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Sampling and Analytical Method Development and Hand Wipe Measurements of Dermal Exposures to Polycyclic Aromatic Hydrocarbons

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This article describes the laboratory assessment of a hand and surface wipe sampling method for polycyclic aromatic hydrocarbons (PAHs). The analytical method employed extraction of the wipe samples into dimethyl sulfoxide (DMSO) and high-performance liquid chromatography (HPLC) flourometric detection of pyrene, a predominant PAH in used gasoline engine oils (UGEO). Recovery of pyrene was evaluated for two different sampling media by first contaminating the hands of a small number of volunteers with UGEO, followed by applying a small amount of corn oil to the palms, and by wiping the skin with a Whatman cellulostic filter paper or a polyester fabric wipe (i.e., Alpha wipes). In summary, using either Whatman or Alpha wipes, the mean recovery of pyrene from the UGEO that was applied to the hands and contained within three consecutive wipes was 69% and 54%, respectively. However, the relative recovery of the first to second wipe was on average 47% and 75% for the two media, respectively. These results indicate that the Alpha wipes were more efficient at recovering pyrene in the first wipe but less efficient overall when all three consecutive samples were included. Even though this sampling was performed in a controlled laboratory environment, the minimum and maximum amount of pyrene recovered in the individual composite samples using either method spanned a range of twofold. Overall, intra- and interpersonal variability, as measured by coefficient of variation, were 22% and 19%, respectively, and were not statistically different by type of media used. This method was used in a pilot field survey to sample the hands of 18 automotive repair technicians and 18 office workers. Detectable amounts of pyrene (>0.2 μg/sample) were found on the hands of 61% and 0% of these two groups, respectively, with the highest measured quantity equal to 1.06 μg. Samples from the upper surfaces of automobile motors were generally low to nondetectable (<0.027 μg/sample), while the median value of 0.047 μg/50 cm^2(CV = 160%) and up to 0.640 μg were found on the drip pans.

Keywords analytical, method, PAH, sampling, skin, wipe

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INTRODUCTION

Workplace exposure to mixtures containing polycyclic aromatic hydrocarbon (PAH) compounds is fairly common. Some examples of the diverse occupational groups that are potentially exposed include asphalt (road) workers, roofers, automotive technicians, used oil recycling workers, foundry workers, cooks, and aluminum reduction workers. In general, workers exposed to environments containing PAHs are at an increased risk for lung, urinary tract, brain, and skin cancers.[1,2] Strategies for assessing occupational exposure to these materials should be influenced by the fact that they have a low vapor pressure (i.e., tend not to become airborne through volatilization) and may be absorbed through the skin.[3] For many occupational exposures (pavers, foundry and aluminum workers, for example), the skin is the major exposure route for PAHs.[4–6] For these workers, air sampling alone probably would miss important exposure information.

Surface and skin contamination sampling can help to characterize the potential or actual magnitude of skin exposure. Having a method to measure PAH skin exposures provides a number of advantages, including being able to rank job types by potential for exposure, relate exposure to morbidity and mortality in epidemiology studies as well as to other potential biological effects, and measure the outcome of exposure reduction interventions.

Used gasoline engine oil (UGEO) contains PAHs that are products of incomplete combustion. PAHs comprise a diverse class of chemicals characterized by two or more fused aromatic rings made up of hydrogen and carbon atoms. It has been clearly demonstrated that the PAH content of gasoline engine oil increases proportionally with increasing driving distances between oil changes.[7] More than 200 PAHs have been structurally identified. Several of them (i.e., benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo (a)pyrene (BaP), dibenzo(a,h)anthracene, and indeno(1,2,3-cd)) are known carcinogens to humans, animals, or both.[8] Pyrene, typically a prominent PAH, has not been found carcinogenic by itself but may promote the carcinogenic activity of other PAHs when it is present.[3]

Several studies have examined the carcinogenicity of UGEO after long-term topical application in experimental animals. The findings indicate increased incidence of dermal papillomas and carcinomas among male C3H/HEJ mice and female CFLP mice after chronic-duration exposure to UGEO.[9−13] No tumors were observed in mice exposed to unused motor oil, confirming that the carcinogens accumulate in the oil during driving. To date, only a few epidemiologic assessments have addressed UGEO as an exposure, and additional larger studies are needed. However, some relationships have been suggested between bladder cancer,[14] lung cancer,[15−17] and astrocytic brain cancer[18−19] among automotive technicians and to other workers who are regularly exposed to lubricating oils containing PAHs.

Pyrene, the most abundant PAH in UGEO, makes up between 10–20% of the PAH content in these samples. Because of its abundance, it has been used previously as a surrogate marker of exposure to PAHs in general.[20,21] From one sampling survey, the average reported concentration of pyrene in UGEO was 430 mg/kg.[20]

Approximately 775,000 persons were employed as automotive repair technicians in the United States in 1996.[22] In the greater Cincinnati area alone, where the present study was conducted, there are over 300 automotive repair shops and 44 oil and lube shops. An additional unknown number of persons may periodically receive skin exposure to UGEO and PAHs when performing work on their own vehicles and when changing engine oil. To date, only one unpublished report has attempted to quantify PAHs on the skin after exposure to UGEO.[23]

Sampling and Analytical Method Development

A method was developed to assess the PAH dermal exposure of workers exposed to UGEO using pyrene as a marker compound. Corn oil was used as a transfer vehicle from the workers' hands to a wipe material. The wipes were extracted with dimethyl sulfoxide (DMSO) and analyzed by high-performance liquid chromatography (HPLC) with fluorescence detection. The authors also evaluated the performance of two types of potential wipe media for surface sampling. These sampling and analysis aspects are discussed in detail below.

Corn oil is used commonly as an innocuous vehicle in various toxicological bioassay dosing studies and is a common human food item.[24,25] By application of corn oil to the hands and rubbing vigorously, the grime is extracted from the skin. The corn oil containing the grime is transferred to the wipes by physical action. Previous skin decontamination studies that compared different vehicles show that oily vehicles such as corn oil are superior to aqueous soap solutions in removing substantial portions of lipophilic compounds up to 8 hr after contamination.[26−28] Literature searches did not reveal any allergenicity or skin irritation from corn oil use. The viscosity allows a liberal and evenly dispersed distribution to be applied over the whole hand when rubbed. It did not contribute any response to the fluorescence detector and was not extremely deleterious to the HPLC column.

A number of wiping materials were considered for use to remove UGEO and PAHs from the skin and other surfaces. The potential wiping materials considered included Whatman cellulostic filters (Whatman, Maidstone, Kent, U.K.), as had previously been used by National Institute for Occupational Safety and Health (NIOSH) researchers during a road paving study and with automotive repair technicians. Other media that were considered include paper towels, cotton gauze, and a nonwoven polyester fabric (TX1004 Alpha7 wipes; Texwipe Corporation, Saddle River, N.J.). All these materials are readily available and potentially could serve as sampling wipes for the intended purpose. After extraction of unused media, no detectable PAHs were found in any of these tested materials, so blank substraction was not necessary.

The authors intentionally chose dry wiping medium because of the belief that by first using a lipophilic vehicle such as corn oil to mobilize the PAH contaminants, followed by removal into an absorbing medium, was an efficient approach. Thus, premoistened wipes such as Wash'n'Dry or Ghost® Wipes were not considered because of their relatively small size and premoistened state. Paper towels and cotton gauze also were not evaluated further because of possible inconsistencies in their manufacture.

From previous experience using Whatman filter papers for wipe sampling, it was observed that these are not rugged; they easily tear and are not designed to be absorptive. The Alpha wipe was designed for wiping purposes; they are much more absorbent and are resistant to tearing and abrasion damage. Another attractive feature is that this fabric is made of pure polyester with no binders added and, therefore, is chemically inert to most chemicals. Because of these attractive properties, Alpha wipes were chosen as a second medium for comparison.

Pyrene was found to provide the best measurement sensitivity among the most common PAH analytes. Because it is also usually the most abundant PAH in mixtures, it is the rational choice as a principal marker of exposure to PAHs in general. Optimizing the detection of pyrene was accomplished with the fluorescence detector by using the maximum excitation and emission frequencies of 328 nm and 394 nm, respectively. Other compounds that may be monitored at these wavelengths are anthracene, fluoranthene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene, but with less sensitivity and selectivity. The injection volume of samples varied with the sample concentration to keep within the linear range of the detector. Corn oil gave no response at the retention time of pyrene.

Sample analysis was performed using reverse phase HPLC and fluorescense detection. A systems controller (Waters 600; Waters Corp., Milford, Mass.) maintained a flowrate of 0.5 mL/min and a binary gradient to enhance separation. The initial conditions of the mobile phase were 40% acetonitrile/

60% water held for 4 min. The gradient was ramped to 100% acetonitrile over 60 min. A 30-min hold time was added to void the column of late eluting peaks before returning to initial conditions. Separation was achieved using a Supelco Supelcosil (Bellefonte, Pa.) LC-PAH column (15 cm × 4.6 mm) packed with 0.5 micron polymeric C18 and the reverse phase gradient program. The eluting signals were monitored by a fluorescence detector (Shimadzu RF-551; Shimadzu, Kyoto, Japan) with the excitation and emission frequencies set at 328 nm and 394 nm, respectively. The light source was a xenon lamp, and the bandwidths were 15 nm. Identification of pyrene was determined by its retention time and the specificity of the fluorescence detector. The detector signal was collected and processed using a chromatogram data handling system (Thermo LabSystems Atlas, Altrincham, Cheshire, U.K.).

Extraction efficiencies for both the Alpha and Whatman media were determined for a variety of fortifications including only PAHs in DMSO, PAHs with corn oil, or UGEO containing PAHs. Extraction efficiency was calculated by dividing the analytical result by the known amount of pyrene added. For the PAH standard fortification evaluation, six of each medium were spiked at concentrations of 62.5, 125, 250, and 500 ng pyrene per wipe.

The various extraction studies performed included:

1. Direct spiking of a PAH standard onto the dry wipe.
2. Direct spiking of a PAH standard onto the dry wipe, followed by the addition of 2 mL of corn oil.
3. Spiking a PAH standard into corn oil, followed by adding 2 mL of the spiked corn oil to the wipe.

Calibration curves were established using serial dilutions with DMSO of EPA 610 PAH Calibration Standard (Supelco) to encompass the sample concentration range. This standard mixture contains 1000 micrograms pyrene per 1 mL along with 15 other priority pollutant PAHs. Using a least squares program, a typical calibration curve shows good linear instrumental response for pyrene as shown in Figure 1. The range studied was from 1 to 5000 ng sample. Since the fluorescence detector had a high and low sensitivity setting, the range was 1 to 100 ng per sample for the high setting and 100 to 5000 ng for the low setting. If any samples exceeded the 5000 ng per sample standard, dilutions with DMSO were made until it fell within this curve. With some experience, segregation of the samples collected in this study into a likely concentration range could be determined visually by the brownish color exhibited within the extraction solvent of each sample.

Using the calibration curve, the instrumental limit of detection (LOD) and limit of quantitation (LOQ) were defined as 3 times and 10 times the standard deviation divided by the slope. With the optimized conditions for detection of pyrene, the LOD and LOQ of pyrene was determined to be approximately 0.02 μg and 0.076 μg per sample using a 20-mL extraction volume. The method LOD and LOQ are calculated in the same manner but are made from low level media spikes.

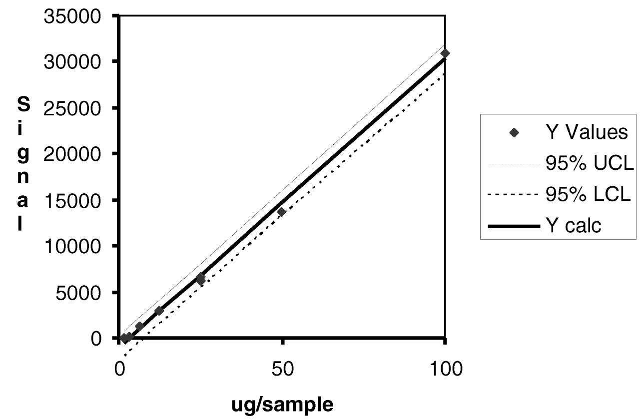

FIGURE 1. Pyrene calibration curve using seven concentrations ranging from 1.6 to 100 μg per sample

Rather than spiking directly onto the wipe medium, the pyrene was spiked into corn oil and 100 μL of the corn oil applied to the wipe followed by DMSO extraction. The method LOD and LOQ for pyrene was approximately 0.6 ng and 2 ng per 100 μL corn oil, or 6 μg and 20 μg/L, respectively. Based on the extraction solvent dilution, this is equivalent to an LOD and LOQ of approximately 0.1 μg and 0.4 μg per wipe sample. LOD and LOQ values were determined for each set of samples that were analyzed during the course of this study by the recovery and variability of spiked media samples. Because of changes in the analytical equipment the LOD and LOQ varied somewhat for each sample set.

Sample preparation of field samples was straightforward, since the sampling media arrived in capped, plastic, conical tubes. Twenty mL of DMSO was added to each sample and rotated at least 12 hr using a rotator. An aliquot of each sample was transferred into a WISP-style vial for autoinjection into the HPLC system.

Hand Wipe Study Design

Once the analytical method was optimized to quantify pyrene, the study was designed to determine and compare the recovery of UGEO from hands using the chosen sampling media and pyrene as the specific analyte. Sampling recovery was performed by applying a known amount of PAH-containing UGEO to the hands, then immediately adding corn oil and wiping the hands with the sampling media. Of several different media initially chosen for evaluation, only Whatman filter papers or Alpha wipes were selected for this final evaluation of the wipe sampling method because (1) Whatman filters have been previously used to measure PAHs on skin in other studies; and (2) the Alpha wipe fabric is highly absorbent, is rugged, is an analytically clean material, and because its chemical inertness was believed to be ideal for sampling and extracting PAHs. The dimensions of the square Alpha wipes were 10 cm × 10 cm (100 cm^2) and the Whatman filters were round with a 10.5 cm diameter (87 cm^2).

UGEO was obtained from a 1997 Mercury Sable with 48,000 miles and had been driven for about 3 months with this oil. To compare the performance of the Whatman filter paper and Alpha wipe cloths, the procedure entailed first applying 250 μL of UGEO to the palm of one hand. The UGEO was distributed evenly over both palmar surfaces by rubbing the hands together for 10 sec. Immediately after distributing the UGEO over the palms, 2 mL of corn oil was pipetted onto the palm; the volunteer was asked to rub this over the hands thoroughly for 15 sec. Each volunteer then used one of the two sampling media to carefully wipe both hands. Each wipe sampling was timed to last 30 sec. This was repeated a total of three consecutive times by each volunteer with a fresh wipe medium, and each sample was placed individually into a labeled 50-mL conical polyethylene plastic tube.

The above procedure was considered one complete "trial" in the experiments. Sequential recovery for the three consecutive wipes was determined, relative to the originally applied dose, as well as relative to the total that was recovered. Immediately after sampling was completed, the participants were provided wet towelettes to remove any remaining oil from their skin and instructed to wash their hands thoroughly with soap and water. The entire procedure of applying, sampling, and decontaminating the skin per application took approximately 10 min. The samples were stored at –80°F until analysis.

Historically, other authors have evaluated and reported PAH analyte recovery from sampling media using two possible approaches. To evaluate the recovery of a wipe medium, this can be calculated as the *absolute* recovery if the amount of the target analyte applied is known. In cases where it is not known what the exact amount of the target chemical is in the PAH-containing matrix, the relative amount recovered in each of several consecutive samplings is often reported. The *relative* recovery of an analyte is based on the amount obtained from a subsequent consecutive wipe of the same surface area.[29,30]

When a sampling method recovers a predominant amount of the analyte in the first sampling vs. the second sampling of the same surface, for instance, it is considered efficient. However, this does not always mean that the sampling method actually recovered most of the analyte that was present in the previous wipe, only that it recovered a large amount of the analyte that was recoverable by the chosen sampling method. The absolute recovery is far superior to relative recovery in determining the actual sampling efficiency of a method, but relative recovery is also valuable to know to determine how many consecutive samplings might be needed in a composite sample to obtain a satisfactory cumulative sample, or the point of diminishing return. Thus, to allow comparison of this study's results with other authors' reports, and to provide the most complete information on overall method performance, both approaches are provided here. The two approaches that can be used to express sampling efficiency are presented below.

Percent absolute recovery (Eq. 1) is simply:

$$[\text{mass recovered in wipe}/\text{mass applied}] \times 100 \qquad \textbf{(1)}$$

Percent relative recovery is calculated as shown in Eq. 2:

$$[1.00 - (\text{2nd sample}/\text{1st sample})] \times 100 \qquad \textbf{(2)}$$

An expanded evaluation of the Alpha wipes was conducted using three volumetric loading levels: 50 μL, 250 μL, and 1000 μL UGEO. At each loading level, only one trial per volunteer per day was performed, and this was usually repeated during 3 consecutive days. Only one concentration level was tested per week. Up to four different volunteers participated in the trials, and these volunteers were used consistently throughout the evaluation with each wiping medium. During the following weeks, the next higher applied volume was used as described above. Before each reapplication, the participants were asked if they perceived any effect on their skin at the application site due to the previous exposure. No adverse effects were noted. Table I depicts the study design with the number of samples collected.

Separately, wipe samples from 18 nonexposed individuals (both smokers and nonsmokers) were collected and combined with the present study to depict concentrations of the target PAH analytes on the skin of persons not exposed to PAHs in their workplace. Additional hand wipe samples of 18 automotive technicians with possible exposure to PAHs in UGEO were collected randomly at local automotive service businesses during normal business hours. Between two and four samples were collected on different days from each of the

TABLE I. Study Design per Sampling Medium

Week	Medium^A	Volume Applied (mL)	Pairs of Hands (persons)	Replicate Trials	Consecutive Wipes per Trial	Analyses Performed
1	1	0.05	3	3	3	27
2	1	0.25	4	3	3	36
3	1	1.00	3	3	3	27
4	2	0.25	4	3	3	36
					Total in study (2 media)	126

^{A}Medium 1 = Alpha wipes; Medium 2 = Whatman filter paper.

technicians. Surface wipe samples were also taken from within the engine compartment of 10 older automobiles at locations likely to have deposits of oil, such as the drip pan, as well as various other sites where engine oil was not likely to be present but which might be touched during service work, such as oil filler cap, spark plug wires, air filter cover, distributor cap, etc. Surface wipe samples from the drip pan consisted of areas as close as possible to 50 cm^2 were taken, but due to the irregularity of other surfaces, only a rough approximation of this size was practical.

The procedures used in this study were reviewed and approved by the CDC Institutional Review Board for the protection of human subjects in research prior to conducting this study. All NIOSH staff volunteers participating in the hand wipe tests provided informed consent.

RESULTS

Investigations as to the best extraction solvent to use showed that dimethyl sulfoxide (DMSO) was superior to acetonitrile, ethanol, isopropanol, or hexane that was back extracted into DMSO. The various analytical recovery studies were conducted at six levels for each wipe and extracted with 20 mL DMSO. No concentration effects were exhibited by the results. The recovery of pyrene from both wipe media that were directly fortified with a known PAH-containing standard showed almost complete recovery of pyrene (99% ± 3%). The addition of the corn oil to the spiked wipe media did not affect the recovery but increased the variability (98% ± 5%). Addition of the pyrene to the corn oil before application showed minor reductions in both recovery and precision (95% ± 10%). During the course of analysis, both blind and analyst spikes were analyzed with the samples for quality assurance purposes. These QA samples ranged from 0.06 to 0.6 μg per wipe and were analyzed over the course of several months. All QA samples that were run were within the control boundaries of less than ± 25% of the known value.

For the hand wipe recovery evaluation, Alpha wipe medium and Whatman filter paper were directly fortified with 250 μL of the same UGEO that was put onto the volunteers' hands. In this way, the absolute sampling efficiency of the analyte from the skin could be calculated, and the analytical recovery from the wipes was automatically accounted for. The mean amount of pyrene measured on the fortified Whatman filters was 17.17 μg (coefficient of variation [CV] = 0.09), and on Alpha wipes 15.76 μg (CV = 0.03). Therefore, the recovery of pyrene from the Alpha wipe medium was 92% of the recovery from the Whatman filters.

Eleven sets of hand wipe samples were collected for the Whatman medium hand wipes. Three replicate sets of wipes were collected from three of the volunteers, but it was possible to obtain only two replicates from the fourth. The results of these samples are reported in Table II.

The mean total amount of pyrene recovered from the hands with Whatman filter paper was 11.81 μg. Therefore, the absolute recovery of pyrene from the hands was 11.81/17.17 or 69% (CV = 0.20). The results in Table II also can be used to calculate the relative recovery using the consecutive wipes as described in Eq. 2. For the first two wipes, the relative recovery of the first wipe compared with the second wipe was 47% (i.e., the first wipe had 47% more pyrene than the second wipe), and for the second wipe compared with the third it was 54% greater. Thus, it can be inferred that this medium was still effectively removing pyrene from the skin after multiple consecutive wipes. While this might be considered good in terms of absolute recovery, this medium is relatively inefficient in removing most of the PAH contamination in the first wipe.

TABLE II. Recovery of Pyrene from Hands Using Whatman Filter Papers

Descriptive Statistics	Wipe 1	Wipe 2	Wipe 3	Total
Mean	6.68	3.52	1.61	11.81
Median	6.65	3.43	1.41	11.60
Standard error	0.38	0.26	0.19	0.71
Coef. variation (CV)	0.19	0.25	0.40	0.20
Minimum	3.84	1.93	0.80	6.87
Maximum	8.84	5.09	2.69	14.69

Exactly the same procedure was used to determine the hand wipe recovery of the Alpha wipe medium as described for the Whatman filters. Four volunteers performed the hand wipe sampling protocol of three consecutive wipes, and three replications were obtained from each individual. When 250 μL of the UGEO was applied to the hands, a mean total of 8.56 μg pyrene was recovered in the three consecutive wipes. Thus, the absolute recovery was 8.56/15.76, or 54% (CV = 0.30). The relative recovery of the first vs. the second wipe was 75%, and for the second vs. the third it was 59%. Details of the individual sample results are provided in Table III.

A depiction of the pyrene measured on each of the three consecutive wipes and total for each wipe medium is presented in Figure 2. The horizontal lines of a box plot mark the minimum point, the 10th, 25th, 50th (median), 75th and 90th percentile points, and the maximum point of the data. The dashed line is the mean. The two circles mark the 5th and 95th percentile of the data. Apparent in this data is the large amount of variation in the results, which typically span at least a twofold range. Also, it should be noted that the median

TABLE III. Recovery of Pyrene from Hands Using Alpha Wipes

Descriptive Statistics	Wipe 1	Wipe 2	Wipe 3	Total
Mean	6.34	1.57	0.64	8.56
Median	6.39	1.36	0.65	8.43
Standard error	0.69	0.22	0.05	0.75
Coef. variation (CV)	0.37	0.48	0.29	0.30
Minimum	3.28	0.57	0.43	5.50
Maximum	11.61	3.55	1.15	14.24

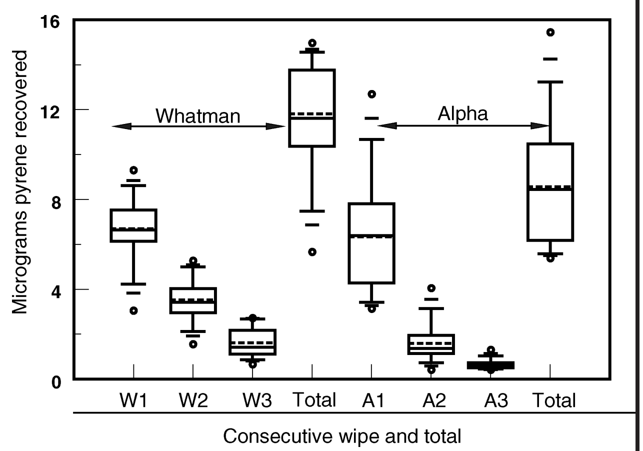

FIGURE 2. Percentile box plot of three consecutive individual wipes and total pyrene recovered with Whatman filter and Alpha wipe medium used to wipe hands

TABLE IV. Total Micrograms of Pyrene Recovered, and Replicate After 250 μL of UGEO was Applied to the Hands

Whatman Filters Participant Number				Alpha Wipes Participant Number			
1	2	3	4	1	2	3	4
13.75	11.60	9.89	14.01	14.24	5.50	5.79	6.78
10.55	13.60	6.87	10.35	7.90	10.19	9.01	8.96
11.17	13.48	—	14.69	10.83	6.95	5.97	10.55
Mean Recovered (μg)				Mean Recovered (μg)			
11.82	12.90	8.38	13.02	10.99	7.55	6.92	8.76
Intrapersonal CV				Intrapersonal CV			
0.14	0.09	0.25[A]	0.18	0.29	0.32	0.26	0.22

[A]Not reliable due to small sample size.

amount of pyrene from the first wipe of the Whatman and Alpha media are approximately equal (6.65 and 6.39 μg; Mann-Whitney two-tail test, $\rho = 0.56$), but the subsequent recovery using the Alpha medium rapidly declined. Thus, the relative recovery when using the Alpha wipes was high for the first vs. subsequent wipe results, but when including all three consecutive samples the total amount was less. The total recovery of pyrene for the Whatman and Alpha media (11.60 and 8.43 μg, respectively) is statistically significantly different (Mann-Whitney test, two-tail, $\rho = 0.01$).

In addition to the 250 μL loading level evaluations conducted to compare the Alpha medium with the Whatman filter medium, the Alpha medium was tested against a 50 μL and 1000 μL application of UGEO to the hands. The absolute recovery of pyrene from the 50 μL loading and for the 1000 μL loading was 57% and 60%, respectively. These absolute recoveries are roughly consistent with the 54% recovered at the 250 μL loading level. At the 50 μL level, the relative recovery of the first vs. second wipe was 85%, and second to third wipe 78%. Again, at the 250 μL loading the relative recoveries were 75% and 59%. At the 1000 μL level, the relative recoveries were 84% and 59%, respectively. Since there seems to be no discernable pattern of differences in relative recovery over the selected range of loading levels, the method seems robust in terms of independence from effects of skin surface loading, at least over the range studied.

Since each person participating in the hand wipe trials was asked to perform the procedure themselves, individual aggressiveness could play a role in analyte recovery. Table IV summarizes the total pyrene recovered for each participant when using the Whatman and Alpha media after 250 μL UGEO was applied to the hands.

Table IV shows that the overall intra-personal variation, when combining the results for the two media, was typically high among the four study participants (mean CV = 22%). The intra-personal variation for the alpha wipes was higher than for the Whatman medium. The mean inter-personal CV was 19%. Although the number of replicates is small and an analysis of variance (one-way ANOVA) by person does not indicate statistically significant differences between results by person for either the Whatman ($\rho = 0.10$) or Alpha media ($\rho = 0.24$), a closer inspection of the results suggests that person #3 typically recovered less of the analyte than the other participants.

As a confirmation of the above findings, a small laboratory hand wipe recovery evaluation using the Whatman and Alpha wipe media was repeated. Another UGEO sample was obtained (1989 Chevy Corsica, 32,000 miles; 4000 miles since last oil change). Next, five 250 μL aliquots of UGEO that had been added to 2 mL corn oil were quantified for pyrene. The average quantity of pyrene was 3.41 μg (CV = 0.12). Next, five replicate hand wipe recovery trials were performed for each wipe medium as described before. The average quantity of pyrene found using Whatman filter paper was 2.36 μg (CV = 0.8), or 69% of the corn oil extract recovery. The average quantity of pyrene found on the Alpha wipe samples was 2.01 μg (CV = 0.14%) or 59% of the corn oil extract recovery. Thus, these results are consistent with the original findings that indicated higher absolute PAH recovery from the hands using the Whatman filter paper but incomplete recovery of PAHs from both sampling media.

Again, the Alpha wipes visually appeared to be superior in removing UGEO from the hands than the Whatman filter paper yet produced lower results when analyzed. It was also noted that a higher recovery was achieved when a known PAH standard was extracted directly from corn oil without the wiping medium being present. Therefore, an additional hand wipe recovery evaluation was performed. This time, the two wipe media were extracted as before, but an aliquot was removed at 36 hr and 3 months later. This was done to determine if the recovery of pyrene was reduced by leaving the Alpha medium in the extraction solution for extended periods of time. The same bulk UGEO was used as described above for this small confirmatory evaluation.

The average quantity of pyrene recovered at 36 hr and 3 months for the Whatman medium was 79% and 76%, and for the Alpha wipes recovery was 73% and 71%, respectively. Similar to before, recovery of pyrene was about 6% less from the Alpha wipes than for the Whatman filter paper. There does appear to be a slight but not statistically significant decrease in pyrene recovery if the medium is left in the extraction vial for a long period of time, but the decrease occurred equally for both media types. Thus, this finding does not help to explain why hand wipe recovery of pyrene from Alpha wipes is lower than the recovery from Whatman filter paper.

Field Samples

Field sampling results collected during this study were unremarkable. Among the hand wipe samples taken from 18 individuals who were not exposed to UGEO, all were below the limit of detection for pyrene. The results were not adjusted for incomplete sampling recovery. Among the automotive repair technicians, 7 of 18 individuals had nondetectable results. Among the 59 samples collected, 18 of the results were above the LOD (0.20 μg/sample) but below the LOQ (0.58 μg/sample), and 8 results were above the LOQ. The highest measured pyrene result was 1.06 μg per pair of hands. Because at the present time there is no known risk estimate for dermal exposure to PAHs and carcinogenesis, the potential long-term health consequences from such exposures is not known.

The engine wipe samples produced detectable pyrene measurements on all the drip pan samples, as might be expected since this is the area where slow leakage of UGEO might occur or otherwise become contaminated during an oil change. The results were highly variable with a median of 0.047 μg and a CV of 160%. One sample contained 0.640 μg pyrene from a car that had been driven awhile without its oil cap in place. A few of the samples (8 of 17) taken from the top areas of the motors also contained detectable amounts of pyrene, but only two were above the LOQ ($>$0.027 μg/sample).

DISCUSSION

Sampling efficiency is an important consideration of any sampling method, yet such information is often lacking for sampling such surfaces as the skin. Without this information, a possible default assumption is that the sampling procedure collected 100% of the analyte that was present. This assumption would underestimate what is actually present if the sampling efficiency is much less than complete.

The sampling method described here extends the approach used by some previous researchers to sample PAHs on skin. Jongeneelen et al.[31] used sunflower oil and paper tissues to wipe the skin of paving workers exposed to road tars derived from coal distillates. In their report, 5 mL of sunflower oil were pipetted onto the hands, followed by 1 min of rubbing, and finally wiping with a tissue. Comparing the amount of pyrene in the first wipe with a second wipe, the relative removal efficiency was determined to be 38%, i.e., the first wipe removed only 38% more pyrene than the second. This poor relative recovery is not too surprising given the large amount of sunflower oil used and limited sorbent capacity of most tissues. In a study by van Rooij et al.,[32] an estimate of the relative sampling efficiency from two consecutive samples was 62% for pyrene on the skin of creosote workers (n = 13), and 48% for pyrene and benzo(a)pyrene on skin of aluminum workers (n = 10 $\times$ 2).

Exposures primarily resulted from aerosol deposition that perhaps may be more easily removed. Large ranges of relative sampling efficiencies were observed (7.1% to 78.9%), which the authors partly attributed to differences in wiping technique used by field staff personnel. By comparison, the mean relative removal efficiencies for the first vs. second wipe as reported in the authors' laboratory evaluation for either the Whatman filter paper or Alpha wipes were 47% and 75% respectively, and the variances were smaller. The rather low relative recovery with the first wipe justifies the collection of at least two consecutive wipes for a composite sample.

The extraction method previously reported by Jongeneelen et al.[31] to recover pyrene from tissue samples also involved more processing steps. First, the tissue was extracted for 15 min with 50 mL dicloromethane. Then a portion of the extract was taken to dryness under nitrogen flow and heating. The residue was dissolved in 5 mL methanol and centrifuged. The supernatant was finally injected into a HPLC. The authors' approach involved only extraction of the wipe media with 40 mL DMSO and injecting an aliquot into the HPLC.

Recently, Vaananen et al.[33] reported results of a field survey of paving workers' exposures to PAHs that included hand wipe sampling by a modified method of Jongeneelen et al.[31] To sample, 3 mL of sunflower oil were placed onto the hands, which were rubbed together for 1 min. The oil was then wiped with a single Kleenex tissue. A 10-mL aliquot of the extract was evaporated to a volume of 1–2 mL and acetonitrile added to a final volume of 3 mL. Instrumental analysis was conducted using a HPLC equipped with a two-channel fluorescence detector. The LOQ was about 0.08 μg/sample and absolute recovery about 60% for pyrene (CV = 0.09). Postshift field sample results for pyrene ranged from about 0.2–20 μg/sample.

The results of the authors' comparison of the Whatman filter paper and Alpha media were unexpected. As stated previously, the Alpha wipes possessed a number of physical attributes that suggested they would generally surpass the performance of the Whatman paper as sampling medium. Notably, when using the Alpha wipes during the laboratory evaluations, they appeared to remove more of the UGEO from the skin than the Whatman paper, which lacks absorbent characteristics. The superior relative recovery for the first vs. second consecutive wipes supports the observation that the Alpha wipes seemed more absorbent than the Whatman paper (Figure 2).

Also, when known standards of PAHs were fortified onto the Alpha wipes either alone or in corn oil, high recoveries were obtained as anticipated due to the relatively inert matrix of the fabric. But when UGEO was used to fortify the two media, the recovery from Alpha wipes was about 6−8% lower than for the Whatman filters. Also, when the results from three consecutive wipes were combined as performed in the hand wipe study, the total analyte recovered by the Whatman paper consistently surpassed the Alpha medium. The reason for the poorer recovery of pyrene in UGEO when using the Alpha wipes is presently unknown.

The variability of the hand wipe sampling results was also somewhat high. It might be expected that interpersonal sampling variation might be greater than intrapersonal variation based on differences in applied pressure and speed of wiping. However, this was not demonstrated in this study, where intra- and interpersonal variability were quite similar. The variability seen occurred in spite of the facts that the participants were all instructed on how to perform the hand wipes, each wipe was supervised by a study author (MB), and the procedure was standardized as much as possible. What was again somewhat unexpected was that the overall variability of the total PAH seen with the Alpha wipes, i.e., CV = 0.30, was higher than for the Whatman filter papers (CV = 0.20), whereas again the latter had been considered to be the poorer wiping medium. However, this difference in variability was not statistically significant (F-test, 2 sample for variances, $\rho = 0.38$). The heterogeneous nature of the hand surface is perhaps responsible for this overall variability.

Time is a performance variable that could potentially affect the recovery of PAHs from the skin. Previous researchers have reported decreasing recovery with the passage of time when known amounts of other analytes were deposited onto the skin and a hand wash sampling method was used.[34–35] This is due presumably to chemical interactions of the analytes with skin constituents and to migration of the analyte from the surface to deeper within the skin. Since certain PAHs have been shown previously to slowly penetrate the skin, it is expected that decreasing sample recovery over time would also have been found here if this evaluation had been performed.[3] This type of an assessment that included several postexposure time points would have involved considerably more sample analyses.

Such an effort was beyond the scope of the present study. In addition, often it is not precisely known during field sampling when the skin initially became contaminated, so an adjustment of the sampling result would probably not be feasible to accurately perform in most cases. Nevertheless, the user of any skin wipe method should be cognizant of this potential loss, and how the sample result may underestimate the amount of analyte that was initially present on the skin surface.

Another issue regarding potential health risks is that of bioavailability of PAHs from UGEO. For example, Sartorelli et al.[36] found the absorption of PAHs from lubricating oil to be less than when the same PAHs were applied to the skin in synthetic sweat. Vehicle partitioning effects are likely to be the cause. Thus, for this and other reasons mentioned, the interpretation of health risks from skin wipe sample analyses are presently difficult to determine.

Although the Whatman filter paper performed better than the Alpha medium in terms of analytical quantification, the Alpha wipes still retain certain characteristics that might persuade the user to adopt this medium for field sampling purposes. User acceptance in the field is an important consideration, and the soft yet rugged nature of the polyester medium is an attractive attribute. The main finding of this evaluation was the quantification of the skin wipe recovery efficiency that can provide a better estimation of the actual amount of PAHs that are present on the skin surface during sampling. However, it should be remembered that wipe sampling for organic compounds may not recover analytes that have already penetrated the skin and would therefore be missed by sampling. Another concern is the twofold range of results found, even under controlled laboratory conditions of use. To somewhat compensate for this variability, larger numbers of samples could be taken to provide greater statistical power.

In conclusion, the collection of skin wipe samples can provide valuable exposure information regarding the skin route, but interpretation is likely to remain challenging, especially in regards to its use in quantitative risk assessment.

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