Biomonitoring of Benzene and Effect of Wearing Respirators during an Oil Spill Field Trial at Sea

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Abstract

Objectives: The main aim of this study was to assess the biological uptake of benzene and polycyclic aromatic hydrocarbons (PAHs) for subjects exposed to fresh crude oil released at sea.

Methods: The study included 22 subjects participating in an «oil on water» field trial in the North Sea. Over two consecutive days there were six releases with two different types of fresh crude oils. Exposed subjects (*N*=17) were either located in small, open air boats downwind and close to the released oil (<50 m) or on the main deck of two large vessels further from the released oil (100-200 m). Subjects assumed to be unexposed (*N*=5) were located indoors on the command bridge of either vessel. Full-shift personal benzene exposure was monitored with passive thermal desorption tubes (ATD-tubes) packed with Tenax TA and subsequent gas-chromatographic analysis. Urine samples were collected before and after work-shift on both days and analyzed for urinary markers of benzene (S-Phenyl Mercapturic Acid, SPMA) and PAHs (1-Hydroksypyrene, 1-OH). Information about the

use of personal protective equipment, smoking habits, location, work tasks and length of work-shift was recorded by a questionnaire.

Results: Subjects located in the small boats downwind and close to the released oil were exposed to relatively high concentrations of benzene (arithmetic mean=0.2 ppm, range 0.002-1.5 ppm) compared to the occupational exposure limits (OELs) for 8 h (1 ppm) and 12 h (0.6 ppm). Although respirators were available to all exposed subjects, SPMA was detected in post-shift urine (0.5-3.3 μmol/mol) of five exposed subjects reporting not wearing respirators, all located in the small boats downwind and close to the released oil. For exposed subjects wearing respirators (*N*=12) the post-shift urinary SPMA was below the detection limit (0.8 μmol/mol) even when the benzene exposure exceeded the OELs. Urinary levels of PAH were within the reference range of what is considered as background levels (<0.4 μmol/mol).

Conclusions: During the initial stages of a bulk oil spill at sea, when the evaporation of benzene is at its highest, it is important to use appropriate respirators to prevent biological uptake of benzene.

Keywords: marine oil spill; benzene exposure; biological monitoring; SPMA; respirators

Introduction

There is a potential health hazard for personnel participating in oil spill operations because of carcinogenic compounds that are present in fresh crude oil including benzene and polycyclic aromatic hydrocarbons (PAHs). Benzene is causally associated with hematotoxic and leukemogenic effects, also after exposure to low concentrations (Vlaanderen et al., 2010; IARC, 2012; Health council of the Netherlands, 2014; ECHA, 2017; Loomis et al., 2017), while PAHs are causally associated with lung, skin and bladder cancer (IARC, 2010; Siddens et al., 2012).

The main routes of human uptake for benzene and PAHs after occupational exposure are inhalation and skin contact. S-Phenyl Mercapturic Acid (SPMA) is a minor metabolite of benzene (about 1 % of total urinary benzene metabolites), but it is a specific and well-known urinary marker of benzene exposure (van Sittert et al., 1993; Farmer et al., 2005). SPMA is also reported to be a sensitive biomarker of benzene exposures as low as 0.1 ppm measured as a time-weighted average (TWA) of 8 h (Boogaard and van Sittert, 1996; Qu et al., 2000). Urinary 1-hydroxypyrene (1-OH), a metabolite of pyrene, has been widely used as a biomarker of exposure to PAHs (Jongeneelen et al., 1985; Jongeneelen, 2001).

Information about benzene and PAH exposure levels during oil spills and cleanup operations and following biological uptake is scarce. Urinary measurements are easy to carry out and preferred over blood and exhaled air, however previous measurements during oil spills have been performed with metabolites that are poor markers of exposure to low concentrations (<1 ppm) of benzene (Lee et al., 2009; D'Andrea and Reddy, 2013). Lee et al. (2009) also measured metabolites of PAHs, but the urine samples were collected two weeks after the spill and therefore the measurements are not valid. In the most recent review of health effects after exposure to oil spills by Laffon et al. (2016) the authors conclude that

there is also a lack of information about the effects of personal protective equipment (PPE) in preventing exposure to carcinogenic compounds.

More knowledge is needed on benzene and PAH exposure levels, biological uptake and the effect of wearing PPE during cleanup of oil spills at sea. Thus, the aim of this study was to assess the concentration of urinary metabolites of benzene (SPMA) and PAHs (1-OH) before and after work-shift among personnel participating in an oil spill field trial at sea.

Methods

Oil release strategy

The field trial was carried out during two consecutive days in June 2016 at the Frigg field in the North Sea, 150 km northwest of Stavanger, Norway. Two different types of fresh crude oils were released, one paraffinic 'light crude oil' (50°API) containing 1.02 wt% benzene, and one 'heavy crude oil' (26°API) containing 0.15 wt% benzene. In total there were six releases of oil with a minimum of 1 h between each release. Two releases of light crude oil (10 m^3 each) followed by three releases of heavy crude oil (4-6 m^3 each) were performed on Day 1 in calm winds (2-5 m/s) and no breaking waves. One release of light crude oil (10 m^3) was performed on Day 2 in moderate winds (6-7 m/s) and breaking waves.

Study population and location

All personnel performing tasks in open air on either of the five boats and vessels involved in the trial (Fig. 1) were invited to participate in the study (Exposed, *N*=18). However, one subject declined to participate, while two exposed subjects only participated on Day 1. A

few subjects not assumed to be exposed to benzene because they stayed indoor on the command bridge on the two vessels were also recruited (Background, *N*=5).

Assumed personal exposure to benzene was assessed *a priori*, based on the location of the boats and vessels, resulting in three exposure groups; 'High exposure', 'Low exposure' and 'Background'. Subjects in the 'High exposure' group (*N*=14) were located in three small, open air 'Man Overboard' (MOB) boats (5-8 m) located downwind and close to the released oil (<50 m). Personnel in these boats were in charge of air and oil sampling or navigation of the boats. Subjects in the 'Low exposure' group (*N*=8) were located on the main deck of two large vessels (75-93 m) in charge of oil release (Norwegian Coast Guard vessel) and oil spill response (Oil Recovery vessel). These vessels were located further from the released oil (100-200 m) and personnel on these vessels performed oil application and ship maintenance. Subjects in the 'Background' group (*N*=5) were located indoors on either of the large vessels doing observations and directing the trial. Five subjects were located a different place on Day 1 than on Day 2.

PPE including disposable chemical protective overalls, nitrile gloves and half-face air purifying respirators with a combination of particle filter (P3) and organic vapor cartridge (A2) were available to all exposed subjects. Use of PPE was recommended by the company in charge of the field trial for personnel assumed to be exposed, but not mandatory. Smoking was prohibited in open air, but each vessel had a designated smoking room. The study was approved by the Western Norway Regional Committee for Medical Research Ethics. Informed written consent was obtained from all subjects.

Sample collection

Air samples

On both days the full-shift personal exposure to airborne benzene was measured with passive Tenax thermal desorption tubes (TD-tubes, Markes int/PerkinElmer, Boston, US-Ma). The methods and results are described by Gjesteland et al. (2017).

Urine samples

Each subject contributed with two urine samples on Day 1 and two urine samples on Day 2. One sample (pre-shift) was collected just before the work-shift started as a baseline measurement, while the other sample (post-shift) was collected just after the work-shift ended. Work-shifts varied between subjects from 5.2 to 14.6 h. Urine was collected in sample tubes (5 mL) containing concentrated hydrochloric acid (HCL, 5 µl) as a preservative and placed in protective cases. All samples were stored at 4°C, and shipped to the analyzing laboratory on ice. All subjects also answered a daily questionnaire before and after work-shift about location, work tasks, length of work-shift, smoking habits and use of PPE.

The urine samples were analyzed for SPMA, a urinary marker of benzene exposure, by high performance-liquid chromatography-mass spectrometry (HP-LC-MS-MS) with a limit of detection (LOD) of 10 nmol/L urine and roughly 0.8 µmol/mol creatinine (method OTOP37). The urine samples were also analyzed for 1-OH, a urinary marker of PAH exposure, by HP-LC-fluorescence with an LOD of 1 nmol/L urine and roughly 0.1 µmol/mol creatinine (method OTOP09). Because benzene and PAHs are present in the main- and side stream cigarette smoke (Darrall et al., 1998; Johnson et al., 2007; Tombolini et al., 2018), cotinine, a urinary marker of direct or passive exposure to tobacco (Haufroid and Lison, 1998), was analyzed by LC-MS-MS with an LOD of 0.06 µmol/L urine. All samples were analyzed by the Health and Safety Laboratory (HSL), England.

Occupational exposure limits (OEL) and biological exposure indices (BEI)

The Norwegian occupational exposure limits (OELs) for benzene is 1 ppm (8-h TWA) and 0.6 ppm (12-h TWA) and the recommended biological exposure index (BEI) is 11.8 µmol SPMA/mol creatinine in end of shift urine (The Norwegian Oil and Gas Association, 2014). No Norwegian OEL for PAHs exist, but the ACGIH (2005) recommends a benchmark guideline of 0.49 µmol 1-OH/mol creatinine in end of shift urine.

Statistical analysis

The results for the two days of sampling were merged in the descriptive analysis. SPMA was below the LOD in the majority of both the pre- (93%) and post-shift (83%) urine samples. Still, these samples were included in the analysis as the LOD/2 (0.4 µmol/mol creatinine), based on the average level of creatinine in urine (Hornung and Reed, 1990). The personal benzene exposure for the three exposure groups (High, Low and Background) is given as the arithmetic mean (AM) and range (minimum and maximum), in addition to the geometric mean (GM) and geometric standard deviation (GSD) due to highly skewed data. Differences between the three exposure groups were analyzed using one-way, independent measures, ANOVA. The correlation between airborne benzene exposure and post-shift urinary SPMA was analyzed by Pearson correlation after log transformation of the data. The results were also stratified by the use of respirator (yes/no) and tested for correlation. The level of significance was set to 0.05. SPSS Statistics 25 for Windows were used for analyzing the data (IBM Inc., Chicago, IL, USA).

Results

A total of 22 subjects participated in the study, of whom 20 subjects participated both days (Table 1). The unexposed subjects were slightly older than the exposed subjects, and only one subject was female. Three subjects reported smoking, between 3 and 10 cigarettes/day. A total of 14 exposed subjects reported wearing respirators during the first hour after release of light crude oil, while five subjects, all located in the small boats downwind and close to the released oil, reported not wearing respirators. Subjects collecting oil samples reported use of gloves. On average the work-shifts lasted for about 10 h in each group, but with large variation within each group (Table 1).

Personal benzene exposure and biological uptake

A total of 42 full-shift personal air samples were collected and the AM and GM benzene exposure of all subjects was 0.2 ppm (range 0.002–1.5 ppm) and 0.05 (GSD=0.006), respectively (Table 2). The benzene exposure differed significantly between the three exposure groups ($p<0.001$), and the mean exposure (GM) was ten times higher for subjects in the 'High exposure' group compared to the 'Low exposure' group.

One subject did not deliver a pre-shift urine sample on Day 1, resulting in a total of 41 pre-shift and 42 post-shift urine samples (Table 2). Urinary SPMA was below the LOD in the majority of the samples (89%) and was only detected in pre-shift urine of three subjects (0.8–1.2 µmol/mol) and post-shift urine of five subjects (0.5–3.3 µmol/mol). The airborne benzene exposure and post-shift urinary SPMA concentration was not correlated ($r= –0.005$, $p=0.98$).

Urinary 1-OH was detected in the urine of only three subjects (0.1–0.3 µmol/mol), all smokers. Cotinine was detected in both pre- (4.6–14.2 µmol/L) and post-shift (1.2–16.0 µmol/L) urine of these smokers. For all non-smokers the cotinine levels were below the LOD.

Effect of respirators

In Figure 2 the personal benzene exposure (upper three plots) and urinary concentration of SPMA (lower three plots) were stratified by the use of respirators (yes/no). The benzene exposure was significantly higher ($p<0.02$) for exposed subjects wearing respirators (AM=0.4 ppm, range 0.003-1.5 ppm) compared to exposed subjects not wearing respirators (AM=0.1 ppm, range 0.002-0.3 ppm).

The benzene exposure on Day 1 and 2 for subjects wearing respirators (*N*=12) were merged (Fig. 2, upper left plot). Two subjects exceeded the 8-h OEL, while four subjects exceeded the 12-h OEL. Urinary SPMA was below the LOD for all of these subjects in both pre- and post-shift urine (Fig. 2, lower left plot), also for the cigarette smoker.

For exposed subjects not wearing respirators (*N*=5) the benzene exposure (Fig. 2, upper right plots) was considerably higher ($p=0.002$) on Day 1 (AM=0.2 ppm, range 0.1–0.3 ppm) than on Day 2 (AM=0.006, range 0.002–0.01 ppm), but the OELs for benzene was not exceeded on either day. Urinary SPMA was detected in the post-shift urine (AM=1.9 µmol/mol, range 0.5–3.3 µmol/mol) for all of these five subjects (Fig. 2, lower right plot), but was not significantly correlated ($r=0.54$, $p=0.14$) with the corresponding airborne benzene exposure.

The highest concentration of SPMA (3.3 µmol/mol) was detected on Day 1, in the post-shift urine of a non-smoker not wearing a respirator. Pre-shift urinary SPMA was detected for this subject on Day 2 (0.8 µmol/mol) and for the two smokers (10 cigarettes/day) not wearing respirators on Day 1 (range 0.9–1.2 µmol/mol).

Discussion

Personnel participating in the oil spill field trial were exposed to relatively high concentrations of benzene compared to the OELs with associated biological uptake. For exposed subjects wearing respirators SPMA was not detected in post-shift urine, even when the benzene exposure exceeded the OEL.

For the five exposed subjects not wearing respirators in the small boats downwind and close to the released oil the full-shift exposure to benzene was below both the 8- and 12-h OEL and the urinary SPMA levels were below the recommended end of shift BEI. The average (AM) post-shift urinary SPMA concentration of these five subjects was 1.9 µmol/mol, while the average benzene exposure was 0.2 ppm. According to ECHA (2017) a benzene exposure of 0.2 ppm should correspond to a post-shift urinary SPMA concentration of about 3.9 µmol/mol based on the mean half-life (9–13 h) of urinary SPMA (van Sittert et al., 1993; Boogaard and van Sittert, 1995; Qu et al., 2000). Although SPMA is suitable for biological monitoring of work-shifts longer than 8 h, the discrepancy between the SPMA levels of this study and the literature may be explained by that for some subjects the urine samples were collected more than 4 h after the exposure to benzene ended.

Although previous studies have shown a correlation between benzene exposure and SPMA levels in post-shift urine (Qu et al., 2003; Farmer et al., 2005) there was no significant correlation in this study because of the use of respirators. There was a positive correlation ($r=0.54$) between benzene exposure and post-shift urinary SPMA among the five subjects not wearing respirators, but presumably due to low statistical power this association was not significant.

Smoking may be a confounding factor at very low benzene exposure levels, but previous results are conflicting. A marked effect of smoking on urinary SPMA (0.2 µmol/mol per cigarette) in subjects not occupationally exposed to benzene was reported by Ghittori et al.

(1999), while other authors report that smoking (up to 30 cigarettes/day) has no influence on the SPMA concentration (van Sittert et al., 1993; Waidyanatha et al., 2004; McNally et al., 2017). Two exposed subjects not wearing respirators with detectable levels of SPMA in post-shift urine were smokers (10 cigarettes/day). However, SPMA was also detected in the post-shift urine of non-smokers, indicating that smoking was not the main source of urinary SPMA.

For subjects wearing respirators the full-shift exposure to benzene ranged from 0.004 to 1.5 ppm. However, the urinary SPMA levels among these subjects were below the LOD (<0.8 µmol/mol), including the subject who reported smoking. This suggests that half-face air purifying respirators with a combination of particle filter (P3) and organic vapor cartridge (A2) prevents biological uptake of benzene during exposure to airborne benzene evaporating from a fresh crude oil released at sea.

Only a few studies have investigated the biological uptake of benzene and PAHs during oil spill cleanup. Significant amounts of urinary phenol was measured among subjects participating in the cleanup of the Deepwater Horizon oil spill (D'Andrea and Reddy, 2013), however, phenol is an unspecific biomarker of benzene exposure (Boogaard and van Sittert, 1995). No significant difference was found in metabolite levels of benzene and PAHs between subjects who wore respirators and those who did not during cleanup of the Hebei Spirit oil spill (Lee et al., 2009). However, urine samples were collected two weeks after the spill and not during the initial high exposure period. Also, the oil was a mix of heavy and medium crude oil (<32°API) with a low content of benzene.

The sampling strategy was among the strengths of this study. The benzene exposure and uptake of individual subjects participating in the field trial was well documented by collecting full-shift air samples in addition to urine samples before and after work-shift. The

study population was small due to a limited number of personnel participating in the field trial. However, repeated measurements of the study subjects over the two consecutive days allowed assessment of the urinary concentration over the time course of the sampling period. Furthermore, the questionnaires provided good documentation of the use of PPE (including respirators, gloves and disposable overalls), work tasks, beginning and end of work-shift, location and smoking habits. The results are relevant for personnel located downwind and close to a bulk spill of fresh oil during oil spill cleanup.

The relatively long work-shifts (up to 15 h) may have affected the post-shift urinary SPMA levels because for some subjects the urine samples were collected several hours after the exposure to benzene ended. Also, the high number of samples with SPMA below the LOD, due to the use of respirators and low personal benzene exposure on Day 2, made it difficult to find significant trends between the benzene exposure and biological uptake.

Conclusions

The results of this study show that during the initial stages of a bulk oil spill at sea, when the evaporation of benzene is at its highest, it is important to use appropriate respirators to prevent biological uptake of benzene.

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Figure 1. Overview of the two large vessels and one of the small, open air boats participating in the field trial.

Table 1. Review of questionnaire presented by the three exposure groups.

	Exposure group	**Location**	**Subjects (*N*)**		**Average age (*range*)**	**Smokers (*N*)**	**Respirator (*N*)**	**Average work-shift, h (*range*)**
			Day 1	**Day 2**				
Unexposed (*N*=5)	Background	Indoors	5	5	45 (*32-63*)	0	0	10.1 (*5.3-14.6*)
Exposed (*N*=17)	Low	Vessels	4	7	34 (*18-57*)	1	5	9.8 (*5.2-12.5*)
	High	Small boats	13	8	38 (*18-58*)	2	9	10.6 (*5.2-14.3*)

N represents number of subjects

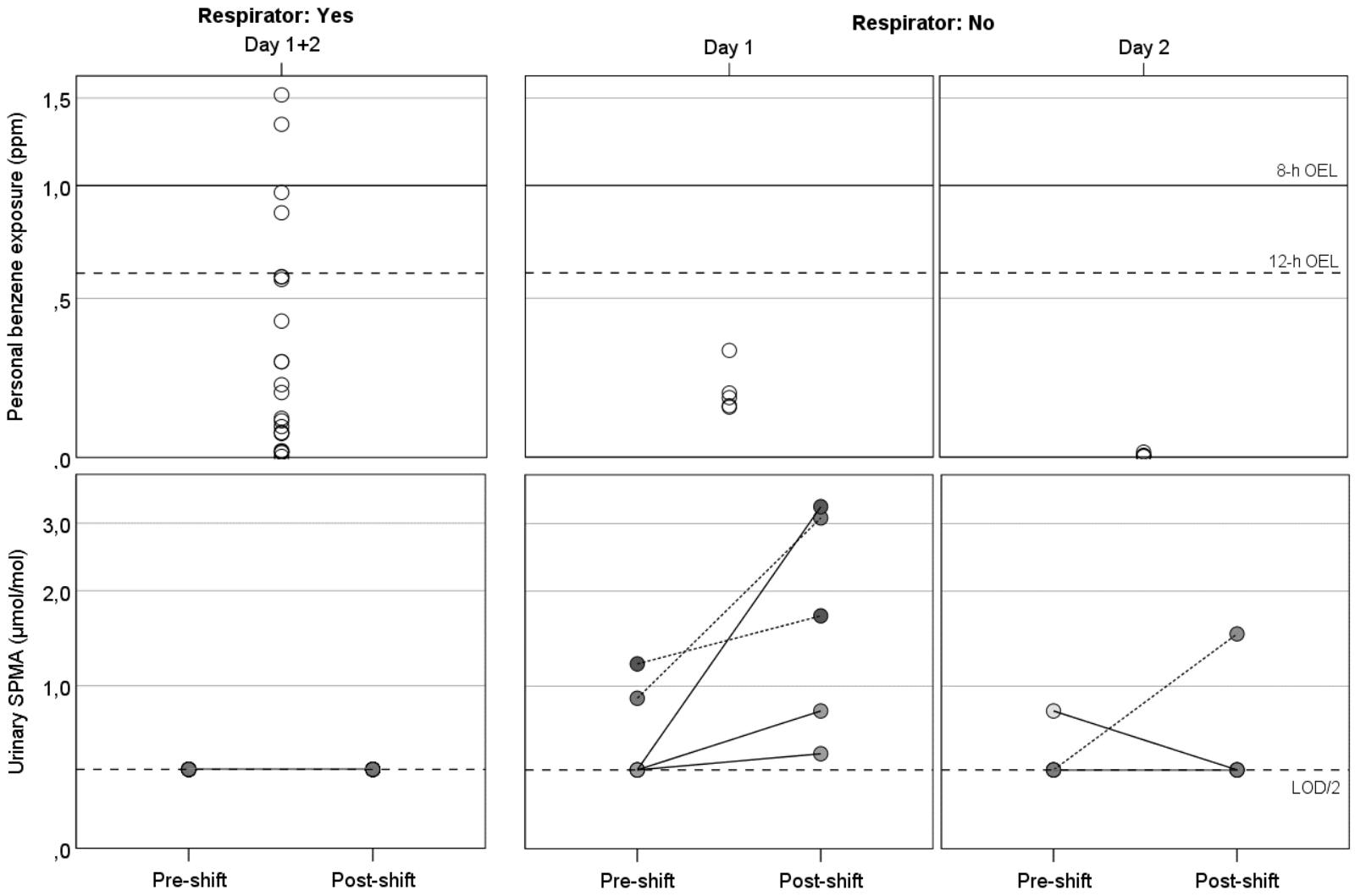

Figure 2. Upper three plots: Full-shift benzene exposure (ppm) on a log scale measured with ATD. The OEL for benzene of 8 h (solid line) and 12 h (stapled line) is included. Lower three plots: Concentration of SPMA in pre- and post-shift urine (µmol/mol) on a log scale. The LOD/2 is included (stapled line) and cigarette smokers are indicated by dotted interpolation lines. The 'Background' group is not included.

Table 2. Environmental and urinary concentration of benzene (ppm) and SPMA (µmol/mol creatinine), respectively, for study subjects (*N*=22) stratified by exposure group.

	Exposure group	**Subjects[a] (*N*)**	**Samples (*n*)**	**<LOD**	**AM[b] (*range*)**	**GM**	**GSD**	***p***
Full-shift	Background	5	10	0	0.01 (*0.004-0.03*)	0.007	0.006	
personal benzene	Low	8	11	0	0.05 (*0.002-0.1*)	0.02	0.07	0.001
exposure (ppm)	High	14	21	0	0.4 (*0.01-1.5*)	0.2	0.4	
Pre-shift	Background	5	10	10	ND	-	-	
urinary SPMA	Low	8	11	11	ND	-	-	0.23
(µmol/mol)	High	14	20	17	0.5 (*ND-1.2*)	0.5	0.4	
Post-shift	Background	5	10	10	ND	-	-	
urinary SPMA	Low	8	11	10	0.5 (*ND-1.5*)	0.5	0.4	0.31
(µmol/mol)	High	14	21	16	0.8 (*ND-3.3*)	0.5	0.5	

LOD: limit of detection, AM: arithmetic mean, GM: geometric mean, GSD: geometric standard deviation, SPMA: S-phenylmercapturic acid, ND: not detected

[a]A few subjects were located a different place on Day 1 than on Day 2, see table 1 for distribution.

[b]The AM, GM and GSD was calculated by including samples with SPMA below the LOD as the LOD/2.