Original article

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The occupational exposure of dermatology nurses to polycyclic aromatic hydrocarbons – evaluating the effectiveness of better skin protection

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Objectives We studied the uptake of polycyclic aromatic hydrocarbons (PAH) in nurses who apply ointments containing coal tar to patients and investigated the effectiveness of skin protection methods.

Methods We determined gas-phase PAH on XAD-2 and particle-associated PAH on filters. We also used pads to determine PAH on the skin. Pyrene and benzo(a)pyrene were analyzed by gas chromatography/mass spectrometry and gas chromatography/tandem mass spectrometry; their respective urinary metabolites 1-hydroxypyrene and 3-hydroxybenzo(a)pyrene were analyzed using high performance liquid chromatography with fluorescence detection.

Results We ruled out the inhalation of airborne pyrene and benzo(a)pyrene as the sources of PAH exposure. However, substantial amounts of pyrene and benzo(a)pyrene were observed on the hands of the nurses (median 33.0 and 16.4 ng/cm^2, respectively). Excretion of urinary 1-hydroxypyrene indicated an increased uptake of pyrene in 8 out of 12 nurses. We asked 35 nurses to perform a treatment with gloves followed by a second treatment without gloves. The use of gloves changed the excretion of 1-hydroxypyrene by -0.58 μmol (range -5.1–1.0 μmol), corresponding to a median reduction of 51.5% ($P<0.001$). Based on this finding, a new protocol was adopted, involving the permanent use of vinyl gloves and Tyvek sleeves. The effectiveness of this protocol was tested against pre-existing work practices and showed a 97% reduction in skin contamination with pyrene and benzo(a)pyrene, and a lowering in urinary excretion of 1-hydroxypyrene of 57%.

Conclusion Protecting the skin more stringently reduced pyrene and benzo(a)pyrene contamination of the hands, and lowered urinary excretion of 1-hydroxypyrene.

Key terms coal tar; benzo[a]pyrene (BaP); pyrene (PYR); skin absorption; urinary excretion; biological monitoring; gloves.

Coal tar has been used in dermatology for many decades. Although evidence from controlled trials is scarce, dermatologists consider (short-term) coal tar treatment of psoriasis and atopic dermatitis to be relatively safe and efficacious (1–3). Coal tar is a complex mixture of organic compounds that is not completely chemically characterized. It contains polycyclic aromatic hydrocarbons (PAH) including some human carcinogens (4). Coal tar is a second choice topical therapy. It is used to support first line treatment with dermatocorticosteroids or topical calcineurin inhibitors (in case of eczema), and dermatocorticosteroids and/or vitamin D derivatives (in case of psoriasis). Dutch dermatologists mainly use coal tar in the treatment of eczema (72% versus 48% in Belgian Flanders), whereas in Flanders, coal tar is mainly prescribed for psoriasis (60% versus 41% in The Netherlands) (5–6).

Patients treated with coal tar ointment (CTO) excrete mutagenic metabolites in urine (7). A component of CTO, pyrene is metabolized to 1-hydroxypyrene (1-OHP) and has been observed in high concentrations in the urine of patients treated for psoriasis (8). Pyrene and

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1-OHP, although not mutagenic, can be used as markers for total poorly volatile (particulate) PAH exposure and as indicators for systemic exposure to mutagenic PAH (9). Also benzo[a]pyrene (BaP) and 3-hydroxybenzo(a)pyrene (3-OHBaP) have been proposed as markers for external and internal exposure to carcinogenic (high molecular weight) PAH, respectively (10).

Several studies have shown an increased risk of skin and internal cancer in subjects with long-term coal tar exposure (8–18). An increase in cancer risk following long-term occupational exposure, although not established for dermatology nurses, cannot be ruled out per se. Therefore, exposure to PAH in nurses applying tar products to patients' skin must be limited as much as possible. In our hospital, we advise our nursing personnel to wear gloves when applying tar. However, applying tar with gloves is not always convenient for the patient and the nurses are inclined, especially in the treatment of children, to ignore this advice.

We did not know to what extent wearing gloves protects against coal tar exposure. To investigate the possible uptake of PAH when administering CTO, we studied the exposure of nurses involved in the topical application of CTO in the dermatology clinic. This paper summarizes our efforts to determine pyrene and BaP levels in the air around and on the skin of nurses, and the excretion of their respective metabolites 1-hydroxypyrene (1-OHP) and 3-hydroxybenzo[a]pyrene (3-OHBaP) in urine as biomarkers of internal exposure. This study was aimed at investigating a possible increased occupational uptake of PAH and finding out about the effectiveness of a more adequate and rigorous protocol of skin protection.

Study population and methods

Subjects

The nurses who volunteered to participate in the study signed a consent form stating they confirmed to have received information about the objectives of the study. The results of the urine analyses were given to the occupational physician, along with a list of the names of the workers and the personal codes. This was so that the results of the biological monitoring study could be added to the medical files and be available to the nurses on request.

The subjects were studied on the day that they treated one or more patients and during the following day(s). During the sampling process, the nurses were asked to refrain from consuming smoked, fried, grilled, or barbecued fish or meat in order to prevent the influence of diet on the pattern of urinary excretion of 1-OHP and 3-OHBaP (8–9, 19–20).

Study design

Four different studies were performed. In each study, the nurses were observed following the administration of one or more treatments of CTO per shift. A single treatment normally takes 10–30 minutes. The first study (A) was designed to investigate the contribution of inhalation and skin absorption as the two most obvious routes of uptake of PAH due to occupational exposures. During a subsequent study (B), we followed three nurses during a prolonged period of time and collected spot urine samples in order to study the relationship between work tasks and urinary excretion pattern of PAH-metabolites. In a third study (C), we followed 35 nurses on two separate occasions involving CTO applications. This treatment was carried out on the same patient, so the amount of applied CTO, body parts treated, and the duration of the treatment were similar. On the first occasion, protective gloves were used and, during the second occasion, no gloves were used. By comparing these two situations, we were able to quantify how wearing gloves can reduce the skin absorption of PAH. In a fourth and last study (D), a new work procedure was tested for its effectiveness in protecting the nurses from the exposure of hands and forearms by using vinyl gloves (Flexam vinyl, supplied by Cardinal Health, Dublin, OH, USA) combined with Tyvek® narrow sleeves (model PS32LA, Du Pont Personal Protection, Amsterdam, The Netherlands). The effectiveness of this new work procedure was tested against results of urinary excretion of 1-OHP obtained in study A. An overview of the study components is presented in table 1.

Questionnaires

Using a questionnaire, we collected information on each nurse's occupational history and smoking habits, and uptake of PAH from the diet and exposure to sources of indoor and outdoor open fire, among others. This questionnaire also contained a number of questions generally describing working practices. A second questionnaire was completed on each day of urine collection. This questionnaire covered diet, active smoking, consumption of alcoholic beverages, use of medication, and some more specific questions about daily work practices (eg, the amount and type of CTO administered to the patient, the use of gloves and protective garments, and personal hygiene such as the time between CTO administration and washing of hands).

Treatment with coal tar ointment

Coal tar is applied either directly to lesions, in the case of limited disease, or diffusely if the lesions are disseminated. In the dermatology clinic, two different

Table 1. Overview of study parameters. (AM = arithmetic mean, PAS = personal air sampling)

Study	N			Inhalation exposure	Skin exposure	Type of skin protection	Treatments AM		Urine collection	
	Total	Women	Men				N	Range	Period	Type of sample
A	12[a]	9	3	PAS	Pads	With loose-fit polyethylene gloves	1.2	1–2	48 hours	Total void
B	3[b]	3	0	·	·	With and without loose-fit polyethylene gloves	7.0	6–8	23 days	Spot sample
C	35[b]	30	5	·	·	With and without loose-fit polyethylene gloves	1.0	··	10 hours	Total void
D	16[a]	12	4	·	Pads	With tight-fit vinyl gloves and Tyvek® narrow sleeves	2.4	2–4	24 hours	Total void

[a] Three smokers.
[b] All non-smokers.

types of CTO are applied in various formulations. Used in the treatment of chronic eczema, "dark" colored CTO contains 5% crude coal tar, 965 μg/g pyrene and 455 μg/g BaP (the ratio of BaP to pyrene is 0.47). "Light" colored CTO, use to treat mild dermatitis and psoriasis, contains an alcoholic extract of crude coal tar (10% solution liquor carbonis) with a much lower PAH content (3.0 μg/g pyrene and 0.13 μg/g BaP, with a BaP to pyrene ratio of 0.043). The nurse noted the type of CTO used and determined the amount applied by weighing the container of CTO before and after each single treatment. From the total amount of CTO and the type of CTO used, the total amount of pyrene and BaP was calculated.

Air samples

Task-based measurements of volatile and particulate PAH were conducted only during the treatment of the patient (approximately 10–60 minutes per patient). Volatile PAH were collected on Amberlite XAD-2 (Sigma-Aldrich Chemie, Zwijndrecht, the Netherlands) placed in the breathing zone of each nurse. The cartridge was connected to an automated electronic personal air sampling pump (DuPont Alpha-1, Deha International, Huizen, the Netherlands) operated at a flow of 0.20 l/min. Airborne particle-associated PAH were monitored using a Teflon filter (Type TE38, Schleicher and Schüll, Dassel, Germany) placed in an air sampling probe for the collection of inhalable particles (according to EN481). Air was drawn through this filter at a flow of 23.6 l/min using a medium volume air sampling pump (Environmental Monitoring Systems, Charleston, SC, USA).

Skin pads

Skin pads were homemade using polypropylene membrane filters embedded in self-adhesive Fixomull stretch elastic bandage (BSN Medical, Almere, the Netherlands); see Jongeneelen et al (21) for a more detailed description. These pads were attached to the skin on three standardized places on the dominant hand: (i) the lateral side of the index finger, (ii) the hand palm, and (iii) the lateral side of the wrist. The pads were attached just before the start of the treatment of the patient with CTO and removed again directly upon completion of the treatment. The pads were wrapped in aluminium foil and kept at -20° C until analysis.

Collection of urine samples

Urine was collected in 250 ml polyethylene (plastic) bottles with screw caps and a label registering the name and date of birth of the subject, and the date and time of the sample collection. Each subject received a sheet with instructions for urine collection. Urine was first collected in a measuring beaker (500 ml) and the total volume of the void was written down on a registration form. An aliquot of the sample was transferred to the bottle and the residue was flushed away. Urine samples were kept in a cool and dark place, and taken to a central storage facility at the Dermatology department. Three different schemes for urine collection were used. In study A, we collected all the urine samples on the same and consecutive day until 48 hours after the time of CTO application. In study B, spot urine samples were collected between 9:00–22.00 hours (post-shift). In study C, a pre-shift spot sample was collected as the first void after awakening. To reduce the workload for the volunteers in this study, urine samples were collected between 8.00–18.00 hours following the application of the CTO. In study D, we used the design of study A and modified only the period of urine collection to 24 instead of 48 hours since these samples were not analyzed for 3-OHBaP.

Analysis from air filters and skin pads

The polypropylene filters were punched from the skin pads and extracted in 10 ml of dichloromethane (high performance liquid chromatography grade purchased from Lab-Scan, Gliwice, Poland). The filters were vortexed for 1 minute followed by 15 minutes in an ultrasonic bath. The Teflon air filters and XAD-2 solid sorbent cartridges were extracted following the same procedure. After removing the filters, the extracts

were evaporated under a gentle stream of nitrogen at 25°C. The residue was re-dissolved in toluene (Merck, Darmstadt, Germany) and injected on the gas chromatography/mass spectrometry and gas chromatography/tandem mass spectrometry (GC/MS/MS) system (consisting of a Varian Star 3400 CX GC system with a Chrompack CP-Sil 8 low bleed/MS column of 30 m and internal diameter of 0.25 mm with a film thickness of 0.25 μm). Pyrene and BaP were detected on a Saturn 4D, ion trap MS/MS (Thermo Varian, Palo Alto, USA), using the following ions: (i) m/z of 202 for pyrene and (ii) m/z 252 and 253 for BaP. The limits of detection were 5.3 and 10.0 pg for pyrene and BaP, respectively. This resulted in limits of quantification for pyrene and BaP in the gas phase of 0.5 ng/m^3 and 1.0 ng/m^3, and in the particulate phase of 0.02 ng/m^3 and 0.04 ng/m^3, respectively. The limits of quantification for the skin pads were 5 pg/pad for pyrene and 10 pg/pad for BaP. Pyrene (purity >99%) and BaP (purity 98%) were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). The analysis was calibrated using standards prepared in toluene in the range 5.87–938 ng/l for BaP and 1.48–237 ng/l for pyrene.

Analysis from coal tar ointment

After diluting CTO in tetrahydrofurane, we injected the sample directly into the chromatographic system without pre-treatment. The analysis was performed with an high performance liquid chromatography method with a gradient dilution and with a detection by fluorescence (excitation and emission wavelengths of 296 and 405 nm, respectively, for BaP and 335 and 385 nm, respectively, for pyrene).

Urine analysis

Urine was analyzed for both glucuronide-conjugated and free 1-OHP in glucuronidase/sulfatase hydrolyzed samples using the method of Jongeneelen et al (22) and urinary creatinine was analyzed using the Jaffé method. 1-OHP was analyzed on a high performance liquid chromatography system with a Lichrospher 100 RP-18 column with a particle size of 5 μm (Merck, Darmstad, Germany) using fluorescence detection with a limit of detection of 2 μg/l and an interday repeatability of 12.6%, based on an analysis of 24 samples in duplicate within a period of 12 months. Only the urine samples that were collected during study A were analyzed for 3-OHBaP. This analysis was performed according to the method described by Simon et al (23) with a limit of detection of 0.1 ng/l, an intraday repeatability of less than 3%, and an interday repeatability of less than 6%.

Calculations and statistical analysis

The amounts of pyrene and BaP used in each patient treatment were calculated from the type and registered amount of CTO used. The concentration of 1-OHP in each urine sample was corrected by the urine creatinine content. Baseline excretion of 1-OHP was determined in the first void of urine after awakening, following a period of 48 hours not working with CTO.

The total excretion of 1-OHP was calculated from the recorded volumes for each void and the concentration was determined in an aliquot. In the analysis, no distinction was made between female or male nurses or smokers and non-smokers. Smoking is estimated to be of limited influence due to the study design (ie, comparison of urinary PAH metabolite excretion within each subject over time). Statistical testing was performed using SPSS version 14.0 (SPSS Inc, Chicago, IL, USA) using a paired Student's t-test for the results of study C. An unpaired Student's t-test was used if different nurses were compared before and after intervention (studies A and D). For comparison of the urinary excretion of 1-OHP in study A with the results of study D, only the results of urine samples collected during the first 24 hours were used.

Results

Study A

The levels of airborne pyrene and BaP were all below the limits of quantification, suggesting that inhalation exposure of pyrene and BaP due to the application of CTO is very unlikely. Therefore, inhalation of airborne pyrene and BaP can be ruled out as a source of occupational exposure.

During this study, all the nurses wore loose-fitting polyethylene gloves during at least part of each CTO treatment. Despite this good work practice, after one working shift substantial amounts of pyrene and BaP were observed on the nurse's hands. The distribution of the contamination of pyrene and BaP over three different pads on the same hand shows considerable inter-individual differences, indicating variations in the PAH content of different CTO products used and possible differences in working methods.

Table 2 presents the results of pyrene skin contamination testing and urinary excretion of 1-OHP. For nurses A and K, the amount of CTO used is the same as they were treating the same patient. This is also the case for nurses H and J. Nurses F and G were involved in the treatment of two patients resulting in the highest amount of administered CTO. However,

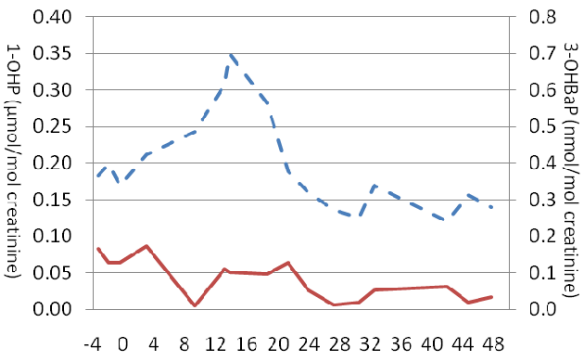

Figure 1. Pattern of excretion of 1-OHP (dotted line) and 3-OHBaP (straight line) in a male nurse (person A) who reported smoking 11-20 cigarettes/day and who treated one patient at 09:47 (time in hours; treatment started at t = 0). During the treatment, 55.8 g of dark CTO was applied during 20 minutes of working without gloves.

these two nurses did not have the highest skin contamination values or the highest excretion levels of 1-OHP in urine.

Pyrene applied as part of the CTO varies widely due to the use of different types of CTO with PAH contents ranging over three orders of magnitude. Similarly, the amount of BaP applied ranged from 1.7–25 200 µg, with a median of 2758 µg (not presented).

The highest level of excretion of 1-OHP was found in nurse A. The urine samples of this nurse were the only samples in which levels of 3-OHBaP were detected (figure 1). Considering the amount of pyrene applied and the contamination of skin pads, this is certainly not the person with the highest external exposure to pyrene. The slight downward trend in both 1-OHP and 3-OHBaP excretion patterns may be related to the use of CTO in treatment of a patient on the day before urine collection. Nurse A reported this exposure (not in line with the instructions) in the questionnaire. Since nurse A is also an active smoker, inhalation of tobacco smoke may have contributed to the excretion of 1-OHP and 3-OHBaP.

The individual with the lowest urinary excretion of 1-OHP (nurse K) was treating the same patient as nurse A, and had lower levels of contamination on the skin (except for contamination of the finger of 118 ng/cm^2). It is likely that these two nurses divided the tasks during treatment of the patient in such a way that nurse A had more direct contact with CTO than nurse K.

We also investigated the contamination of skin pads with BaP. The levels observed on the pads were (median and range): (i) wrist: 6.5 ng/cm^2 (<0.01–105.1), (ii) palm: 3.9 ng/cm^2 (1.2–79.7), and (iii) finger: 23.3 ng/cm^2 (<0.01–98.0). The pyrene and BaP levels on the wrist and palm were highly correlated (R^2=0.95 and 0.97, respectively). The correlation between pyrene and BaP on the finger pad was weaker (R^2=0.43) but still statistically significant (P<0.05).

We did not find statistically significant correlations between the urinary excretion of 1-OHP and work characteristics such as the total amount of CTO applied, the contamination of PYR on the skin pads,

Table 2. Amount of pyrene (PYR) used in coal tar ointment (CTO), skin contamination and excretion of 1-hydroxypyrene (1-OHP) during a period of 48 hours following the administration of CTO. (ND = not detected)

Person code	Age (years)	Smoking status	Number of treatments	Total treatment time (minutes)	PYR in CTO applied (µg)	Skin exposure to PYR					Excretion of 1-OHP			
						Wrist (ng/cm^2)	Palm (ng/cm^2)	Finger (ng/cm^2)	Mean (ng/cm^2)	Total (ng)	Total excretion (nmol/48 hours)	Baseline excretion level [a] (µmol/mol)	Highest excretion level (µmol/mol)	Increase from baseline (µmol/mol)
A	51	Smoker	1	20	112	17.5	6.85	112	45.6	254	7.7	0.21	0.35	0.14
B	39	Non-smoker	1	60	438	0.43	9.33	15.9	8.55	51.9	3.8	0.09	0.21	0.12
C	28	Non-smoker	1	60	22 **000**	173	15.6	189	126	762	1.5	0.01	0.18	0.18
D	43	Non-smoker	1	20	6 **230**	14.6	1.46	56.0	24.0	146	3.9	0.11	0.15	0.04
E	39	Smoker	1	5	36.2	2.71	2.02	18.8	7.84	47.6	2.2	0.10	0.13	0.03
F	34	Smoker	2	90	31 **500**	43.9	3.12	52.1	33.0	201	4.0	0.07	0.13	0.06
G	40	Non-smoker	2	70	445 **000**	165	28.4	9.58	67.5	410	2.2	0.06	0.11	0.06
H	50	Non-smoker	1	20	83.8	300	202	49.0	184	1110	1.7	0.08	0.10	0.03
I	22	Non-smoker	1	30	4 **970**	0.19	0.32	9.4	3.30	20.0	1.7	0.19	0.10	-0.09
J	49	Non-smoker	1	20	83.8	68.0	19.1	55.2	47.4	287	2.4	0.09	0.09	-0.01
K	44	Non-smoker	1	20	112	0.25	1.6	118	40.1	67.9	1.2	0.03	0.04	0.01
L	29	Non-smoker	1	35	455	0.34	0.17	ND	0.17	1.10	1.3	0.05	0.04	-0.01

[a] Baseline excretion was determined in the first void of urine after awakening following a period of 48 hours not working with CTO.

the duration of the treatment, or time elapsed between CTO application and washing hands.

Study B

The results from the three-week follow-up study are presented in **figure 2**. Nurse 1 reported working a total accumulated period of 202 minutes without wearing gloves. Dark CTO was applied for only 22 minutes in total (on days 2, 3, 16, 17, and 19) while light CTO was used for the remaining 180 minutes (days 2, 3, 9, 12, 16, 17, and 19). Of the time used to apply CTO, 92% was spent without protection. During a shift where a significant amount of time was spent working unprotected (>30 minutes per shift), the urinary excretion of 1-OHP increased (days 3 and 17). However, on other days with a similar duration of working unprotected, no increase in urinary excretion of 1-OHP was observed (days 2, 9, 16, and 19). On day 12, working 10 minutes with unprotected hands resulted in a decrease of the urinary excretion of 1-OHP. The elevated level of excretion in the pre-shift sample on day 19 may be the result of a continued excretion of a relatively high skin absorption of pyrene on day 17.

For nurse 2, the reported duration of working with unprotected hands amounted to 93 minutes (15 minutes of which were spent using dark CTO and 78 minutes using light ointment). This nurse worked unprotected during 26% of all the time engaged in CTO application. Urinary excretion did not appear to be influenced by the application of CTO.

Nurse 3 reported the lowest total duration of work spent applying CTO (45 minutes) and only light CTO was used during this time. This nurse worked unprotected during 38% of the time spent applying CTO. The urinary excretion of 1-OHP also showed hardly any significant rise.

The total number of hours spent in unprotected direct handling of CTO led to higher levels of urinary excretion in nurse 1 who worked most of the time (and also a high percentage of "CTO application time") without gloves. However, this increase did not occur on all days where significant time was spent handling CTO. Use of (primarily) light CTO by nurses 2 and 3 did not result in a consistent rise in urinary excretion of 1-OHP.

Study C

Since studies A and B did not show a consistent elevated excretion of 1-OHP following skin exposure to CTO, a third study was conducted to find out if protecting hands with gloves would lead to a statistically significant lower uptake of PAH in a large group of nurses. Wearing loose-fitting polyethylene transparent gloves (King Gloves, Braintree, MA, United States), 35 nurses were asked to treat a single patient and to repeat the treatment in the same patient without the gloves. Repeating the topical application of CTO is part of the normal procedure in the clinical practice.

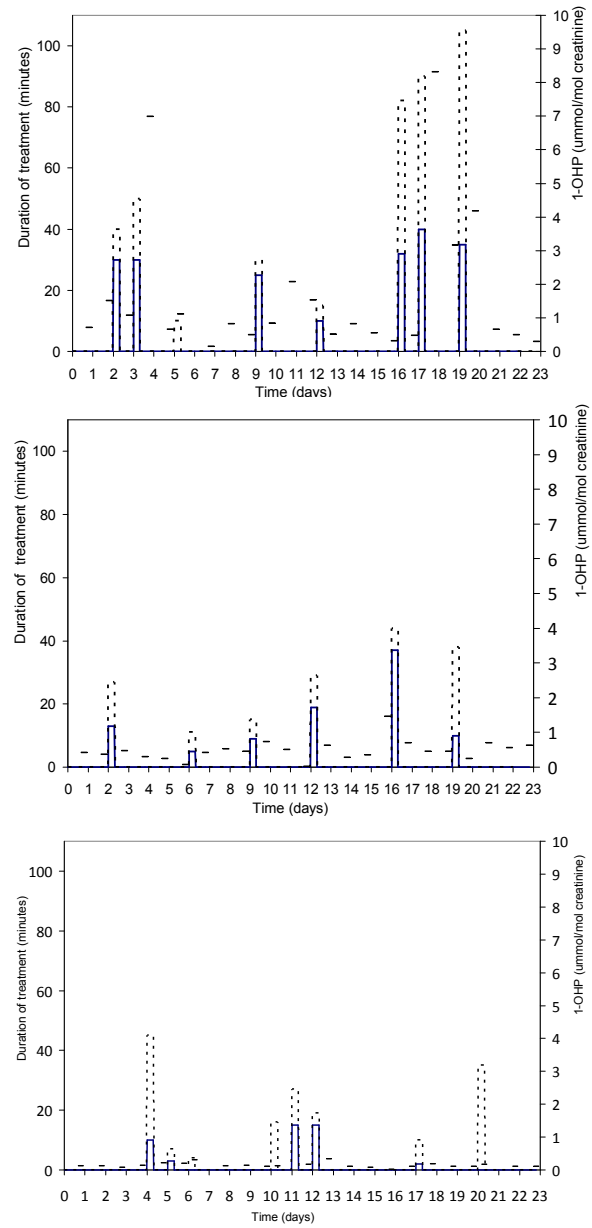

Figure 2. Duration of CTO treatment with gloves (dotted line) and without gloves (solid line) and excretion of 1-OHP in a post-shift (evening) urine sample (dashes). Results of three different nurses (non-smokers) are presented: nurse 1 upper panel, nurse 2 middle panel, and nurse 3 lower panel.

The results of the paired observations are presented in table 3. The wearing of gloves during a single treatment resulted in an average reduction of 51.5% in the total excretion of 1-OHP. The highest level of 1-OHP in a single spot urine sample decreased by 61.2%. As

Table 3. Median (range) or urinary excretion of 1-OHP in nurses working with and without gloves (study C).

CTO application	Excretion of 1-OHP							
	Total amount excreted (µmol/24 h)		Highest post-exposure level (µmol/mol creatinine)		Baseline (µmol/mol creatinine)		Change from baseline (µmol/mol creatinine)	
	Median	Range	Median	Range	Median	Range	Median	Range
Without gloves	1.34	0.19–5.6	0.31	0.06–0.98	0.05	0.01–0.28	0.23	0.00–0.74
With gloves	0.69	0.17–3.3	0.12	0.04–0.70	0.04	0.01–0.13	0.06	0.00–0.57
Change	-0.58	-5.1–1.0 [a]	-0.18	-0.81–0.04	0.00	-0.14–0.09	-0.16	-0.74–0.02

[a] P<0.001

Table 4. Contamination of skin pads before and after introduction of a protocol for use of gloves and sleeves (studies A and D). (PYR = pyrene, BaP = benzo[a]pyrene, ND = not detected)

Subgroup	PAH contamination before introduction of skin protection protocol (ng/pad)		PAH contamination after introduction of skin protection protocol (ng/pad)	
	Median	Range	Median	Range
Same nurses (paired observations, N=6)				
PYR	349	146–1110	0.9 [a]	ND–35.6
BaP	184	60.5–394	4.6 [b]	0.2–31.7
Different nurses (unpaired observations, N=10–12)				
PYR	173	1.1–1110	3.7 [b]	ND–93.5
BaP	107	5.5–394	3.2 [b]	0.5–171

[a] P<0.001.
[b] P<0.005.

Table 5. Excretion of 1-hydroxypyrene (1-OHP) before and after introduction of a new protocol for the use of gloves and sleeves.

Subgroup	Before introduction of skin protection protocol		After introduction of skin protection protocol	
	Median	Range	Median	Range
Paired observations (N=6)				
Total urinary excretion of 1-OHP (nmol in 24 hours)	1.6	0.84–5.0	0.69 [a]	0.45–3.4
Increase of urinary excretion of 1-OHP from baseline (µmol/mol creatinine)	0.05	-0.01–0.18	0.03	0.01–0.17
Unpaired observations (N=10–12)				
Total urinary excretion of 1-OHP (nmol in 24 hours)	1.5	0.74–5.0	0.64 [b]	0.15–2.8
Increase of urinary excretion of 1-OHP from baseline (µmol/mol creatinine)	0.04	-0.09–0.18	0.02	-0.01–0.05

[a] P<0.005.
[b] P<0.05.

expected, the urinary excretion of 1-OHP at baseline (as observed from the first voids after awakening) was not different when working with or without gloves. The change from baseline to the maximum excretion of 1-OHP within 10 hours after CTO application was 74% lower when using gloves compared with working unprotected, but this result was not statistically significant.

Study D

A new work practice was introduced based on the use of latex-free polyvinyl chloride gloves and 50 cm Tyvek® narrow sleeves with elastic on the wrist. With this new set of skin protection equipment, study A was repeated to verify if the reduction in uptake shown in study C would be feasible under more realistic exposure conditions. Since only six nurses were still working in the department, a paired analysis was performed in this subgroup. In addition, an unpaired statistical analysis was performed involving ten nurses from study A and 12 other individuals from study D. In both subgroups, a substantial and statistically significant reduction of skin contamination of more than 97% was observed for both pyrene and BaP (table 4). The total urinary excretion of 1-OHP was reduced by 56.9% in the paired analysis (P<0.05) and 57.3% in the unpaired analysis (P<0.05). The increase from baseline to the highest recorded value within 24 hours after the CTO showed a similar trend but no statistical significant reduction was observed (table 5).

Discussion

Pyrene and BaP were not detected in the gas phase and particle phase. For BaP, the air concentration was apparently lower that the previously reported background value for BaP in the particulate phase of outdoor air in the Netherlands of 0.05–0.15 ng/m^3 (24). For the gas phase, our method was less sensitive (due to the lower air flow used during sample collection) but it is unlikely

that BaP would be present in the gas phase when this substance was not observed in the particulate phase.

In contrast, significant amounts of pyrene and BaP were observed on skin pads attached to the dominant hand. The (calculated) amounts of BaP and pyrene applied to each patient using the different types of CTO showed a high correlation (R^2=0.97). On skin pads, correlations were low for the finger but higher for the wrist and the palm of the hand. This suggests that pyrene is useful as a chemical marker of PAH exposure while 1-OHP is a suitable biomarker of internal exposure to BaP in the case of CTO applications by nurses. This is important in this study because we were unable to detect urinary excretion of 3-OHBaP in all but one nurse in study A.

No significant correlation was observed between the contamination of pyrene on the skin pads and the total amount of 1-OHP excreted in urine (R^2=0.11). This may be due to inter-individual differences in metabolism or skin absorption but also to the limited capability of estimating total skin exposure using skin pads. The skin contamination with CTO was not diffuse but showed some patchiness. This heterogeneity in skin contamination is difficult to assess when using skin pads.

Study B showed that working without gloves is associated with increased urinary excretion of 1-OHP in particular when using dark CTO (eg, nurse 1 in figure 2). This pattern is less pronounced in nurses 2 and 3 who worked fewer hours unprotected and used smaller amounts of dark CTO. A limitation to this finding is that the urinary excretion of 1-OHP is indicated by only one single post-shift urine sample per day.

In study C, working with gloves showed that the group median 1-OHP excretion was 0.12 and the P95 value was 0.30 μmol/mol creatinine. When the nurses did not wear gloves, the median and P95 were 0.31 and 0.83 μmol/mol creatinine, respectively. The P95 values of 0.76 and 0.24 μmol/mol creatinine proposed by Jongeneelen et al (9, 25) were used to classify respectively smoking and non-smoking nurses as being occupationally exposed to PAH. In study C, 17 out of 35 workers would be classified as "occupationally exposed" when not wearing gloves, whereas only 2 workers would be classified as being exposed when wearing protective gloves. Therefore, wearing gloves created a situation that would be very close to what would be considered as "no occupational exposure" for the group of 35 nurses.

It is also interesting to compare the level of observed exposure with known sources of non-occupational "background" exposure (20, 26). The group median uptake of 1-OHP of 2.2 nmol in 24 hours as calculated from table 2 is approximately 21.6% of what is estimated to be taken in on a daily basis from the diet of non-smokers, or only 23.9% of the amount estimated for active smokers if a complete conversion of pyrene to 1-OHP is assumed (20).

In 11 of the 12 nurses in Study A, there was no detection of 3-OHBaP. In part, this may be due to the method of collection and storage of urine leading to loss of 3-OH-BaP glucuronide. In one nurse, 3-OHBaP was detected in collected urine samples. This subject smoked 0.5–1 pack of cigarettes per day. The urinary excretion for 3-OHBaP in nurse A (figure 1) was somewhat higher than the level that is normally seen in smokers (26), but well below the biological limit value of 0.45 nmol/mol creatinine proposed by Lafontaine et al (10). The highest value at 4 hours prior to the application of CTO and the gradually decreasing pattern of values suggests an uptake of BaP on the previous day. The latter was confirmed by a report from nurse A who acknowledged that she had treated a patient with CTO on that day. Moreover, excretion of 1-OHP showed a pattern indicating a possible work-related uptake of pyrene. This is consistent with a substantial skin contamination as indicated by a total 254 ng of pyrene on all pads. The latency time between the moment of application (09:47 hours) and the highest value of urinary excretion (approximately 23:32 hours), suggests a delay of 13:45 hours, indicating skin absorption as the primary route of uptake rather than smoking (27).

The results of study B showed that especially the use of dark CTO when wearing no gloves might contribute to an uptake of PAH, as indicated by the increase of urinary excretion of 1-OHP from pre- to post-shift. These results indicated that the use of light CTO did not (in most cases) lead to such an increase and contributed to the explanation of the results observed in study A, which showed that an increase in uptake of PAH is not observed in all subjects. Furthermore, these findings suggested that the role of skin protection should be further investigated.

The results of study C were related to a task-based study of exposure rather than a study of a real life work shift, which may involve the treatment of two or more patients during the course of a shift. The introduction of a new protocol for skin protection resulted in an almost complete elimination of skin exposure (<3% residual exposure for pyrene and BaP). In this work situation, skin absorption is considered to be the primary route of uptake since both inhalation and oral exposure are not very plausible routes of exposure. So it may be concluded that most of the occupational exposure to PAH due to the use of CTO was virtually eliminated. This major reduction in exposure did not lead to a complete reduction in the excretion of urinary metabolites due to the contribution of non-occupational sources of exposure to PAH.

Study C was performed because it was still uncertain how effective a more stringent regime of skin protection

would be in terms of reduction of PAH uptake. It was of particular interest to involve the nurses in this intervention study, comparing working with and without gloves under more or less standardized conditions. This participatory study design helped to convince the nurses of the added value of protecting their skin. The result of this study showed a substantial reduction that was also statistically significant based on the findings for the entire group. However, it should be made clear that, when looking at the results on an individual level, it appears the effectiveness may vary substantially from person to person: from a 5-fold increase to a 15-fold reduction.

In study D, we showed that a twofold reduction of uptake of PAH (as indicated by excretion of 1-OHP) is feasible for a group of nurses working with CTO on a day-to-day basis inspite of the greater number of CTO treatments in study D (arithmetic mean 2.28, range 2–4) than in study A (arithmetic mean 1.17, range 1–2). It is obvious that this substantial reduction in the contamination of skin pads, would lead to a decrease in urinary excretion of 1-OHP, but the excretion observed was very close to levels seen in the general population at baseline values (9).

The influence of smoking on this result was evaluated by reanalyzing the data after excluding the smokers. The removal of the two smokers from the paired observations reduced the level of significance (the P-value changed from 0.007 to 0.022). The removal of three smokers from the unpaired observations caused the P-value to change from 0.037 to 0.214, suggesting that interference of changes in smoking patterns on the excretion of 1-OHP cannot be excluded in this part of the analysis.

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