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ORIGINAL ARTICLE

Urinary 1-hydroxypyrene levels in offshore workers

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Abstract
Objectives To compare differences in pre- and post-shift urinary 1-hydroxypyrene (1OHP) levels as a measure of internal dose of polycyclic aromatic hydrocarbons (PAHs) between two groups of oil production workers offshore assumed to be exposed to PAH, and to compare the exposed group to an unexposed control group.
Methods Participants' ($n = 42$) urine samples, collected over a study period of three consecutive 12-h work days (pre-shift on the first day and post-shift on the third day), were analyzed using high performance liquid chromatography (HPLC) with fluorescence detection. Analysis of covariance was used in the statistical models.
Results (1) Post-shift 1OHP levels were significantly higher in the exposed workers compared to the controls. (2) Tank workers and process operators did not show statistically significant different post-shift 1OHP levels.
Conclusion Altogether, this study indicates the presence of a low level PAH exposure among offshore oil production workers.

Keywords Polycyclic aromatic hydrocarbons · PAH · 1-Hydroxypyrene · Offshore · Biological monitoring · Crude oil exposure

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Introduction

Crude oil contains carcinogenic polycyclic aromatic hydrocarbons (PAHs) (International Agency for Research on Cancer (IARC) 1983) to a variable degree depending on the geographic source of the oil. After arriving at an offshore petroleum production facility, crude oil is piped through a closed system where it is separated into oil, gas, water and solid waste (sand and sediment). The amount of PAH in crude oil is generally low, but the crude oil residues in sand/clay (sludge) may contain higher amounts of PAHs (Wang and Fingas 1999). Fossil water trapped with oil and gas in the geologic reservoir and pumped up with the oil also contains PAHs (Faksness et al. 2004; Durell et al. 2006). Although most of the processes on offshore production facilities are performed in closed systems during ordinary operations, the oil production workers may be at risk of exposure to PAH and other aromatic hydrocarbons whenever the system is opened for surveillance and maintenance. Exposure to other aromatic hydrocarbons has been demonstrated among oil production workers (Kirkeleit et al. 2006; Bråtveit et al. 2007). In spite of PAH being a known constituent of crude oil, PAH exposures among these workers have never been investigated before.

The PAHs can enter the body through ingestion, inhalation, and percutaneous absorption. It is therefore advantageous to use biological monitoring of PAH exposure over environmental monitoring to estimate the internal dose from all routes of exposure (Lauwerys and Hoet 1993). The internal dose can be estimated using urinary 1-hydroxypyrene (1-OHP) as a surrogate for PAH exposure. 1OHP is a metabolite of pyrene, which is one of many compounds in the PAH mixture. 1OHP is easily detected in urine and has been extensively studied as a biomarker for monitoring PAH exposure (Jongeneelen et al. 1987; Buchet et al. 1992;

Boogaard and van Sittert 1994). 1OHP urinary elimination in humans is triphasic with half-lives of 5 h, 22 h, and 17 days, and urinary 1OHP level in post-shift urine samples mainly reflect an individual's daily variable PAH exposure, while a pre-shift, before work-week, of urinary 1OHP levels reflect chronic exposures (ACGIH 2005).

The objective of this study was to compare measured urinary 1OHP-levels before and at the end of a 12-h work shift among two groups of oil production workers assumed to be exposed to PAH, and to compare the exposed group to an unexposed control group.

Methods

Study population

The study population included 42 offshore petroleum production workers employed on the Norwegian continental shelf. One of the two exposed groups was tank workers ($n = 13$) recruited from a crude oil production vessel. They performed maintenance work in crude oil cargo tanks (volume 5,000–7,800 m^3) and performed tasks including tank cleaning, tank inspection, scaffold construction, and welding to mend leaks. Before maintenance work started, the tanks were cleaned with hot crude oil and sea water and purged with inert gases and fresh air. However, the empty storage vessels ready for inspection and maintenance had sludge lining the inside of the tank. The tanks were continuously ventilated with forced air. Use of protective suits (Tyvek®) and half-mask air-purifying respirators (APR) with a combination of particle filter and organic vapor cartridge (OV) were mandatory during cleaning and inspection, but not during scaffold building and welding. The protective suits were worn over the cotton coveralls and discarded twice during each shift (before lunch and at the end of the shift). Tank workers were both contractors and permanent employees. While the permanent employees did not perform tank work prior to our study, we do not have information regarding the work that contractors performed before participating in our study. However, the contractors would not perform tank work at least 2 days prior to the pre-shift urine sample because it would take 1 day to get to the installation and 1 day offshore to plan the tank work.

The second exposed group, the process operators ($n = 12$), were recruited from a fixed oil and gas installation, and ran the day-to-day operations on the oil and gas installation. Tasks associated with potential PAH exposures were: (1) sampling and analysis of crude oil, condensate and produced water, (2) sending and receiving pipeline cleaning devices to clean out the sludge lining the inside of the vessels and (3) inspection of and work on the flotation package (handling of produced water) (Bråtveit et al. 2007). The process operators used cotton coveralls during work hours. The process operators were recommended, but not required to use APRs with OV cartridges during tasks associated with open hydrocarbon leading systems (e.g. maintenance, analysis, etc.).

The control group ($n = 17$) was recruited from the same installations as the exposed workers and was matched on shift schedule. All controls worked in the living quarters; two controls surveyed the production from a central control room, while the remaining controls worked in catering or housekeeping. Although a few of the controls could have been exposed to PAH during cleaning of dirty suits, none of the controls were in the area where crude oil or produced water was present.

All participants completed a self-administered questionnaire including questions on age, gender, and whether they were current smokers (yes/no) during the study period. Information on grilled food was not included in the questionnaire. In addition, the process operators maintained a logbook where they recorded their job tasks during the respective shifts.

Informed written consent was obtained from all participants. All subjects were informed about their own results. The study protocol was approved by the Western Norway Regional Committee for Medical Research Ethics, the Data Inspectorate, and the Institutional Review Board of the University of Cincinnati. The Ministry of Health and Care Services in Norway gave permission to transfer urine samples to the University of Cincinnati for analysis.

Urine samples

Urine samples ($n = 81$) used for this analysis were collected as part of a biomonitoring study on benzene and the sampling of urine is described in detail elsewhere (Kirkeleit et al. 2006; Bråtveit et al. 2007). In short, two urine samples were collected from each participant at two points in time: (1) Pre-shift: the morning void before the tank workers entered the tank and in the morning at the heliport before departure to the oil- and gas installation for process operators, and (2) Post-shift, collected immediately at the end of the 12-h shift on the third day of tank work for tank workers, and immediately after work on the 13th day of the offshore work period for process operators. The reason for including a delay between the pre-shift and post-shift samples of at least 3 days was that one of the aims of the original study was to assess whether benzene accumulated during the extended work schedule experienced by offshore production workers. Urine collection times for the controls were the same as for their matched exposed subjects. Pre-shift urine samples were not collected from two process operators due to late enrollment into the study.

Table 1 Descriptive urinary 1OHP (not log-transformed, creatinine adjusted given in μg/g creatinine and un-adjusted values given in μg/l of urine) statistics by exposure group

1OHP variable	Creatinine	Controls ($N = 17$)				Process operators ($N = 12$)				Tank workers ($N = 13$)			
		n	>LOD (%)	GM	GSD	*n*	>LOD (%)	GM	GSD	*n*	>LOD (%)	GM	GSD
Pre-shift	Adjusted	17	24	0.058	0.101	10	40	0.068	0.123	13	69	0.137	0.265
	Un-adjusted			0.014	0.009			0.031	0.038			0.034	0.042
Post-shift	Adjusted	17	12	0.034	0.059	12	17	0.201	0.608	13	69	0.281	0.872
	Un-adjusted			0.016	0.022			0.027	0.041			0.059	0.069

N number of workers, *n* number of urine samples analyzed, *GM* geometric mean, *GSD* geometric standard deviations

Laboratory analysis

The laboratory analyst was blinded to the exposure category and previous results for each individual until the analysis was completed. Urinary 1OHP was analyzed by high performance liquid chromatography (HPLC) (Waters 680 Automated Gradient Controller) with fluorescence detection (Waters 730 Data Module), after enzymatic hydrolysis according to the method of Jongeneelen et al. (1987). The excitation wavelength of the fluorescence detector was set to 242 nm and the emission wavelength to 388 nm. Creatinine was measured in urine samples using a creatinine kit (Stanbio Direct Creatinine LiquiColor Procedure No. 0420) and a spectrophotometer (Beckman Coulter DU800).

Statistical analysis

In the statistical models, the value limit of detection (LOD)/$\sqrt{2}$ was used for results below the LOD (Succop et al. 2004). To account for differences in urine volume between study participants, urinary 1OHP values were creatinine-corrected (μg/g creatinine). The corrected 1OHP levels were not normally distributed (Shapiro–Wilks $P < 0.0001$), and therefore we used a log-transformation using the natural log. Although log-transformed 1OHP levels also failed the Shapiro–Wilks test, the normality of this variable greatly improved. The Studentized residual test was used to detect possible outliers. No statistical outliers were observed when the magnitude of the Studentized residuals was compared to the Bonferroni adjusted value. Tank workers and process operators were grouped into a single exposed group for comparison with the controls. Smoking can give rise to increased urinary 1OHP levels, and was included as a dichotomous covariate (current vs. never or former smokers) in the statistical model. Additional covariates included in the model were age (continuous variable) and pre-shift urinary 1-OHP.

Analysis of covariance (ANCOVA) was performed using the PROC MIXED procedure in SAS version 9.1 (SAS Institute Inc. 2000–2003) to account for repeated urine measurements collected for each study participant. All covariates and interactions of interest were included in the initial models (e.g. smoking × exposure group, age × exposure group). Covariates and interaction terms that were not statistically significant ($P > 0.05$) were removed in a stepwise fashion, beginning with the most complex interaction term. To test the assumption of equal variances among the three exposure groups, the Brown–Forsythe test was performed**.** No statistically significant variance heterogeneity between the three groups ($P > 0.05$) was found.

Pearson's correlation coefficient was calculated to evaluate the relationship between smoking and urinary 1OHP level.

Results

The majority of the workers were men: controls (62.5%), process operators (66.7%), and tank workers (100%). Less than 1/3 of all workers were smokers (controls: 29%, tank workers: 31%, and process operators: 25% smokers). The controls (median age 45 years; range 29.0–60.0) were older than both process operators (median age 44.5 years, range 22.0–59.0) and tank workers (median age 30.8 years; range 27.0–55.0).

Urinary 1OHP data are presented by exposure group in Table 1 both adjusted and not adjusted for creatinine to allow for comparison with other studies. The LOD for the urine analysis was 12 ng/l urine, which is similar to what has been reported for this method by others (Jongeneelen et al. 1987). Although the LOD was low, a large number of samples were still below the LOD (Table 1).

Post-shift 1OHP levels were statistically significantly higher in the exposed workers compared to the controls, after controlling for age and pre-shift 1OHP levels. The final model consisted of exposure group ($P = 0.014$), age ($P = 0.014$), pre-shift 1OHP level ($P = 0.008$), and the interaction of age and exposure group ($P = 0.0004$).

Tank workers and process operators did not show significantly different post-shift 1OHP levels, after adjusting for age and pre-shift 1OHP levels. The final model

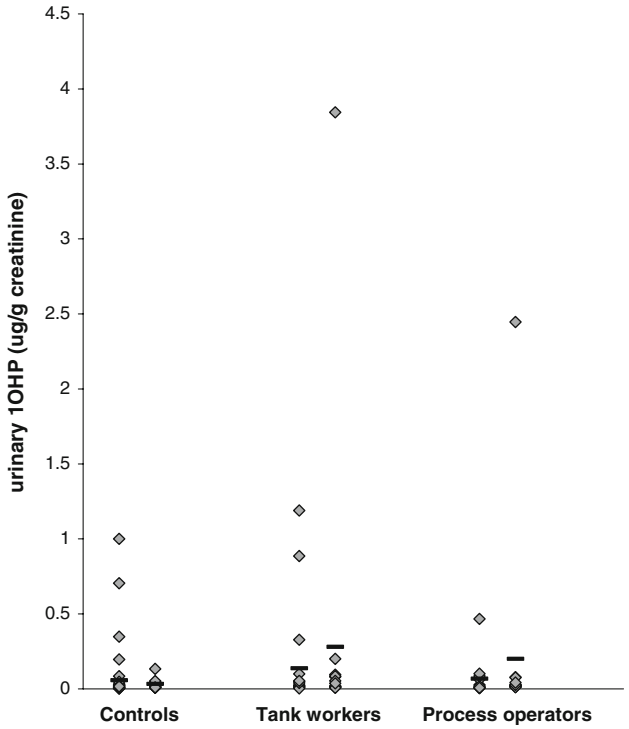

Fig. 1 Urinary 1OHP (μg/g creatinine) by exposure group (controls, tank workers, and process operators) and shift (pre- and post-shift)

consisted of the non-significant exposure group ($P = 0.827$), age ($P = 0.007$), and pre-shift 1OHP level ($P = 0.026$).

Pre-shift 1OHP levels were not statistically significantly different between exposed and control workers, and the final model consisted of the non-significant exposure group ($P = 0.18$) and smoking ($P = 0.02$). In this model, pre-shift 1OHP levels were only associated with smoking; not the exposure group (control, process operator, or tank worker).

Smoking status was statistically significantly correlated with pre-shift urinary 1OHP (Pearson correlation 0.32, $P = 0.04$), but not with post-shift urinary 1OHP (Pearson correlation: 0.27, $P = 0.08$).

Two workers had considerably higher 1OHP levels than the rest (Fig. 1); however, they did not meet statistical criteria to be considered outliers. The tank worker with the highest post-shift urinary 1OHP level was a non-smoker. He had performed miscellaneous tasks in the tank such as scaffold building and being a safety guard for approximately 6–7 h on the last day with no use of respiratory protection. The process operator with the highest post-shift urinary 1OHP level was a smoker performing ordinary process work, but was not reporting any task with a potential for hydrocarbon exposure.

Three of the tank workers were scheduled to perform tank work for all 3 days, but only performed tank work the first of these 3 days. These workers' urinary 1OHP levels did not reflect possible PAH exposures associated with tank work because most of urinary 1OHP would have been excreted post-shift the first day. Excluding these three tank workers from the statistical analysis did not change the outcome (data not shown).

Discussion

Exposed workers had statistically significantly higher urinary levels of urinary 1OHP than the control group. The two exposure groups, process operators and tank workers, did not show differences in 1OHP urinary levels. The internal exposure of PAH, measured as urinary 1OHP not corrected for creatinine, was low compared to the biological exposure index (BEI) for urinary 1OHP (1 μg/l) (ACGIH 2005) in both process operators and in tank workers. The urinary 1OHP levels ranged between 1.6 and 5.9% of the BEI.

All of the offshore-workers' exposure levels were well below what has been reported for many other occupational groups exposed to PAH (Hansen et al. 2008). The controls had similar urinary 1OHP levels as those reported in the German general population (Schulz et al. 2007), while tank workers mean post-shift levels corresponded to levels reported for a general population of smokers in Germany (Wilhelm et al. 2008).

A high percentage of 1OHP levels were below the LOD among all exposure groups (Table 1). Occupational exposures might have been sufficiently controlled by the use of personal protective equipment (PPE) where needed. The highest 1OHP level was measured in post-shift urine belonging to a non-smoking tank worker who did not use respiratory protection. Also, environmental PAH sources such as vehicle exhaust and passive cigarette smoke were practically non-existent due to the location of the oil production facilities on the open sea with no nearby traffic, and to the restrictive smoking enforcement due to fire hazards. Therefore, the high number of non-detects could likely be due to lack of occupational and environmental PAH exposures. In future studies, PPE should be recorded and included in the statistical models as a covariate that may very well be associated with non-detected or very low 1OHP levels.

In our study, post-shift urine samples were collected 3 days after the collection of the pre-shift samples for tank workers, which would presumably maximize the urinary 1OHP concentrations. The time of the peak urinary 1OHP levels is relative to the time of the tank work performed during the shift. If tank work was performed earlier in the shift, then peak 1OHP excretion would be during mid-day and could have been missed. However, due to the ambient temperatures and low vapor pressure of PAH, dermal

exposure probably predominates and this is associated with a slower appearance of urinary 1OHP (Lafontaine et al. 2002).

Age played a significant role in both models. It is not known if the metabolism of pyrene to 1OHP significantly changes with age. Therefore, the importance of age may be better explained by jobs performed: (1) older workers are experienced workers, knowing how to minimize their PAH exposures or (2) younger workers perform the dirtiest jobs. The age relationship could not be further explored due to the small sampling size.

The association between post-shift urinary 1OHP levels and smoking was lower than pre-shift and not significant, implying that urinary 1OHP levels are related to occupational exposures and less to smoking. An explanation for the higher association between pre-shift urinary 1OHP levels and smoking might be that, although the participants were asked to refrain from smoking prior to sampling, smokers among process operators and their controls had unlimited access to cigarettes before collection of the pre-shift urine samples.

Sampling of urine is an efficient and very reliable way of assessing exposure to PAHs, and this method may be an effective way of assessing internal exposure to crude oil derived PAH in this industry. Importantly, the internal dose of pyrene reflects all routes of PAH-exposures, extended work schedules, increased uptake due to physical activity, as well as the inter-individual variations in absorption, metabolism, and excretion of this PAH compound.

Conclusion

Although mean post-shift 1OHP levels among oil production workers was well below the BEI and levels reported from other industries, the urinary 1OHP levels were statistically significantly higher than the levels found among the controls. The low 1OHP levels imply either a low PAH exposure in oil production offshore, that the PPE were sufficiently used where needed and/or a low background levels of PAH offshore. Future studies among offshore oil production workers should include detailed information on PPE, diet, smoking and PAH concentrations in air, crude oil and sludge.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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