hospital designed a health surveillance plan for the workers, collecting urine samples post-shift to assess urinary *trans,trans*-muconic acid (*t,t*-MA). The purpose of this study was to expand the laboratory analysis to include an internal dose biomarker of PAHs, 1-hydroxypyrene-glucuronide (1-OHPG) and to re-analyze the t.t-MA measurements as continuous data (including values below 500 ug/gCr). These results should expand our understanding of the exposures sustained by these workers and lay the groundwork for further assessment of potential acute and chronic health effects.

Materials and Methods

The urine samples were first collected as part of the health surveillance for oil spill cleanup workers. The consent for use of urine samples for scientific study was obtained by the Rayong Hospital and the Thai Naval Medical Department. Approval for the analysis of de-identified urine samples and data in our study was approved by the institutional review board of the Johns Hopkins Bloomberg School of Public Health, and the ethical committees of the Prince of Songkla University, Rayong Hospital, and the Thai Naval Medical Department.

Study Population and Urine Samples

Our study used the available data and frozen urine samples previously collected by Rayong hospital. The urine samples were transported to our laboratory in Baltimore, MD, USA, on dry ice. The total number of oil spill cleanup workers with available questionnaire and urinary *t,t*-MA data was 2118. Of the 1486 urine samples available to our research team, 1343 samples had sufficient volume (≥ 2 mls), for measuring urinary 1-OHPG and cotinine. Creatinine was previously measured in 1282 of those samples by Rayong Hospital, and we measured creatinine in the remaining 61 urine samples in our laboratory. The 1343 urine samples were collected from 1262 workers. Most of the workers provided only 1 sample; 80 workers provided 2 samples and 1 worker provided 3 samples.

The urinary creatinine measurements previously performed by Rayong Hospital used an enzymatic assay (OSR 61,204) using creatinase enzyme on a Beckman Coulter AU analyzer (Beckman Coulter, Inc., Brea, CA), while our laboratory used Jaffe's kinetic reaction (Cayman Chemical Company, Ann Arbor, MI) to measure the remaining 61 urine samples plus the 60 repeat measurements of urines already assayed by Rayong Hospital. Because the two methods gave slightly different results on assays of the same 60 samples, we adjusted the results of the 61 samples assayed in our laboratory to be consistent with the Rayong Hospital sample set.

Characteristics, Job Descriptions and Personal Protective Equipment (PPE) Use of the Rayong Oil Spill Cleanup Workers

The date that the on-land cleanup began (29 July 2013) was counted as Day 1 of cleanup in our study. The health surveillance protocol, including questionnaire and urine sample collection, began the next day (Day 2). A calendar depicting the cleanup sequence and our study time periods is shown in Fig. 1. Demographic factors and their distribution are shown in Table 1. Of 1343 usable urine samples, 93.2% were provided by male workers. The median age was 27 years (Interquartile Range (IQR) = 18.0) and the majority (55.3%) of the urine samples were provided by workers whose background occupation was military personnel. Forty percent of the urine samples were provided on Day 2 to Day 4 of the oil spill cleanup.

The urine samples were provided by workers who performed various oil spill cleanup jobs. Of 1343 usable urines, 57.9% were provided by workers whose cleanup job was to manually remove oil-contaminated sand, rocks, and trash (Supplementary Table S.1), and 23.5% were from workers whose job description was to vacuum or manually remove the oil slick from water. Workers who provided more than 1 urine sample were classified by the task they performed on the day they provided the urine sample. All shifts/samples were included in the data analysis. Workers who provided urine samples were also asked about their personal protective equipment (PPE) use. They were asked if they wore any PPE, an N95 mask, an R95 mask, any mask with filter, coveralls, gloves or boots. The mask questions were grouped as "any mask use" if the workers answered "yes" to at least one of the questions, regarding the use of N95, R95 or mask with filter. Most of the workers (84%) self-reported using at least one piece of PPE (either mask, coveralls, gloves or boots) during their shifts (Table S.2). However, only 16.8% of the workers wore the complete set of PPE, and 31.7% reported that they **"**often**"** wore at least one piece of PPE.

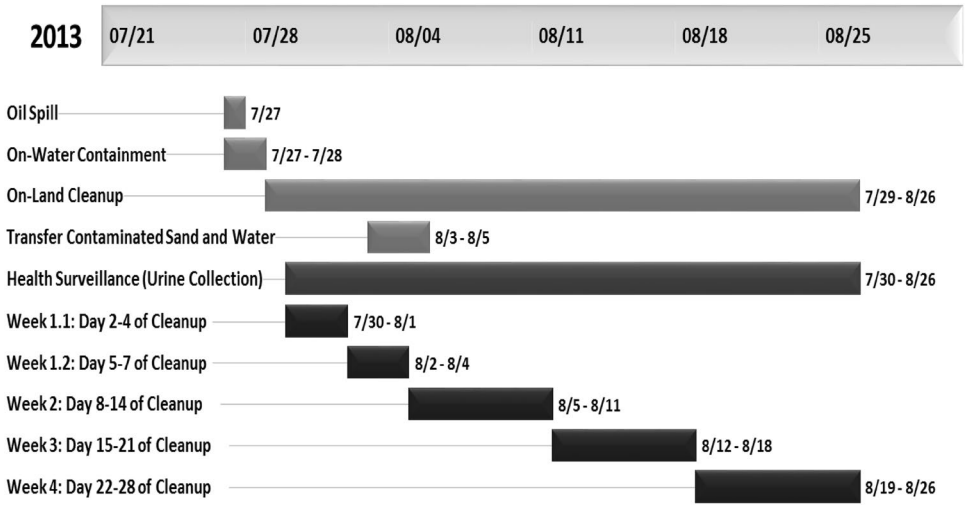

Fig. 1 Rayong oil spill cleanup study (27 July 2013–26 August 2013)

Table 1 Demographic factors of cleanup workers

Demographic factors	Descriptions	Number of workers	Percent
Total		1343	100.0
Age	Median (1st–3rd quartiles)	27.0 (22.0–40.0)	
	Unknown age	9	0.7
Sex	Male	1252	93.2
	Female	90	6.7
	Missing	1	0.1
Background	Military personnel	743	55.3
	Oil company employees	408	30.4
	Citizen volunteers	183	13.6
	Unknown	9	0.7
Days of cleanup	Day 2–4	537	40.0
	Day 5–7	328	24.4
	Day 8–14	282	21.0
	Day 15–21	115	8.6
	Day 21–28	81	6.0

Laboratory Methods

Urinary 1-Hydroxypyrene-Glucuronide (1-OHPG) Analysis

To quantify the PAH exposure in cleanup workers, 1-OHPG, a metabolite of pyrene measurable in urine, was used as the surrogate biomarker for the whole group of PAHs. Urinary 1-OHPG was measured using immunoaffinity chromatography and synchronous fluorescence spectroscopy (SFS), as modified from Strickland et al. (1994). The final urinary 1-OHPG fractions from immunoaffinity columns were eluted with 55% methanol (in PBS; 4 ml) and collected for synchronous fluorescence spectroscopy analysis (Perkin Elmer LS50B Luminescence spectrometer, Norwalk, CT, USA) using a wavelength difference of 34 nm. The limit of detection (LOD) of the assay was 0.04 pmol/ml; the recovery was 82% and the coefficient of variation was 5.6%.

Urinary Creatinine Analysis

As mentioned above, 61 urine samples did not have available urinary creatinine data from Rayong Hospital. Therefore, we randomly selected 60 urine samples with available urinary creatinine measurements from Rayong Hospital as a validation set to compare and quantify the differences in the urinary creatinine levels measured by our laboratory and the Rayong Hospital laboratory. Our laboratory used an assay based on Jaffe's kinetic reaction (Creatinine urinary colorimetric assay kit #500,701, Cayman Chemical Company, Ann Arbor, MI). The coefficient of variation was 5% and the limit of detection was 0.1 mg/dl. Rayong Hospital used a creatinase enzymatic assay using reagent OSR 61,204 on a Beckman Coulter AU analyzer (Beckman Coulter, Inc., Brea, CA).

The differences between creatinine from our laboratory and Rayong hospital's laboratory were assessed using linear regression analysis. The coefficient and intercept from the linear regression model were used to convert the creatinine concentrations (mg/dl) measured in our laboratory to that of the Rayong Hospital laboratory (Supplementary Figure S.1). before further statistical analysis.

Urinary Cotinine Analysis

We used a solid phase competitive ELISA (No. CO096D; Calbiotech, El Cajon, CA) assay to measure urinary cotinine. The coefficient of variation was 8% and the limit of detection was 2 ng/ml. Generally, a cut-off of 50 ng/ml is recommended to differentiate between nonsmokers and passive or active smokers (Zielinska-Danch et al. 2007; SRNT Subcommittee on Biochemical Verification 2002).

Urinary *t,t*-MA Data

Urinary *t,t*-MA data from Rayong Hospital was retrieved and linked to the questionnaire data. Urinary *t,t*-MA from Rayong Hospital was measured using high performance liquid chromatography with fluorescent detection (Intawong and Sithisarankul 2015). The limit of quantitation (LOQ) was estimated to be 0.01 mg/dl or 0.10 µg/ml. These samples were analyzed in several government laboratories in Thailand, however, the results were only partially reported as categories (> or < 500 ug/gCr),[12] rather than as continuous values. In the current study, we have re-examined these t.t-MA measurements as continuous data (including values below 500 ug/gCr).

Statistical Methods

All available questionnaire data was linked to the 1-OHPG, *t,t*-MA, and cotinine measurements. Non-detectable measurements of urinary 1-OHPG and cotinine were replaced with the value of the LOD/$2^{1/2}$, assuming log normal distributions. For descriptive analysis, continuous variables, including urinary 1-OHPG, urinary cotinine, and age were presented as median (1st–3rd quartile) values due to non-normal distributions. Categorical variables, such as number of workers by days of cleanup, PPE use, or job description were presented as number (%).

For inferential statistics, log-linear regression models were used to compare the levels of 1-OHPG among days of cleanup (days 2–4, days 5–7, days 8–14, days 15–21 and days 22–28), adjusting for age of workers, urinary cotinine, and/or creatinine. To adjust for workers' dehydration status, creatinine concentration was added as a covariate in the log-linear regression models. Finally, the log-linear regression models were used to compare levels of 1-OHPG among job description categories, adjusting for days of cleanup and cotinine concentration. *P* values for trends of the geometric difference ratios were calculated using Rao's score test (Radhakrishna Rao 1948). Generalized estimating equations (GEE), as described in Liang and Zeger (1986), were used to account for multiple samples from the same workers. Because most of the workers (1141 workers) provided their urine sample only from their first work shift, we did not adjust for consecutive shifts in the regression models.

Detailed *t,t*-MA data was not reported in the previous two published papers from the Rayong oil spill (Sithisarankul and Intawong 2015; Rheanpumikankit et al. 2015) Therefore, we re-analyzed the complete urinary *t,t*-MA data set, previously measured by the Rayong Hospital, and adjusted these results with our cotinine measurements. Because of the large proportion of non-detectable samples (67.5%), the urinary *t,t*-MA data was analyzed as a binary variable (detectable vs non-detectable). In addition, due to the relatively smaller sample size, *t,t*-MA data from the 3rd and 4th weeks of cleanup were combined before the statistical analysis. To further adjust for smoking, stratification by nonsmokers and smokers (urinary cotinine ≤ 50 ng/ml and > 50 ng/ml) and logistic regression was used to assess the association between odds of having detectable *t,t*-MA in urine and days of cleanup, job descriptions and PPE use, adjusting for age of workers, cotinine, and/or creatinine. All statistical analysis was completed using R version 3.2.4. (R Development Core Team, Vienna, Austria, 2016).

Results

Urinary 1-OHPG

In the 1343 urine samples analyzed, the median level of urinary 1-OHPG was 0.79 pmol/ml (Q1–Q3 0.31–1.81). The number of urine samples with the non-detectable levels was 94 (7.0%). Using the suggested categorical values from Kang et al. (1995), 57.6% of the urine samples had "low" levels of 1-OHPG (< 1.0 pmol/ml), 36.5% had "moderate" levels (1.0–5.0 pmol/ml) and 5.9% had "high" levels (> 5.0 pmol/ml) as shown Figure S.2.

1-OHPG exhibited a decreasing trend by days of cleanup as shown in Fig. 2. We assigned the starting date of on-land cleanup (29th July) as "day 1 of cleanup" in our study. Urine samples from day 1 of cleanup were not available because the health surveillance protocol was not implemented until day 2 of the study. The median of urinary 1-OHPG on days 2–4 of the Rayong oil spill cleanup was 0.97 pmol/ml, and the levels decreased by 66.7% to 0.32 pmol/ml by day 22–28 of cleanup. This was consistent with our hypothesis that the exposure levels of PAHs would be the highest in the first week of cleanup and decline thereafter.

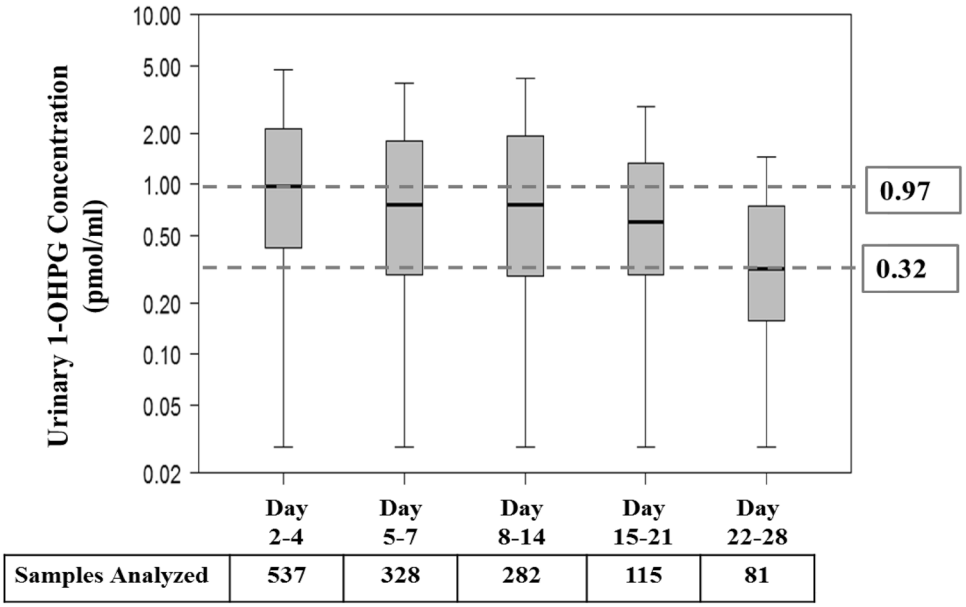

Fig. 2 Urinary 1-OHPG (Log-scale) by days of cleanup

Urinary Cotinine

Since smoking status of cleanup workers recorded on questionnaires was limited (only 387 workers had available smoking status) we measured urinary cotinine as a biomarker of tobacco smoke exposure. Overall, the median level of urinary cotinine was 37.3 ng/ml (Q1–Q3 3.0–1229.5) and urinary 1-OHPG was 2.8 times higher in the 4th cotinine quartile than in the 1st quartile (1.65 vs. 0.58 pmol/ml) (Figure S.3). The median level of urinary cotinine in nonsmokers was 3.1 ng/ml (Q1–Q3 1.4–5.9), whereas the median level in smokers was 1240.6 ng/ml (Q1–Q3 699.8–1841.3).

Using a urine cotinine cutoff concentration of 50 ng/ml to distinguish between smokers and non-snokers (Zielinska-Danch et al. 2007; Srnt Subcommittee on Biochemical Verification 2002), we observed that the median urinary 1-OHPG concentration of smokers was 2–3 fold higher than that of nonsmokers, by days of cleanup. In nonsmokers, urinary 1-OHPG exhibited a clearly decreasing trend by days of cleanup, as shown in Fig. 3. The median concentration of urinary 1-OHPG on days 2–4 in nonsmokers

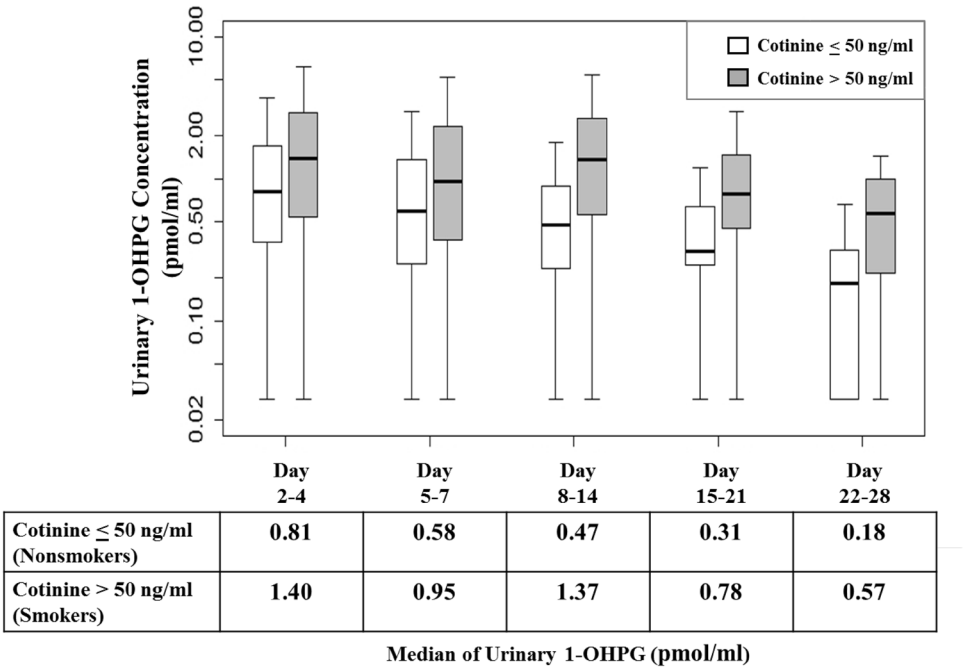

Fig. 3 Urinary 1-OHPG (Log-scale) by days of Cleanup in Smokers and Nonsmokers

was 0.81 pmol/ml, decreasing by 79% to 0.18 pmol/ml by day 22–28 of cleanup. Whereas in smokers, the median of urinary 1-OHPG on days 2–4 was 1.40 pmol/ml, decreasing by 59% to 0.57 pmol/ml by day 22–28 of cleanup (Fig. 3).

Regression Analysis of 1-OHPG by Days of Cleanup

We performed 3 different log-linear regression models for 1-OHPG and days of cleanup (Table 2). For Model 1, the association between 1-OHPG and days of cleanup was adjusted by age and urinary creatinine. For model 2, age and urinary cotinine were used as an adjusting variable. For model 3, age, urinary creatinine and cotinine were used as adjusting variables. All the models showed significantly decreasing trends in 1-OHPG geometric mean (GM) ratio over time (P-trend < 0.001) (Table 2). The GM of urinary 1-OHPG increased by 7% (GM ratio 1.07, 95% CI 1.06–1.07) per 100 µg/ml increase in urinary creatinine; and the GM of urinary 1-OHPG increased by 71% (GM ratio 1.71, 95% CI 1.57–1.86) per 1 µg/ml increase in urinary cotinine (data not shown).

Urinary 1-OHPG and Job Descriptions

The urinary 1-OHPG levels were stratified by job description of cleanup workers (Table 3). Contaminated sand and trash removal (57.9%) was the most common job description. The highest GM level of urinary 1-OHPG was found in urine samples from oil dispersant applicators who sprayed oil dispersants (GM 1.79, IQR 0.31–1.81 pmol/ml). The second highest level was found in urine samples from workers who removed contaminated sand and trash (GM 0.75, IQR 0.32–1.87 pmol/ml). The lowest 1-OHPG level was found in support personnel (coordinators, PTTGC corporate representatives, visitors, photographers, and journalists) (GM 0.44, IQR 0.25–1.04) (Table 3 and Fig. 4).

In the log-linear regression analysis with GEE of 1-OHPG by job descriptions, support personnel, with the

Table 2 Log-linear regression with GEE* of urinary 1-OHPG by days of cleanup ($n = 1343$)

Weeks of study	Days of cleanup	Geometric mean ratio of 1-OHPG (95% CI)			
		Univariable Model	Model 1	Model 2	Model 3
Week 1.1	Day 2–4	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Week 1.2	Day 5–7	**0.77 (0.64–0.93)**	0.91 (0.77–1.07)	**0.69 (0.57–0.82)**	**0.78 (0.67–0.92)**
Week 2	Day 8–14	**0.80 (0.65–0.98)**	1.05 (0.88–1.25)	**0.68 (0.57–0.82)**	0.86 (0.73–1.01)
Week 3	Day 15–21	**0.69 (0.54–0.87)**	0.84 (0.66–1.07)	**0.55 (0.44–0.77)**	**0.66 (0.53–0.83)**
Week 4	Day 22–28	**0.32 (0.23–0.44)**	**0.52 (0.38–0.71)**	**0.32 (0.23–0.43)**	**0.46 (0.35–0.62)**
P-trend		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

Model 1: Adjusted by Urinary Creatinine and Age

Model 2: Adjusted by Urinary Cotinine and Age

Model 3: Adjusted by Urinary Creatinine,Cotinine and Age

Bold numbers indicate statistically significant results ($P < 0.05$)

*Generalized estimating equation with exchangeable correlation structure

Table 3 Urinary 1-OHPG by job descriptions of cleanup workers ($n = 1343$) (descending order by geometric mean of 1-OHPG)

Job Descriptions	Urinary 1-OHPG (pmol/ml)				
	Numbers (%)	Geometric mean	Median	1st Quartile	3rd Quartile
Total	1343 (100.0%)	0.72	0.79	0.31	1.81
Oil dispersant applicator	17 (1.3%)	1.79	3.02	0.97	6.98
Contaminated sand/trash removal	778 (57.9%)	0.75	0.81	0.32	1.87
Environmental sampling personnel	9 (0.7%)	0.72	1.42	0.54	1.91
Oil vacuum/oil slick removal	315 (23.5%)	0.70	0.70	0.32	1.72
Supervisor/health care professional	38 (2.8%)	0.68	0.69	0.35	1.80
Transport driver/ship pilot	23 (1.7%)	0.61	0.88	0.26	2.31
Support personnel*	61 (4.5%)	0.44	0.45	0.25	1.04
Others	44 (3.3%)	0.64	0.55	0.34	1.67
Missing	58 (4.3%)	0.72	0.90	0.32	1.66

*Coordinators, PTTGC corporate representatives, visitors, photographers, and journalists were grouped as support personnel

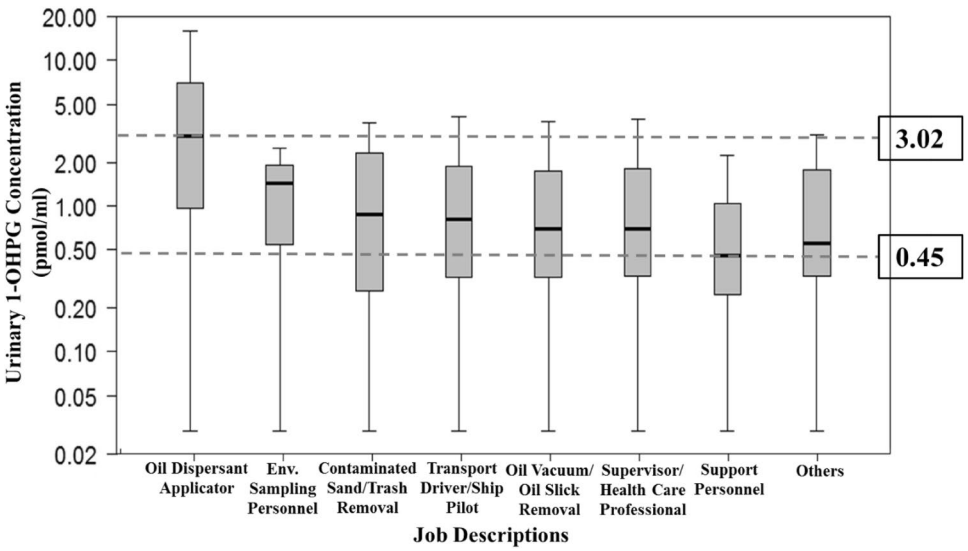

Fig. 4 Urinary 1-OHPG (Log-scale) by job (descending order by median of 1-OHPG)

lowest GM of urinary 1-OHPG, was used as the reference group (Table 4). In the univariable model, compared to support personnel, oil dispersant applicators had the highest 1-OHPG GM ratio (4.1; 95% CI 1.57–10.69). Contaminated sand/trash removal and oil vacuum/oil slick removal were two other job groups with significantly elevated GM ratios (95% CI) of 1-OHPG, compared to the support personnel (1.71 (1.24–2.36) and 1.61 (1.15–2.67), respectively). The other job groups exhibited non-significantly elevated GM ratios compared to the support reference) group—perhaps due to small sample sizes. After adjusting for cotinine (Model 3), the GM ratios (95% CI) of the transport driver/ship pilot group decreased from 1.40 (0.65–3.00) to 1.06 (0.55–2.07), suggesting that this group of workers might include a high proportion of smokers. After adjusting for days of cleanup, urinary creatinine and urinary cotinine (Model 4), only oil dispersant applicators and contaminated sand/trash removal workers demonstrated significantly elevated 1-OHPG, compared to support personnel ((GM ratio 2.33, 95% CI 1.29–4.21) and (GM ratio 1.33, 95% CI 1.02–1.75), respectively).

Table 4 Log-linear regression with GEE* of urinary 1-OHPG by job descriptions ($n = 1285$)**

Job Descriptions	Geometric mean ratio of 1-OHPG (95% CI)				
	Univariable model	Model 1	Model 2	Model 3	Model 4
Support personnel***	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Oil dispersant applicator	**4.10 (1.57–10.69)**	**4.39 (1.68–11.48)**	**3.06 (1.39–6.70)**	**3.20 (1.34–7.64)**	**2.33 (1.13–4.83)**
Contaminated sand/trash removal	**1.71 (1.24–2.36)**	**1.85 (1.33–2.58)**	**1.56 (1.16–2.10)**	**1.55 (1.14–2.09)**	**1.33 (1.02–1.75)**
Environmental sampling personnel	1.65 (0.62–4.36)	1.71 (0.64–4.56)	1.58 (0.70–3.58)	2.08 (0.80–5.46)	1.90 (0.84–4.31)
Oil vacuum/oil slick removal	**1.61 (1.15–2.67)**	**1.56 (1.10–2.21)**	**1.37 (1.00–1.86)**	1.36 (0.99–1.87)	1.21 (0.91–1.61)
Supervisor/health care professional	1.54 (0.94–2.51)	1.58 (0.99–2.54)	1.26 (0.84–1.88)	1.63 (0.99–2.57)	1.31 (0.89–1.92)
Transport driver/ship pilot	1.40 (0.65–3.00)	1.48 (0.68–3.21)	1.55 (0.79–3.03)	1.06 (0.55–2.07)	1.14 (0.66–2.00)
Others	1.46 (0.88–2.43)	1.28 (0.76–2.15)	0.91 (0.58–1.43)	1.14 (0.71–1.84)	0.84 (0.55–1.28)

Model 1: Adjusted by days of cleanup (day 2–4, day 5–7, day 8–14, day 15–21 and day 22–28)

Model 2: Adjusted by days of cleanup and urinary creatinine

Model 3: Adjusted by days of cleanup and urinary cotinine

Model 4: Adjusted by days of cleanup, urinary cotinine and creatinine

Bold numbers indicate statistically significant results ($P < 0.05$)

*Generalized estimating equation with exchangeable correlation structure

**58 Unknown job description

***Coordinators, oil company corporate representatives, visitors, photographers, and journalists were grouped as the support personnel

Urinary 1-OHPG and Protective Equipment (PPE) Use

Personal protective equipment (PPE) use by oilspill cleanup workers did not show evidence of protection against PAH exposure as measured by urinary 1-OHPG concentration. This was true for overall PPE use, as well as for use of individual equipment, including N95 and R95 masks, gloves, boots and coveralls. The urinary 1-OHPG levels in cleanup workers who wore PPEs, was not significantly lower than in those who did not wear PPEs (Table S.3). In the univariable model, the GM of 1-OHPG in workers who wore mask or coveralls were higher than the workers who did not wear mask or coveralls (GM ratio 1.27, 95% CI 1.09–1.47 and GM ratio 1.40, 95% CI 1.20–1.63, respectively). This may be because mask and coveralls were used by workers mostly in the early days of cleanup when the 1-OHPG levels were high (data not shown). After adjusting by days of cleanup (Model 1), the GM ratios move toward one (null) (Models 1–3). Although not significant, workers who "sometimes" or "often" used PPE, had lower levels of 1-OHPG, compared to those who never used PPE.

Urinary *t,t*-MA

The distribution of urinary *t,t*-MA among cleanup workers is shown in Figure S.4. There was a large number of samples with non-detectable levels of *t,t*-MA (907 out of 1343) (67.5%). Therefore, we elected to statistically analyze the *t,t*-MA data as a binary variable (detectable vs non-detectable). To increase statistical power when analyzing *t,t*-MA as a binary variable, we grouped data from week 3 (day 15–21) and week 4 (day 22–28) together. Before adjusting for covariates, the proportion of urine samples with detectable levels of *t,t*-MA were not different by days of cleanup (overall *t,t*-MA detectable percentage = 30–34%) (Figure S.5). However, this result was confounded by tobacco smoke exposure, which is known to contain benzene. Detectable *t,t*-MA was more frequent in the urine of smokers (urinary cotinine > 50 ng/ml) than nonsmokers (44.2% vs. 21.2%, respectively, $P < 0.001$). In addition, by quartiles of urinary cotinine, the percentage of urine samples with detectable *t,t*-MA was much higher in subjects with cotinine levels in the 4th quartile than in subjects with lower quartiles (64.9% vs. 21.3%, 21.1% and 22.6%, $P < 0.001$) (Figure S.6). Therefore, we controlled for smoking by stratifying urinary cotinine concentrations in subsequent analyses of the association between *t,t*-MA detectable levels and days of cleanup.

We distinguished presumed smokers from nonsmokers using a urinary cotinine cut-off of 50 ng/ml. The nonsmoker group (cotinine ≤ 50 ng/ml) exhibited a clearly decreasing trend in *t,t*-MA detectable percentage by days of cleanup (P-trend = 0.001) (Fig. 5). The percentages of non-smoking workers with detectable urinary *t,t*-MA were 26.3%, 20.9%, 14.8% and 6.3% on days 2–4, days 5–7, days 8–14 and days 15–28 of cleanup, respectively. While in the smoker group (urinary cotinine > 50 ng/ml), a decreasing trend in detectable *t,t*-MA with days of cleanup was not observed, consistent with our finding that smoking increases the probability of having detectable *t,t*-MA in urine.

By logistic regression with GEE, the odds ratio of having detectable urinary *t,t*-MA among nonsmokers (urinary cotinine ≤ 50 ng/ml) also showed a decreasing trend by days of cleanup work (Table 5). In the univariable model, the odds ratio (95% CI) of detectable *t,t*-MA declined to 0.19 (0.07–0.54) on day 15–28 compared to the reference group (day 2–4) with a highly significant trend ($P < 0.001$). In Models 2 and 3, the decreasing trends remained significant after adjustment for urinary cotinine. Among smokers only, there was no evidence of a decreasing trend in odds ratio of detectable *t,t*-MA with days of cleanup (data not shown).

Urinary *t,t*-MA detectable percentages did not differ substantially among workers by job description ($P = 0.335$ by Fisher's exact test), ranging from 29.4 to 47.8% among job

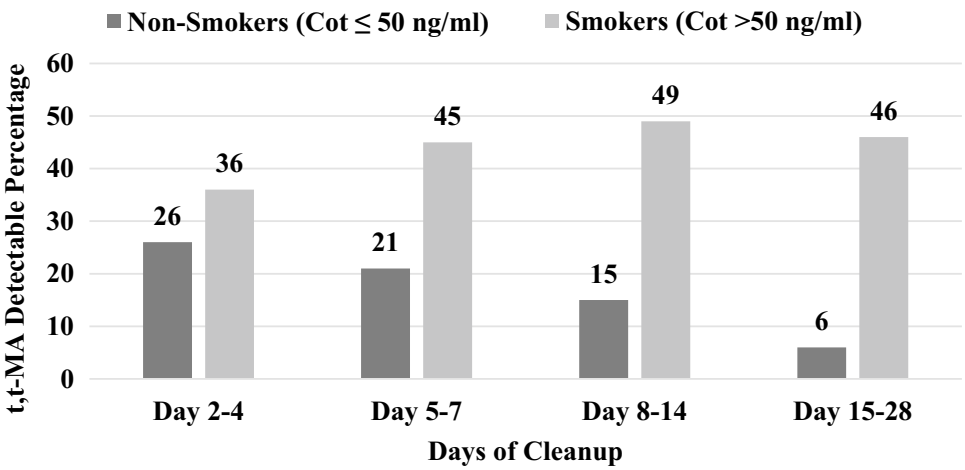

Fig. 5 Urinary *t,t*-MA detectable percentages by days of cleanup in smokers and non-smokers ($n = 1343$). *Smokers were workers whose urinary cotinine was more than 50 ng/ml

Table 5 Logistic regression with GEE* of detectable *t,t*-MA by days of cleanup (nonsmokers: cotinine ≤ 50 ng/ml) ($N = 679$)

Weeks of study	Days of cleanup	Odds ratio of detectable *t,t*-MA (95% CI)			
		Univariable Model	Model 1	Model 2	Model 3
Week 1.1	Day 2–4	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Week 1.2	Day 5–7	0.74 (0.47–1.17)	0.97 (0.57–1.64)	0.66 (0.41–1.09)	0.84 (0.48–1.44)
Week 2	Day 8–14	**0.49 (0.28–0.85)**	0.85 (0.46–1.57)	**0.45 (0.25–0.79)**	0.74 (0.40–1.40)
Week 3–4	Day 15–28	**0.19 (0.07–0.54)**	0.40 (0.13–1.20)	**0.19 (0.07–0.52)**	**0.32 (0.11–0.93)**
P-trend		**< 0.001**	0.077	**< 0.001**	**0.041**

Model 1: Adjusted by urinary creatinine and age

Model 2: Adjusted by urinary cotinine and age

Model 3: Adjusted by urinary creatinine, cotinine and age

Bold numbers indicate statistically significant results ($P < 0.05$)

*Generalized estimating equation with exchangeable correlation structure

groups with 15 or more workers (Table S.4). Similarly, by logistic regression, odds of detectable *t,t*-MA did not differ among non-smoking workers by job description (Table S.5). Also, there was no evidence for a protective effect of PPE use for benzene exposure, assessed by urinary *t,t*-MA concentration. The proportion of urine samples with detectable *t,t*-MA were not different between workers who wore PPEs and those who did not.

Discussion

In our study of Rayong oil spill cleanup workers, we examined internal dose of PAHs and benzene to examine factors related to their exposure. The internal dose of PAHs, as measured by urinary 1-OHPG, was highest in individuals who worked during the first 3 days of cleanup work and was significantly lower among individuals who worked in the final week of the study 3 weeks later. This was consistent with our hypothesis that the exposure levels of PAHs would be the highest in the first week of cleanup and decline thereafter. After adjusting for age, cotinine and creatinine by regression analysis, the decline in urinary 1-OHPG concentration with days of cleanup remained highly significant. Job descriptions with the highest level of urinary 1-OHPG were oil dispersant applicators and contaminated sand/trash handlers. We also observed a decreasing trend by days of cleanup of detectable urinary *t,t*-MA, a biomarker of benzene exposure. These results demonstrate that oil spill cleanup workers can be exposed to PAH and benzene at concentrations sufficient to be measured internally as metabolites. Furthermore, these exposures occurred after a relatively small spill of only about 50–300 barrels of oil, much less than that of the Deepwater Horizon (5 million barrels) (U.S. Coast Guard 2010) or Hebei (80,000 barrels) (Laffon et al. 2016) oil spills.

Previous studies from the Hebei oil spill measured biomarkers of PAHs in urine, as well as biomarkers of benzene, toluene, ethyl benzene, and xylene (BTEX) (Ha et al. 2012; Cheong et al. 2011). They reported elevated levels of *t,t*-MA, mandelic acid (a metabolite of ethylbenzene), and 1-hydroxypyrene in urine samples collected after cleanup, compared to samples collected before participation ($P < 0.05$) (Ha et al. 2012). Comparing another group of Hebei cleanup workers with an unexposed reference group, they found no difference between the groups in concentrations of biomarkers of PAHs or the four BTEX compounds (Cheong et al. 2011). However, they did report a decline in the levels of two PAH biomarkers (1-OHP and 2-naphthol) over the course of several weeks among the cleanup workers. In general, the levels of PAH biomarkers reported in these studies were high overall (1-OHP geometric mean 0.5 µg/gCr; range 0.1–2.4 µg/gCr, approximately equivalent to ~ 0.69–16.5 pmol/ml), even in the unexposed reference group (GM 0.6 µg/gCr; range 0.2–1.7 µg/gCr approximately equivalent to 1.38–11.70 pmol/ml) compared to other studies.

A number of factors could contribute to differences in exposure between spills and between studies. The half-life of PAHs in crude oil in the environment can range from a few hours up to weeks or months depending on the chemical composition of the oil, the molecular weights of the PAHs, bacterial biodegradation and photolysis (Abdel-Shafy and Mansour 2016; Alegbeleye et al. 2017). After a spill and during cleanup, low molecular weight (LMW) PAHs would be expected to evaporate within a few days, resulting in the rapid decline in biomarkers, while the higher molecular weight (HMW) PAHs might take a few weeks to evaporate or degrade. Pyrene, the parent compound of 1-OHPG, is of intermediate MW ($m = 202$) having both rapid and slow evaporation characteristics. The Hebei oil spill workers were recruited for study 2 or more weeks after the oil spill

occurred (Ha et al. 2012; Cheong et al. 2011)thereby reducing expected PAH exposure.

In our study, the median of urinary 1-OHPG among all oil spill workers was 0.79 pmol/ml, with median levels declining from 0.97 pmol/ml when the cleanup began (days 2–4) to 0.32 pmol/ml 4 weeks later (days 22–28). These levels of 1-OHPG are similar to those reported by Kang et al. (1995) for steel plant workers (1.82 pmol/ml) and controls (0.38 pmol/ml), in a study that used the same laboratory and method for 1-OHPG analysis as our study. For comparison, the GM of urinary 1-OHPG in nonsmokers in the US is 0.16–0.25 pmol/ml (Gunier et al. 2006), and 0.025 µmol/molCr (approximately equivalent to ~ 0.38 pmol/ml) in rural nonsmokers in Thailand (Petchpoung et al. 2011). Thus, the 1-OHPG levels we observed were comparable to occupational exposures during the early days of cleanup, and declined to near background (general population) levels by the end of the cleanup operations (0.18 pmol/ml in nonsmokers).

We also examined the levels of urinary 1-OHPG among cleanup workers with different job descriptions. We found that certain types of jobs including, oil dispersant applicators, contaminated sand/trash removal workers and oil vacuum/oil slick removal workers, had higher levels of urinary 1-OHPG than other workers and support personnel. Oil dispersant applicators might be at increased risk of PAH exposure because spraying dispersants on oil–water interfaces generates aerosols that are respirable (Ehrenhauser et al. 2013). Water wave action on the sea while applying dispersants can also facilitate aerosolization and evaporation of PAHs (Ehrenhauser et al. 2013). Workers dealing with contaminated sand/trash removal and oil vacuum/oil slick removal were often in close (or direct) contact with crude oil, thereby enhancing the possibility of dermal contamination. Thus, these workers might be expected to have higher levels of exposure than other workers or support personnel who did not directly contact crude oil. The study of Ha et al. (2012) among the Hebei oil spill cleanup workers explored the association between PAH metabolites and job types, but did not find any differences in PAH metabolite levels between "direct cleanup jobs" and "logistics-related jobs", the only categories reported.

We also examined the potential effect of PPE use on PAH exposure among cleanup workers. Unexpectedly, levels of 1-OHPG were not associated with overall PPE use, consistent with the finding of Lee et al. (2009) from Hebei oil spill (Lee et al. 2009). Furthermore, individual equipment use (masks, gloves, boots, or coveralls) was not associated with a protective effect. This suggests that either the PPE was not used properly, or that the questionnaire data was not reliable, or the PPE was not effective. Also, the masks used would not protect against volatile compounds such as benzene or low molecular weight PAHs. Paradoxically, mask and coverall use were apparently associated with elevated levels of 1-OHPG. This might have resulted from exposure selection bias because of higher hazard recognition (resulting in enhanced PPE use) in the early days of cleanup when the beach was covered in oil, compared to later weeks of cleanup. About 60% of workers who worked in the first 3 days of cleanup (when exposure was high) wore masks or coveralls, whereas only 1–10% of workers during the last 2 weeks of cleanup wore them. In addition to exposure recognition, masks can be contaminated accidentally by direct contact with oil soaked gloves. Another complicating factor is the possible limitation of supply of PPE which would be expected to restrict PPE use. For example, boot and glove use increased over the course of cleanup from 37% (boots) and 47% (gloves) during the first 3 days of cleanup, to 62% and 78%, respectively, during the last 2 weeks of cleanup.

We found that the percentage of oil spill workers with detectable urinary *t,t*-MA decreased from day 2–4 to week 3–4 of cleanup in nonsmokers, but not in smokers. The method used to measure *t,t*-MA in these workers (Intawong and Sithisarankul 2015) had limited sensitivity, with an estimated limit of quantification of 0.10 µg/ml. This compares unfavorably with the background *t,t*-MA level in the general population of 0.07 µg/ml (range 0.02–0.30 µg/ml) (Chanvaivit et al. 2007; Centers for Disease Control and Prevention (CDC) 2015). Because of the high percentage (67.5%) of samples that were not detectable in our study, we analyzed the *t,t*-MA data as a binary variable. This limited our statistical power to detect associations (Altman and Royston 2006) and might partially explain why we did not observe a decreasing trend with time of cleanup in smokers. In addition, tobacco smoke contains benzene and significantly increases concentration of urinary *t,t*-MA in smokers compared to nonsmokers (Melikian et al. 1994). Although we did adjust for smoking by including urinary cotinine concentration in regression models, this adjustment may not have been sufficient to completely control for the confounding effects of benzene from smoking. In addition, sorbic acid-containing foods can artificially increase levels of urinary *t,t*-MA, apart from exposure to benzene, and this was not controlled for in our analysis. Compared to 1-OHPG, the percentage of detectable *t,t*-MA samples among nonsmokers decreased more rapidly with days of cleanup than the levels of 1-OHPG. The rapid decline in detectable *t,t*-MA, is not unexpected as benzene is relatively more volatile than PAHs, and would be expected to evaporate within a few days of the oil spill. Detectable urinary *t,t*-MA was not found to be related to job descriptions as was urinary 1-OHPG. This lack of association could be due to the rapid evaporation of benzene or the lack of statistical power. In addition, urinary *t,t*-MA was not associated with PPE use, similar to our findings on PPE use and urinary 1-OHPG.

Urinary *t,t*-MA measured in other studies of oil spill workers is somewhat limited. Ha et al. (2012) found that levels of urinary *t,t*-MA of workers at the Hebei spill were higher after cleanup participation compared to levels before participation among both smokers (2.5-fold higher) and nonsmokers (3.2-fold higher) (Ha et al. 2012). In contrast, among another group of Hebei spill cleanup workers, Cheong et al. (2011) found no difference in *t,t*-MA levels between workers and unexposed controls, and no change in *t,t*-MA levels between weeks 2–3 and weeks 5–6 of cleanup.

Our study is the first investigation of PAH and benzene biomarkers in cleanup workers' urine samples that were collected within 2 days of a fresh oil spill. In contrast to the studies of cleanup workers at the most intensively investigated spill, the Hebei oil spill, where urine sample collection started 2 weeks after cleanup started, our study assessed internal dose of PAHs and benzene beginning on the 2nd day of cleanup, at which time exposure was expected to be close to maximum. To our knowledge, none of the studies of oil spill incidents that incorporated exposure biomarkers had access to urine samples collected on the first few days of cleanup. In addition, our study had a relatively large sample size ($n = 1343$) compared to the three studies from the Hebei oil spill ($n = 121$, $n = 154$, $n = 724$) (Cheong et al. 2011; Ha et al. 2012; Centers for Disease Control and Prevention (CDC) 2015) and the study of D'Andrea and Reddy (2014) from the Deepwater Horizon oil spill ($n = 117$). In the current study, we also used urinary cotinine to adjust for expected confounding effects of smoking on PAH biomarkers.

The current analysis has several limitations. It employs a cross-sectional exposure analysis, thus limiting our ability to assess causal inference. Second, the questionnaire data were initially designed as part of a health surveillance program initiated by the Rayong Provincial Health office, rather than a formal scientific study. As a result, some of the data, such as hours of cleanup participation, smoking status, dietary patterns, and pre-exposure assessment was not complete or unavailable for statistical analysis. Third, we were unable to assess possible confounding effects due to diet, including sorbic acid-containing foods affecting *t,t*-MA (Weaver et al. 2000) and PAH-containing foods, such as broiled and smoked meats, affecting 1-OHPG (Panalaks 1976; Rothman et al. 1990),that may have resulted in either underestimating or overestimating our results. Urinary S-phenyl-mercapturic acid (S-PMA), which is more specific for benzene than *t,t*-MA, might be a better biomarker, however, due to limited funding, this assay was not completed. Fourth, our study did not have an ideal negative control population that was absolutely unexposed to crude oil, such as pre-cleanup baseline measurements of workers, or non-participants who were not involved in the cleanup. For these reasons, it is difficult to assess the magnitude of the increase in levels of PAH and benzene biomarkers among the oil spill cleanup workers on the first days of the spill. In addition, genetic polymorphisms in Phase I enzymes, such as CYP1A1 and CYP1B1 (Shimada and Fujii-Kuriyama 2004), and Phase II enzymes, such as glutathione S-transferases (GSTs), N-acetyltransferase-1 (NAT1) and epoxide hydrolase (EPHX1), might explain some of the variation in the levels of urinary 1-OHPG and *t,t*-MA that we observed.

This study will serve as the baseline exposure assessment and characteristics of workers for future research from the Rayong oil spill cohort. The health follow-up of these workers at Rayong Hospital is ongoing and planned to last 5 years. Since our study found evidence of moderate to high exposure to carcinogenic substances, PAHs and benzene, we believe that long-term surveillance of these workers is prudent.

In conclusion, Rayong oil spill cleanup workers exhibited evidence of elevated levels of PAH and benzene exposure during the early days of cleanup, compared to near-background levels 4 weeks after cleanup began. Certain types of jobs including, oil dispersant applicators, contaminated sand/trash removal workers, and oil vacuum/oil slick removal workers, were at highest risk of PAH exposure. Long-term health monitoring of oil spill cleanup workers should be implemented.

Acknowledgements This study was supported by the Johns Hopkins NIOSH Education and Research Center [T42 OH008428] through a pilot project grant, and funding from the Faculty of Medicine, Prince of Songkla University [REC 57-278-07-1]. We would like to acknowledge the assistance and support of Dr. Pornchai Sithisarakul, Dr. Pitchaya Phakthongsuk, Dr. Chantana Padungtod, Dr. Chanon Kongkamol and Dr. Sunthorn Rheanpumikankit, during the course of this study.

Compliance with Ethical Standards

Conflict of interest The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

Abdel-Shafy HI, Mansour Mona S M (2016) A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Petrol 25(1):107–123. https://doi.org/10.1016/j.ejpe.2015.03.011

Agency for Toxic Substances and Disease Registry (ATSDR) (1995) Toxicological profile for polycyclic aromatic hydrocarbons. In: Chemical sampling information, 369. Online: U.S. Department of Healthand Human Services. http://www.atsdr.cdc.gov/toxprofiles/tp69.pdf

Aguilera F, Mendez J, Pasaro E, Laffon B (2010) Review on the effects of exposure to spilled oils on human health. J Appl Toxicol 30(4):291–301. https://doi.org/10.1002/jat.1521

Alegbeleye OO, Opeolu BO, Jackson VA (2017) Polycyclic aromatic hydrocarbons: a critical review of environmental occurrence and

bioremediation. Environ Manag. https://doi.org/10.1007/s00267-017-0896-2
Altman DG, Royston P (2006) The cost of dichotomising continuous variables. BMJ 332(7549):1080. https://doi.org/10.1136/bmj.332.7549.1080
Centers for Disease Control and Prevention (CDCP) (2015) Fourth national report on human exposure to environmental chemicals. Department of Health and Human Services, pp 1–1095
Chanvaivit S, Navasumrit P, Hunsonti P, Autrup H, Ruchirawat M (2007) Exposure assessment of benzene in thai workers, DNA-repair capacity and influence of genetic polymorphisms. Mutat Res 626:79–87. https://doi.org/10.1016/j.mrgentox.2006.09.007
Cheong HK, Ha M, Lee JS, Kwon H, Ha EH, Hong YC, Choi Y et al (2011) Hebei spirit oil spill exposure and subjective symptoms in residents participating in clean-up activities. Environ Health Toxicol 26:e2011007. https://doi.org/10.5620/eht.2011.26.e2011007
D'Andrea MA, Reddy GK (2014) Health risks associated with crude oil spill exposure. Am J Med 127(9):886 e9-13. https://doi.org/10.1016/j.amjmed.2014.04.035
Ehrenhauser FS, Avij P, Shu X, Dugas V, Woodson I, Liyana-Arachchi T, Zhang Z, Hung FR, Valsaraj KT (2013) Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: laboratory experimental demonstration of the transport pathway. Environ Sci Process Impacts 16:65–73. https://doi.org/10.1039/C3EM00390F
Gunier RB, Reynolds P, Hurley SE, Yerabati S, Hertz A, Strickland P, Horn-Ross PL (2006) Estimating exposure to polycyclic aromatic hydrocarbons: a comparison of survey, biological monitoring, and geographic information system-based methods. Cancer Epidemiol Biomark Prev 15:1376–1381. https://doi.org/10.1158/1055-9965.EPI-05-0799
Ha M, Kwon H, Cheong HK, Lim S, Yoo SJ, Kim EJ, Park SG, Lee J, Chung BC (2012) Urinary metabolites before and after cleanup and subjective symptoms in volunteer participants in cleanup of the hebei spirit oil spill. Sci Total Environ 429(July):167–173. https://doi.org/10.1016/j.scitotenv.2012.04.036
Intawong C, Sithisarankul P (2015) Interlaboratory comparison for urinary *trans,trans*-muconic acid testing in Rayong Province. Thammasat Med J 15(3):363–376
International Agency for Research on Cancer (IARC) (1983) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol 32. Polynuclear aromatic compounds: part 1. Chemical, environmental and experimental data. World Health Organization, International Agency for Research on Cancer, Lyons
Kang D, Rothman N, Cho SH, Lim HS, Kwon HJ, Kim SM, Schwartz B, Strickland PT (1995) Association of exposure to polycyclic aromatic hydrocarbons (estimated from job category) with concentration of 1-hydroxypyrene glucuronide in urine from workers at a steel plant. Occup Environ Med 52(9):593–599
Laemun N, Mutsuji D, Samana K, Srisang J, Saipang T (2014) Lessons from oil spill incident, Rayong, fiscal year 2014. Environmental medicine section. Thai Bureau of occupational and environmental health report, p 67
Laffon B, Pasaro E, Valdiglesias V (2016) Effects of exposure to oil spills on human health: updated review. J Toxicol Environ Health B Crit Rev 19(3–4):105–128. https://doi.org/10.1080/10937404.2016.1168730
Lee SM, Ha M, Kim EJ, Jeong WC, Hur J, Park SG, Kwon H et al (2009) The effects of wearing protective devices among residents and volunteers participating in the cleanup of the hebei spirit oil spill. J Prev Med Public Health 42(2):89–95. https://doi.org/10.3961/jpmph.2009.42.2.89
Liang K-Y, Zeger SL (1986) Longitudinal data analysis using generalized linear models. Biometrika 73(1):13–22. https://doi.org/10.1093/biomet/73.1.13
Melikian AA, Prahalad AK, Secker-Walker RH (1994) Comparison of the levels of the urinary benzene metabolite *trans,trans*-muconic acid in smokers and nonsmokers, and the effects of pregnancy. Cancer Epidemiol Biomark Prev 3(3):239–244
Murphy D, Gemmell B, Vaccari L, Li C, Bacosa H, Evans M, Gemmell C, Harvey T, Jalali M, Niepa TH (2016) An in-depth survey of the oil spill literature since 1968: long term trends and changes since deepwater horizon. Mar Pollut Bull 113(1–2):371–379. https://doi.org/10.1016/j.marpolbul.2016.10.028
Occupational Safety and Health Administration (OSHA) (1999) Petroleum refinining process. In: OSHA technical manual (OTM). U.S. Department of Labor. https://www.osha.gov/dts/osta/otm/otm_iv/otm_iv_2.html
PTT Global Chemical (2013) Summary of PTTGC oil spill incident and execution. http://www.pttgc-oilspill.com/Uploads/Document/20130802-PTTGC-PROGRESS-EN.pdf
Panalaks T (1976) Determination and identification of polycyclic aromatic hydrocarbons in smoked and charcoal-broiled food products by high pressure liquid chromatography and gas chromatography. J Environ Sci Health B 11(4):299–315. https://doi.org/10.1080/03601237609372045
Petchpoung K, Kaojarern S, Yoovathaworn K, Sura T, Sirivarasai J (2011) The influence of metabolic gene polymorphisms on urinary 1-hydroxypyrene concentration in thai bus drivers. Environ Toxicol Pharmacol 31:160–164. https://doi.org/10.1016/j.etap.2010.10.006
Radhakrishna Rao C (1948) Large sample tests of statistical hypotheses concerning several parameters with applications to problems of estimation. Math Proc Camb Philos Soc 44:50–57. https://doi.org/10.1017/S0305004100023987
Rheanpumikankit S, Chanthip I, Naiyana P (2015) Health surveillance for oil spill responders, Praw Bay, Samet Island, Rayong Province. J Prapokklao Hosp Clin Med Educ Center 32(3):229–243
Rothman N, Poirier MC, Baser ME, Hansen JA, Gentile C, Bowman ED, Strickland PT (1990) Formation of polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells during consumption of charcoal-broiled beef. Carcinogenesis 11(7):1241–1243
Shimada T, Fujii-Kuriyama Y (2004) Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci 95(1):1–6
Sithisarankul P, Chanthip I (2015) preliminary report of health effects among oil spill cleanup workers and volunteers, Thaliand, 2013. J Health Res 3(29):197–201
SRNT Subcommittee on Biochemical Verification (2002) Biochemical verification of tobacco use and cessation. Nicotine Tob Res 4(2):149–159. https://doi.org/10.1080/14622200210123581
Strickland PT, Kang D, Bowman ED, Fitzwilliam A, Downing TE, Rothman N, Groopman JD, Weston A (1994) Identification of 1-hydroxypyrene glucuronide as a major pyrene metabolite in human urine by synchronous fluorescence spectroscopy and gas chromatography-mass spectrometry. Carcinogenesis 15(3):483–487
U.S. Coast Guard (2010) U.S. geological survey: deepwater horizon MC252 gulf incident oil budget. National Oceanic and Atmospheric Administration, p 10
Weaver VM, Buckley T, Groopman JD (2000) Lack of specificity of *trans,trans*-muconic acid as a benzene biomarker after ingestion of sorbic acid-preserved foods. Cancer Epidemiol Biomark Prev 9(7):749–755
Zielinska-Danch W, Wardas W, Sobczak A, Szoltysek-Boldys I (2007) Estimation of urinary cotinine cut-off points distinguishing nonsmokers, passive and active smokers. Biomarkers 12(5):484–496. https://doi.org/10.1080/13547500701421341