Assessment of exposure to polycyclic aromatic hydrocarbons in engine rooms by measurement of urinary 1-hydroxypyrene

Bente E Moen, Ralph Nilsson, Rolf Nordlinder, Steinar Øvrebo, Kristin Bleie, Anne Helene Skørve, Bjørg Eli Hollund

Abstract

Objective—Machinists have an increased risk of lung cancer and bladder cancer, and this may be caused by exposure to carcinogenic compounds such as asbestos and polycyclic aromatic hydrocarbons (PAHs) in the engine room. The aim of this study was to investigate the exposure of engine room personnel to PAHs, with 1-hydroxypyrene in urine as a biomarker.

Methods—Urine samples from engine room personnel (n = 51) on 10 ships arriving in different harbours were collected, as well as urine samples from a similar number of unexposed controls (n = 47) on the same ships. Urinary 1-hydroxypyrene was quantitatively measured by high performance liquid chromatography. The exposure to PAHs was estimated by a questionnaire answered by the engine room personnel. On two ships, air monitoring of PAHs in the engine room was performed at sea. Both personal monitoring and area monitoring were performed. The compounds were analysed by gas chromatography of two types (with a flame ionisation detector and with a mass spectrometer).

Results—Significantly more 1-hydroxypyrene was found in urine of personnel who had been working in the engine room for the past 24 hours, than in that of the unexposed seamen. The highest concentrations of 1-hydroxypyrene were found among engine room personnel who had experienced oil contamination of the skin during their work in the engine room. Stepwise logistic regression analysis showed a significant relation between the concentrations of 1-hydroxypyrene, smoking, and estimated exposure to PAHs. No PAHs were detected in the air samples.

Conclusion—Engine room personnel who experience skin exposure to oil and oil products are exposed to PAHs during their work. This indicates that dermal uptake of PAHs is the major route of exposure.

Key words: engine room; 1-hydroxypyrene; polycyclic aromatic hydrocarbons

Several epidemiological studies have shown an increased risk of both lung cancer and bladder cancer among machinists compared with the general population. This increased risk may be due to smoking, but occupational factors have been suggested as additional aetiological factors. A known occupational carcinogenic factor in engine rooms is asbestos, as several ships have used this type of insulation material. This is hardly a problem today, as other types of insulation material are used. Other possible carcinogenic factors are polycyclic aromatic hydrocarbons (PAHs). Exposure to these compounds may occur during work with oil and oil products in the engine rooms. Fuels used in marine engines can differ greatly in content of PAHs, depending on the origin and contamination of the fuel. Several fuel oils are known to have high concentrations of these compounds.

PAHs can also be formed during combustion in the engine room, and arise from exhaust gases and soot. Exhaust gases may also contain nitroaromatics. Several individual PAHs have been shown to have carcinogenic or mutagenic activity, or both, and these compounds may be related to the increased risk of both lung and bladder cancer.

The risk of cancer associated with work as a machinist may therefore be related to occupational exposure to PAHs in the engine room. Exposure to fuel oils and combustion products probably takes place in engine rooms even today. Therefore, it is of interest to evaluate the present exposure to PAHs.

Polycyclic aromatic hydrocarbons are metabolised to many different compounds. Most of these are excreted in the faeces and only a minor fraction is excreted in the urine. Pyrene, however, is almost completely metabolised to 1-hydroxypyrene and conjugates, which are excreted in the urine. The content of pyrene is often high in PAH mixtures, and 1-hydroxypyrene has been used as a variable for the biological monitoring of exposure to compounds containing PAHs.

Exposure to PAHs may occur by inhalation, by dermal exposure, and by oral uptake. Considerable variation between people has been found in the peak urinary excretion of 1-hydroxypyrene after exposure to compounds containing PAHs, with a mean (range) of 10-4 (3-9–26-7) hours. This makes the exposure to compounds containing PAHs in the past 24 hours relevant to the urinary analysis of this metabolite.

Data on exposure to compounds containing PAHs in engine rooms are scarce. This study was performed to evaluate the exposure to PAHs among engine room personnel on ships today, and the need for improvement of their working environment to reduce this type of chemical hazard.
Material and methods
SELECTION OF SHIPS AND THE STUDY POPULATION
The engine rooms on ships and the working conditions for the personnel in these rooms may differ. In earlier days, the engine room was built as one single room with the engines, the separators, and a small workshop. The engine room crew spent most of their working hours inside this room. In ships built today, the engine room is often divided into different rooms; rooms for the engines, separator room, workshop, and control room. The crew spend most of their working hours in the control room.

Ten ships, five arriving in a large Norwegian harbour, four arriving in a large harbour in Sweden, and one during maintenance work at a shipyard in France, were chosen for the present study. There were five passenger ships, two roll on roll off ships, two product tankers, and a container ship. The ships were built between 1956 and 1993 and the size ranged from 5000 to 50 000 dead weight tonnes.

Four of the ships were chosen because they were old, with a separate control room. The other ships were chosen at random. All ships were visited on randomly selected days. We asked for permission to visit the ships and collect the samples shortly before the ships arrived in the harbour.

All the engine room personnel on the ships were included in the study. A control group was established by including a similar number of men from the crew on each ship who did not work with oil, oil products, or other sources of exposure to PAHs. Nobody refused to participate in the study.

SAMPLING OF URINE AND ANALYSIS OF 1-HYDROXYPYRENE
Each participant of the study answered a questionnaire with questions about age, occupation, exposure to possible sources of PAH the past 24 hours, use of gloves, overalls, and personal respiratory protection equipment. Participants also answered questions about smoking habits, as tobacco smoke may influence the results.

Also, 1-hydroxypyrene may be increased by the intake of food rich in PAHs. However, as the personnel from the engine room and the control group had the same food on the ships, the diet was unlikely to influence the results to any large extent. Therefore, no questions concerning the diet were included in the questionnaire.

A sample of urine (50 ml) was obtained from each person. The samples were immediately frozen at −20°C and remained so until laboratory analysis of the urine. Each sample was coded and analysed without knowledge of the person’s name and exposure status. The method used for measurement of 1-hydroxy-pyrene in urine was that described by Jongeneelen.22 Urine (10 ml) was diluted with acetic acid, the pH adjusted, and incubated for 16 hours after addition of an enzyme mix of glucuronidase and sulphatase. This mixture was applied on a Sep-pak C18 cartridge (Millipore, Milford, MA). The cartridges were washed with water and eluted with 4 ml methanol. The eluate (20 μl) was injected into a high performance liquid chromatograph with a Novapak C18, column. The elution was with a methanol/water gradient and detection with a fluorescence detector (Perkin-Elmer, Beaconsfield, UK) at 242 nm (excitation) and a 288 nm (emission). Five spiked control urine samples were used as standards with addition of 10, 20, 40, 100, and 250 nmol/l 1-hydroxypyrene (Jansen Chimica, Beerse, Belgium). Measurement was performed with Millennium integration software. All measurements were corrected for creatinine.

PAHS IN WORKROOM AIR
The concentrations of PAHs in the engine rooms on two different passenger ships were monitored. In one of the ships, built in 1956, the engine room consisted of a single room where both the engines and the engine room crew were located. The other ship, built in 1993, had a control room where the crew was located most of the time. The monitoring of PAHs was performed during two similar sea voyages along the Norwegian coast.

Engine room personnel on duty during the voyage were monitored for PAHs. Area monitoring was carried out at a minimum of two positions in each engine room, at places were PAH exposure was likely to occur and where the engine room crew used to stay during their work. The sampling was performed for two or three working shifts. The compounds containing PAHs (both gasous and particulate phases) were collected on an acrylic copolymer filter (Versapor 800, Gelman Sciences, Ann Arbor, Michigan) and XAD-2 in series. Medium flow pumps (DuPont a-1 and DuPont P/S 2500, DuPont, Largo, Florida and Casella AFC 123-1S, Casella, London, England) maintained at a flow rate of 2 l/min were used for the sampling.

The compounds containing PAHs were extracted from XAD-2 with dichloromethane after addition of internal standards. The filters were extracted with cyclohexane in an ultrasonic bath after addition on internal standards. Polar compounds and PAHs were transferred to N,N-dimethylformamide with 3% water. After dilution with water, the PAHs were extracted back into cyclohexane. The extracts were dried with water free Na2SO4 and concentrated. Both types of extracts were analysed splitless on an HP 5890 series II gas chromatograph with flame ionisation detector. The column used was a 25 m CP-sil-8 CB (inner diameter, 0.25 mm; film thickness, 0.25 μm) and the oven was programmed from 35 to 310°C, with a run time of 90 minutes. Table 1 shows the analysed components. The detection limit was 13-6 ng/m3 for pyrene and 10 ng/m3 for benzo[a]pyrene (0-4 μm air sample). All of the analysed components had a detection limit <1 μg/m3 (1 m3 sample).

STATISTICAL METHODS
For categorical data, χ2 tests were performed to compare groups. For continuous data, non-parametric tests were used, as the data were not normally distributed. An arbitrary cut off point
for 1-hydroxypyrene (0.2 µmol/mol creatinine) was chosen and a stepwise logistic regression analysis was chosen to evaluate the relation between 1-hydroxypyrene, exposure, smoking, and age.

The exposure of the crew to PAHs was categorized according to their answers on the questionnaire. The categories were unexposed, meaning no known exposure to PAHs in the past 24 hours, exposed degree I, meaning that they had been working inside the engine room during the past 24 hours; and exposed degree II, meaning that they had been working in the engine room during the past 24 hours and had experienced contamination of the skin with oil during this period.

### Results

#### MONITORING OF URINE

A total of 98 samples of urine were collected, 51 from personnel who had been working in the engine room and 47 from a control group on the same ships who had not been exposed to compounds containing PAHs in the engine room. The crew were working for periods of four or six weeks on the ship, and in shifts of four or six hours. The control group was older than both the exposed groups (exposed degree I and exposed degree II: Mann-Whitney rank sum test, P = 0.02). No significant difference was found between the groups for smoking habits (table 2; $\chi^2$ test, P = 0.4).

None of the engine room personnel had used personal respiratory protection equipment in the past 24 hours. Two people had used gloves for a short period during work with oil and grease in a motor, but they had not used gloves all the time during this work. All but two people had used overalls during the work.

A significantly higher urinary concentration of the PAH metabolite 1-hydroxypyrene was found among personnel who had been working in the engine room during the past 24 hours (exposed groups, degrees I and II), compared with unexposed personnel on the ships (Mann-Whitney rank sum test, P < 0.0001). As smoking may interfere with the results, a comparison of exposed (both degree I and degree II) and unexposed non-smokers was performed as well, showing a similar difference (Mann-Whitney rank sum test, P = 0.02). Comparing non-smokers of degree I with non-smokers of degree II, a significant difference was found between the groups (Mann-Whitney rank sum test, P = 0.04). No significant difference was found between the urinary concentrations of 1-hydroxypyrene in the unexposed non-smokers and those of the non-smokers in the exposed group, degree I, (Mann-Whitney rank sum test, P = 0.72). The urinary concentrations of 1-hydroxypyrene in the non-smokers of the exposed group were significantly lower than those of the non-smokers in the exposed group, degree II, (Mann-Whitney rank sum test, P = 0.01). The figure shows the concentrations of 1-hydroxypyrene in the different groups.

Significant relations between the concentration of 1-hydroxypyrene (cut off point set to 0.2 µmol/mol creatinine) and both exposure (P < 0.001) and smoking (P < 0.001) were found by a stepwise logistic regression analysis. No significant relation between age and 1-hydroxypyrene was found (P = 0.8).

The urinary concentrations of 1-hydroxypyrene of both smokers and non-smokers among the exposed personnel on the old ships were not significantly different from those of the exposed personnel on the new ships. Also, no significant difference was found between the non-smokers in these groups.

#### AIR MONITORING

Air monitoring was performed on two passenger ships, built in 1956 and 1993. Table 3 shows the information about the ships and the work performed in the engine rooms during the monitoring period.

Altogether 19 samples were collected. For each series (each ship), there was one blank. Three of the samples were destroyed by a technical failure, giving a total number of 16

### Table 2

<table>
<thead>
<tr>
<th>Smoking habits</th>
<th>No exposure to PAHs (n = 47)</th>
<th>Exposure to PAHs (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6–10</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>11–20</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
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Discussion

This study shows a significant relation between an estimated exposure to PAHs during work in the engine rooms of ships and the concentration of the PAH metabolite 1-hydroxypyrene in urine. The excretion of 1-hydroxypyrene was significantly increased in a group of engine room personnel who had experienced dermal exposure to oil products compared with a group of unexposed seamen. Estimation of the exposure was based on self-observed exposure to the skin and probable inhalation of the PAHs during work with oil and oil products in the engine rooms. Monitoring of PAHs in the air of the engine rooms did not show any such compounds present. This suggests that the engine room crew receive their exposure to compounds containing PAHs mostly by dermal contamination, and less by inhalation. Oil contamination of the skin during special types of work seems to cause the major exposure to compounds containing PAHs in the engine rooms.

Standardised and established methods were used for the sampling and analyses of 1-hydroxypyrene, and methodological errors are unlikely in the present study.

The temperature in engine rooms may be high, and it is probable that this may affect the urinary concentration of the personnel working in the engine room. This may influence the concentration of the urinary metabolite, but was corrected, as the concentration of 1-hydroxypyrene was adjusted for creatinine.

Several factors are known to influence urinary concentrations of 1-hydroxypyrene. Our results confirm the importance of cigarette smoking. The relation between smoking and urinary concentrations of 1-hydroxypyrene has been shown in several studies. Age in our study was not found to have any relation with the concentration of urinary 1-hydroxypyrene. This effect has been suggested, but no significant relations have been found.

The ships included in this study were chosen with no previous knowledge about the engine or the exposures to compounds containing PAHs. All shipowners asked allowed their ships to participate in the study. The information obtained should therefore be without any bias from shipowners or crew.

The overall values of 1-hydroxypyrene found in this study were lower than those found in other occupational groups with exposure to compounds containing PAHs. Workers exposed to creosote have been shown to have concentrations of 1-hydroxypyrene up to 84 μmol/mol creatinine, and coke oven workers up to 11 μmol/mol creatinine. However, our data are similar to values found among car repair workers. The exposure of this type of worker is more comparable with personnel from engine rooms than the exposure of creosote workers or coke oven workers. Both automobile repair workers and personnel from engine rooms perform mechanical repair work, and are known to experience skin contamination by oil and oil products during this work. They may be exposed to compounds containing PAHs by dermal exposure, as well as by inhalation of oil mist or combustion products. The types of compounds containing PAHs present in the environment of car mechanics may also be more similar to those found in the engine rooms than in the environment of creosote workers and coke oven workers.

The concentrations of 1-hydroxypyrene found in the present control group was similar to the values found in unexposed groups from other studies. The control group in the study of car repair workers showed a mean concentration of hydroxypyrene of 0.08 μmol/mol creatinine among non-smokers and of 0.16 μmol/mol creatinine among smokers. In our study, equivalent values were 0.09 and 0.14. In a Canadian study of urinary 1-hydroxypyrene in non-occupationally exposed people, equivalent values were 0.07 and 0.12 μmol/mol creatinine. A Danish study showed a mean concentration of urinary 1-hydroxypyrene of 0.012 μmol/mol creatinine in a population with no occupational exposure to compounds containing PAHs.

To our knowledge, there are no previously published studies of the exposure to compounds containing PAHs in engine rooms. However, monitoring of occupational exposure has been performed in Finland, and researchers have described the exposures to compounds containing PAHs as low. A Norwegian study reports similar information. In that study only traces of PAHs were detected in the air of the engine rooms, although these compounds were shown to be present in the fuel and oil products used in the engine room environment. This supports our findings, with almost no compounds containing PAHs present in the air, not even during maintenance work with visible oil spill in the working area.

On the other hand, the engine room personnel may sometimes perform other types of work which may cause exposure to compounds containing PAHs in the air. These types of work have not been monitored in the present study.

The monitoring periods of the air samples were short, due to the duration of the shifts.

Table 3 Description of two Norwegian passenger ships and their engine rooms where air monitoring of compounds containing PAHs was performed

<table>
<thead>
<tr>
<th>Description of two Norwegian passenger ships</th>
<th>Monitoring of compounds containing PAHs performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date built</td>
<td>1956</td>
</tr>
<tr>
<td>Gross tonnage</td>
<td>2200</td>
</tr>
<tr>
<td>Engines</td>
<td>1 main engine</td>
</tr>
<tr>
<td></td>
<td>3 auxiliary engines</td>
</tr>
<tr>
<td>Control room</td>
<td>No control room</td>
</tr>
<tr>
<td>Room for separators</td>
<td>No</td>
</tr>
<tr>
<td>Fuel</td>
<td>Motor special distillate (MSD)</td>
</tr>
<tr>
<td>Maintenance work performed by a mechanic and</td>
<td>by a mechanic and</td>
</tr>
<tr>
<td>machine during the first shift.</td>
<td>during the first shift.</td>
</tr>
<tr>
<td></td>
<td>the control room. Few and short visits in the engine room</td>
</tr>
</tbody>
</table>

analysed samples. Nine of these samples were stationary, and seven were personal samples. The sampling period was restricted to six hours, as this was the duration of the workshifts on board. No PAHs were detected in any of the samples.
Sampling periods less than eight hours are not unusual in occupational settings, as special working periods may have varying durations. Short sampling periods may reduce the chance of finding compounds in the air. However, the risk of not detecting PAHs in the air of the engine room was reduced in this study by the low detection limits of the PAHs.

Although several compounds containing PAHs are classified as carcinogenic, it is difficult to evaluate the health risk connected to the concentrations of 1-hydroxypyrene found among personnel working in engine rooms. Little knowledge exists as to what concentration compounds containing PAHs actually will cause cancer. However, as exposure to compounds containing PAHs seems to be present in this occupational environment, a small increased risk of developing occupational cancer may be present. It is wise to reduce the present exposure as much as possible. This can probably be done easily in the engine rooms, by reducing the dermal exposure to oil and oil products by improved protection of the skin.

Conclusion

Seamen who had been working in the engine room for the past 24 hours, had a significantly higher concentration of 1-hydroxypyrene in the urine than unexposed seamen. The highest concentrations of 1-hydroxypyrene were found among engine room personnel who had experienced oil contamination of the skin during their work in the engine room. No PAHs were detected in air samples from the engine rooms. As oil contamination of the skin is known to be a possible source of exposure to PAHs, the results indicate that dermal uptake of PAHs may occur during work in engine rooms.

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