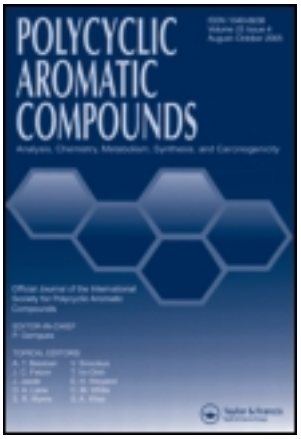

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Study Design and Methods to Investigate Inhalation and Dermal Exposure to Polycyclic Aromatic Compounds and Urinary Metabolites from Asphalt Paving Workers: Research Conducted through Partnership

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Study Design and Methods to Investigate Inhalation and Dermal Exposure to Polycyclic Aromatic Compounds and Urinary Metabolites from Asphalt Paving Workers: Research Conducted through Partnership

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Innovations in science may require crossing traditional boundaries between industry, unions, government, and academia. While such collaborations have the potential to be highly beneficial and productive, opportunities for such collaborations are often missed due to some of the inherent challenges. This collaborative research effort demonstrates an example of how a successful partnership can optimize the ability to answer complicated scientific questions. Specifically, these researchers collaborated to investigate inhalation and dermal exposures to polycyclic aromatic compounds and related urinary metabolites in hot-mix asphalt paving workers. Reported here are details of the partnership process used to create the study design, the review processes, and details of the

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analytical methodologies employed to help attain the study goals related to the identification of the nature, source, pathway, and biological relevance of exposure during hot-mix asphalt paving operations. The actual results of the study are being prepared for future publications.

Key Words: asphalt, dermal, fumes, inhalation, paving, polycyclic aromatic compounds, urinary metabolites

INTRODUCTION

Workers' exposures to polycyclic aromatic compounds (PAC) when placing hot-mix asphalt (HMA) were investigated in this study. HMA is produced by mixing a heated paving asphalt binder with hot aggregate at 130–160°C in a rotating drum. The asphalt binder is a product of the non-distillable fraction of crude oil from petroleum refineries; the binder coats the aggregate with a thin film of asphalt, producing HMA. As needed, the HMA is hauled to the paving site where it passes through the asphalt paver to make the pavement after compaction and cooling.

Paving asphalt binder is a complex mixture of paraffinic and aromatic compounds. A small portion of the aromatic materials are PACs that can be released from the asphalt binder when the asphalt is heated and coated onto the mineral aggregate. The asphalt film is typically only 20 micrometers (or μm) in thickness on the heated aggregate, so volatile and semi-volatile components of the viscous asphalt are released in the form of asphalt emissions. Asphalt emissions are typically comprised of condensed aerosol droplets and volatile gases. Because asphalt emissions occur during paving operations, the workers associated with this process can be exposed to PACs in a variety of ways; this study focuses on inhalation and dermal exposures. In dermal exposures, the PACs can come from condensed fume on the skin, from PACs released from the neat asphalt binder or from other sources such as smoking, contaminated surfaces, traffic (diesel exhaust), and tool/equipment cleaning agents.

The objective of this study is intended to address research gaps identified by the NIOSH Hazard Review (1) on Asphalt and by the International Health Symposium on Bitumen (Asphalt) (2) in Dresden, Germany in 2006. Both of these organizations found that the relative importance of the dermal and inhalation routes of exposure is not clearly understood.

After the Dresden Conference, researchers from academia, government, labor, and industry independently tried to determine how to fill the data gap. It was ultimately determined that no one group was positioned to answer this complex question, and that a collaborative effort by a number of researchers would be advantageous.

As sponsors, the National Asphalt Pavement Association (NAPA) and 37 State Asphalt Pavement Associations (SAPA) (including many manufacturers and contractors) brought the scientific researchers together to address these

complex questions. These organizations provide the funding source for this study and facilitate timelines and contribute ideas, but are not co-authors and allow freedom to the principal scientific investigators to lead the study. The goals of this study were to determine the source, nature, pathway, and biological relevance of PAC exposure in HMA paving workers. Source refers to the identification of the potential sources from which the workers exposure may originate, e.g., asphalt emission, asphalt, diesel oil, and so forth. Nature refers to the physical form of the workers' exposure including (gas, vapor, and particulate) and chemical composition. Pathway is concerned with route of exposure, and biological relevance addresses what are the known potential health outcomes due to these exposures. The partnership, formed around these data gaps, was composed of leading researchers and interested stakeholders/unions.

Within the government sector, the National Institute for Occupational Safety and Health (NIOSH) had originally highlighted the lack of dermal pathway in their Health Hazard Review and was asked to participate. NIOSH has had a continued interest in exploring the dermal pathways. NIOSH researchers also have extensive experience in methods development and field experience in measuring asphalt worker exposure (3–9) and have performed much research on the complex chemistry of asphalt fumes (10–15). NIOSH frequently collaborates with the Centers for Disease Control and Prevention, National Center for Environmental Health/Agency for Toxic Substances and Disease Registry laboratories to conduct biomonitoring studies (16, 17). Historically, NAPA has partnered with NIOSH, the Federal Highway Administration, and the affected labor unions for the development of "Engineering Control Guidelines for Hot-Mix Asphalt Pavers—Part 1, New Highway-Class Pavers" (18–20) issued in January of 1997.

On the academic side of the partnership, the Harvard School of Public Health and Boston University School of Public Health were also asked to participate. Both groups have extensive experience (21, 22) in studying dermal routes of exposure in construction workers, including asphalt pavers. They also have extensive experience in biostatistics, which helped in optimizing the study design, sample size, and data analysis strategies.

The Laborers Health and Safety Fund of North America, and the International Union of Operating Engineers, whose members are involved in placing of HMA, also participated in the design of the study. Industry participation involved three HMA contractors: Mathy Construction (Wisconsin), E&B Paving (Indiana), and Milestone Contractors, L.P. (Indiana); these companies provided access to their jobsites (after safety and proper protection training) and crews whose members were willing to volunteer as study participants.

Finally, the partnership also included two separate research laboratories, both of which service the asphalt industry. These laboratories were selected because of their expertise in the area of asphalt fume research. Heritage Research Group, a laboratory that has extensively studied asphalt fume exposure

and chemical makeup, has developed and published techniques for measuring air exposure to PACs in asphalt workers (23, 24). PetroLabs Inc. has developed techniques for measuring indicators of mutagenicity in complex petroleum mixtures such as asphalt fume condensate (25–27).

This article focuses on the process, design, and methodology for filling the data gaps and is the initial part of a series of articles which will describe the findings of the study goals.

MONITORING CHALLENGES

Inhalation

There is no specific marker compound for exposure to asphalt emissions. Most sampling methods used are nonspecific and cannot be used for total asphalt emissions (1). Work by Brandt et al. (28) and a study summarized in the NIOSH Hazard Review (10) showed that asphalt emissions are chemically complex with thousands of chemical compounds present. Although a standard technique for measuring exposure to asphalt emissions involves drawing air with a sampling pump through a glass fiber filter or membrane filter, it is has long been understood that some asphalt emission components are semi-volatile or volatile and will be easily lost from a glass fiber filter or polytetrafluoroethylene (PTFE) membrane filter during prolonged sampling. This was one of the reasons that the NIOSH recommended exposure limit (REL) was established as a 15-min ceiling limit value (29).

Many studies (3–9, 30, 31) have demonstrated that if a sorbent tube containing XAD−2 is added behind the membrane filter, some of otherwise lost volatile and semi-volatile compounds can be captured, thereby providing a more complete measure of the total exposure. McCarthy et al. (31) also demonstrated that the boiling range of asphalt emissions (fumes) is reasonably similar to diesel oil or kerosene (160–400°C).

The NIOSH Hazard Review (10) summarizes studies along with Reinke et al. (32) and Herrick et al. (33) that demonstrated individual PACs can be measured in asphalt fume condensate and asphalt emissions using GC/MS. While fluorescent techniques have been used to assess unalkylated and alkylated PAC exposures during asphalt paving (3–9, 14, 30, 34), Osborn et al. (35) adjusted the excitation (385 nm) and emission (415 nm) wavelengths to make the technique specific for unalkylated and alkylated 4–6 ring PACs based on actual asphalt fume fractions with known carcinogenicity. These results provide a good indicator of the presence or absence of 4–6 ring PACs in asphalt emissions collected on the combined membrane filters and sorbent tubes containing XAD-2.

An international sampler study was conducted by Ekström et al. (36) using samplers from around the world to measure exposure to asphalt emissions.

This study included the Institute of Occupational Medicine (IOM) sampler suggested by ACGIH (American Conference of Government Industrial Hygienists) for determining whether exposures to asphalt emissions meet the current recommended Threshold Limit Value (TLV) of 0.5 mg/m^3 inhalable benzene-soluble aerosol (37). Kriech et al. (38) demonstrated the method used in the current research correlated with the IOM sampler almost 1 to 1. One improvement to the method described above was the addition of an activated charcoal section after the XAD-2 sorbent section to capture volatile compounds that migrated off the XAD-2 sorbent, thereby insuring a more complete collection of the asphalt emissions. The air collection system used in this study included the PTFE membrane filter in series with a sorbent tube containing 150 mg XAD-2 followed by 50 mg activated charcoal.

Dermal

Methods for assessing dermal exposures can be classified into three groups: surrogate skin techniques, removal techniques (i.e., wipes, tape strips), and fluorescent tracer techniques (39). Väänänen et al. (40) used hand wipes with sunflower oil and tissues to evaluate dermal exposure in road pavers. Väänänen et al. also evaluated polypropylene dermal pads worn on the wrists of road pavers. McClean et al. (22) conducted studies of asphalt paving workers using dermal patches. In that study, polypropylene filters were attached to an exposure pad to create a dermal patch worn on the inside wrists of the asphalt paving workers.

Other existing surrogate techniques, such as the "biologically relevant dermal sampler" for volatile compounds (IOM Dermal Sampler) (41), were all considered, but determined to be unacceptable for sampling dermal asphalt emission. These biologically relevant samplers were not designed to effectively collect and recover PACs. Therefore, this study employed a new dermal sampler utilizing a 5-layer design to allow capture all of the organic compounds present in asphalt emissions even in the presence of diesel oil. The sampler was designed such that compounds will be quantitatively retained on select sorbent layers yet be easily extractable for subsequent analyses. The 5-layer sampler is being referred to as the passive organic dermal (POD) sampler (see Figure 1). The POD sampler has a polypropylene outer layer that acts somewhat like the protective barrier of skin (39) followed by a polyurethane foam (PUF) layer to provide high capacity and reasonable collection efficiencies for most organic compounds and a C-18 solid-phase extraction (SPE) disc to collect most of the remaining organic compounds including PACs. The bottom sorbent layer consists of a carbon cloth to capture of the more volatile compounds that may pass through the other layers. A layer of ethylene tetrafluoroethylene (ETFE) is placed between the C-18 disc and the carbon cloth bottom layer to prevent the transfer of collection media between the two layers.

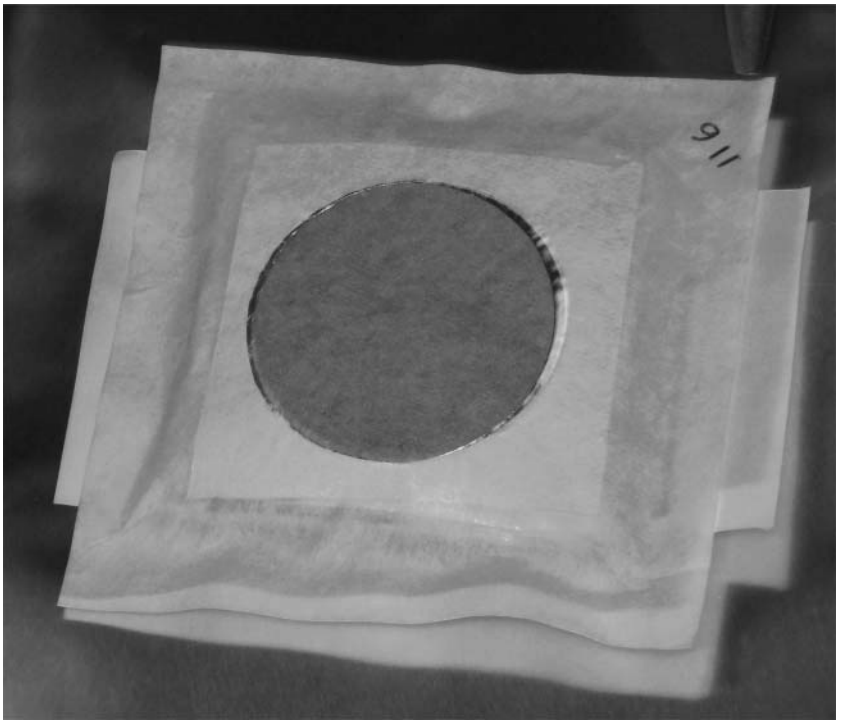

Figure 1: Photo of an assembled five-layer Passive Organic Dermal sampler.

PAC Metabolites in Urine

A study by Walter and Knecht (42) exposed volunteers to asphalt fumes, with and without powered air purifying respirators, and concluded that approximately 50% of measured urinary PACs metabolites (pyrene, chrysene, and phenanthrene) result from dermal absorption. In a study of roofing asphalt workers, Reinhart (43) concluded that exposures by the dermal route were twice as high as those by inhalation, which corresponded to a 40% increase in post-shift 1-hydroxypyrene levels in urine. McClean et al. (21) studied road pavers using a distributed lag model and concluded the impact of dermal exposure on urinary 1-OHP is approximately 8 times greater than that of inhalation exposure.

None of these studies considered other confounding influences such as diesel oil used for cleaning tools and equipment, a common practice in the U.S. today. This study addressed the effect of this confounder in HMA paving workers. Although diesel exhaust fumes from the equipment is also a potential confounder, the effects are minimal compared to the effect of the diesel oil use and were not evaluated as part of this study.

Boogaard (44) suggested the possible influence of co-exposures being contributors to elevated levels of urinary 1-OHP. More recently, Sobus et al. (45) investigated the use of multiple urinary PAC biomarkers rather than just 1-OHP when studying workers exposed to hot asphalt. These included the measurement of unmetabolized naphthalene, and phenanthrene as well as the monohydroxylated metabolites of naphthalene, phenanthrene, and pyrene in each urine sample.

In the current study, 24 PAC metabolites were initially monitored in the urine using the methodology originally developed for the National Health and Nutrition Examination Survey (46); however, because only 10 PAC metabolites are generally found in urine, only these 10 PACs were reported for later samples. Therefore, this study examines the most comprehensive group of PAC metabolites in HMA paving to date.

Although it was hoped that the start of the work week would be Monday after a weekend of non-exposure, this was not always the case and will be considered in the data analysis. The anticipated half-life of PACs in urine varies for the different OH-PACs (44) and will likely carry over from one day to the next. The design was to always start on a Monday such that crews (in theory) had two days away from work and will analyze the accumulation throughout the work week. Pre-shift and post-shift results from the pilot study showed that concentrations would allow the necessary statistical evaluations between the experimental scenarios, especially because the study design each worker acted as their own control.

STUDY DESIGN

After meeting with the principal investigators in the study, a draft protocol was prepared and sent to an autonomous science advisory committee (SAC) for external review. This panel of experts from academia, government, and the private sector peer-reviewed the protocol and made suggestions to improve the design. The SAC included John Cherrie (Institute of Occupational Medicine, UK), Bryan Hardin (Veritox Inc., USA), Hans Kromhout (Utrecht University, NL), Glenn Talaska (University of Cincinnati, USA), and Richard Niemeier (NIOSH, USA).

The study was divided into two phases. The first phase (Phase I) was an efficacy/pilot study to develop and evaluate a set of methods and procedures that would provide a range of responses sufficient to be used in the larger Phase II study. This included development and evaluation of existing dermal methods for determining exposure to potential PAC sources normally encountered when working with HMA. Methods for measuring PAC and their resultant metabolites in urine were also studied. Logistical issues were of concern and Phase I provided information useful in Phase II. For example, it was determined that

at least four field sampling personnel on each crew would be needed to handle the extensive monitoring and worker observations.

The goals of Phase II were to identify the source, nature, pathway, and biological relevance of workers' exposures. In this study, the exposure route could be through the lungs, skin, or consumption from sources found in food. This study investigated the sources of the PACs that are present in the work environment. These sources include the PACs from asphalt emissions, neat asphalt, diesel oil, diesel exhaust or other sources such as smoking or contaminated tools. The biological relevance of these PACs was studied in the PAC metabolites present in worker urine and genotoxic characteristics of those PACs. Both dermal and inhalation exposure to PACs were determined through detailed chemical, biological, and physical analyses (gas, aerosol, particulate). A repeated measures panel study design was utilized to monitor each participant for three days over four different scenarios (e.g., baseline (no interventions), no diesel oil, respiratory protection, dermal protection) for a total of 12 days of monitoring.

The initial "baseline" scenario involved establishing baseline measurements for these workers under routine work practice conditions without interventions. This included standard inhalation exposure measurements, which have been historically used to collect information about paving asphalt workers such as NIOSH Method 5042, as later described. It also included additional methods of collecting and characterizing exposure by inhalation and dermal routes.

In the second "no diesel" exposure scenario, diesel oil normally used to clean tools and equipment was replaced with B-100 Biodiesel fuel (47). B-100 is comprised of fatty acid methyl esters from soybeans or other plants and it contains no PACs. Previous studies by Tompa et al. (48) as well as work by Weker et al. (34) have shown that diesel oil may be a significant source of PAC exposure and a confounding agent in exposure studies in HMA paving. B-100 Biodiesel was found by paving workers to be as effective as diesel oil in cleaning.

The third "respiratory protection" exposure scenario was designed to reduce inhalation sources of PACs using a Powered Air Purifier Respirators (PAPR) (49). PAPRs are full-face protection respiratory devices with a protection factor of 25 in reducing PAC exposures of the wearer. A 3M organic vapor/high efficiency cartridge (R.S. Hughes Company, Inc. Indianapolis, IN, Cat. No. 051131-07196) was used to filter out PAC source exposures from the breathing zone of the worker. Other 3M PAPR parts from the same vendor included a comfort belt (OHESD GVP-CB 051131-52772), a breathing tube (OHESD L-122 051131-37012) lens cover (OHESD L-133-25 051131-37014), a hard hat with face shield (OHESD L-701 051138-66151), and a battery pack (OHESD GVP-111 051138-29208). Each contractor provided respirator training and medical clearance. Since these are loose fitting masks, it was not

necessary that the workers be clean shaven or fit tested. All units were calibrated pre- and post-shift and monitored mid-day to make sure that the airflow was above 6 cfm. During PAPR use, personal observers were provided to insure proper hydration to minimize risk of heat stress, and to help protect the worker due to increased safety concerns (vision and hearing restrictions).

The fourth exposure scenario involved minimizing dermal routes of exposure. During this scenario the workers were provided with a standardized set of clothing. This included long sleeve cotton shirts, cotton painters pants, hard hats, or cloth hats equipped with neck guards and gloves with a latex coated palm and fingertips. Face and ears were not covered, and it was possible for some contamination to occur through the cotton shirts. Because each worker was provided with clean clothes each day, sorption through the cotton clothes did not appear to be a problem. If needed, spare shirts and pants were available for each worker to allow an immediate change of clothing. Again, personal observers were provided during this scenario to ensure proper hydration and to protect against heat stress. During this scenario, the dermal sampler was located underneath the clothing.

Three different paving crews were monitored and each crew consisted of four workers: operator, screed persons, raker and/or shuttle buggy operator (a shuttle buggy is a vehicle for collecting HMA from trucks and transferring it to the paver tractor). Three of the four workers for each crew had common descriptions: operator, screedman, and raker. The fourth person's description varied per crew (foreman/Milestone Inc., laborer/Mathy Construction, and shuttle buggy operator/E&B Paving). Each of the four exposure scenarios conducted were monitored over three consecutive days, such that each worker was sampled 12 days (total of 144 worker-day measurements). For each exposure scenario, over the three consecutive monitoring days, single void urine sample specimens were collected pre-shift, post-shift, and before bed (bedtime) and once upon waking in the morning after the third day for a total of 10 urine samples per worker per scenario. Crews were at different locations: two in Indianapolis, IN, and one in Merrill, WI. Class A pavers with engineering controls were used for the most part, shuttle buggies were used by some crews more than others and HMA types included PG 64-22, PG 58-28, and PG 76-22 with these parameters all variable between and within crews.

Additionally, a control group for comparison of non-asphalt exposed construction workers to asphalt exposed workers was also studied, which included four members of a concrete crew. For the control group, only the baseline work practice monitoring and the "no diesel" scenarios were conducted. Diesel oil that had been used as a release agent for concrete forms was replaced with B-100 biodiesel. No exposure to asphalt or asphalt emissions occurred in this group. Although not measured, the relative amounts of diesel exhaust emissions appeared to be comparable between the control group and the exposed groups. As it turns out, the concrete crew had discontinued the use of diesel oil

after the pilot study results were shared, so both weeks during Phase II were "no diesel" scenarios.

One of the strengths of the repeated measures study is that each subject serves as his own control and a relatively small number of subjects provides sufficient power to detect modest changes in urinary OH-PAC concentrations. For the sample size used in this project, of 10 repeated urinary OH-PAC measurements per subject per scenario, an estimate of the power to detect changes in urinary OH-PAC concentrations associated with exposure control scenarios was made. Accordingly, using 12 subjects provides there is an 80% chance of detecting a 23% change in urinary OH-PACs when comparing each of the controlled exposure scenarios to the baseline (uncontrolled) scenario without interventions (50).

Participant recruitment was based on the ability and willingness of each worker to participate in the study for four weeks. In addition, all workers received information about the study and the role they would play. Crews expressing a willingness to participate were shown and allowed to try the equipment they would wear, such as PAPR, gloves, and monitoring equipment. Each signed an informed consent form approved by the NIOSH Human Subjects Review Board. Standard monitoring data for individuals who participated in the study were shared with each individual worker (only their own results). In addition, a summary report was made available to the construction company management for each site. This report did not include the volunteer names; but rather reported the individual data per scenario, labeled worker 1, worker 2, worker 3, and worker 4. Since this study involved human subjects, the NIOSH human subjects review board (HSRB) was required to review the protocol. The Harvard human subjects review board accepted the approval of the NIOSH HSRB as the designated approval. To encourage participation and retention, each participant received $25 for any amount of participation during any study week. This totaled $100 if they participated in all 4 weeks of the study. Because participants were asked to wear special personal protective equipment (PPE) for 6 days, if they participated in 100% of the study, they received a $50 bonus. This money was distributed on the last day of the study in cash. While it was hoped that participants would stay to the end, they were free to drop out of this study at any time; however, if they dropped out, they did not get any more payments such as those they got when they were in the study. All crewmembers, not just study participants, were provided lunch each day of sampling.

The participants were also given a list of foods containing PACs that they were to avoid during the study (specifically grilled and smoked foods). They were also advised to avoid other non-workplace sources of PACs, e.g., use of coal tar-based driveway sealers. Smoking was not prohibited during this study, but smoking habits were recorded for each worker based on observation. Since smoking-related PACs are a confounder, it was accounted for in the analyses by

measuring cotinine levels in each urine sample. Urine cotinine measurements captured direct and second hand cigarette smoke exposures.

SAMPLING STRATEGY

The strategy on air inhalation monitoring is to fully capture, characterize, and quantify the compounds found in the breathing zone of HMA paving workers. Special emphasis is placed on detection of PACs.

Air Monitoring (Inhalation)

Each worker wore a personal sampling pump which collected air from the breathing zone and that was connected to a membrane filter followed by a sorbent tube for the collection of total particulates (TP), benzene solubles fraction (BSF), and total organic matter (TOM). This arrangement was positioned to collect air in the worker-breathing zone. These samples were also analyzed for PACs, total 4–6-ring PAC content, and other organic sources besides asphalt emissions (12, 14, 23, 35, 51, 52). For HMA workers, 144 full-shift air samples were collected whereas 24 air samples were collected for control group (concrete workers).

One background sample was collected each day, positioned upwind of the paving operation. Descriptive data was collected on potential confounders from the site, e.g., vehicle exhaust, construction dust and any other background interferences. A field blank was collected on each day of sampling for each crew (36 blanks for paving workers, 6 blanks for concrete workers). As required by the TP and BSF methods (NIOSH 5042) (12), 4 additional filter field blanks (5 total) per day were collected and analyzed.

Hand Washing with Sunflower Oil (Dermal)

Wrist and hand washing was performed at the start of the work shift before placement of POD sampler and after removal of the POD sampler at the end of the day. The hand wash extracts were analyzed for TOM, PACs, total 4–6-ring PACs, asphalt content, and other organic compounds besides asphalt emissions. In total, 288 and 48 hand wash samples were collected from HMA and concrete workers, respectively. Thirty-six background hand washing samples and 36 hand washing field blanks were collected in the study.

Passive Organic Dermal (POD) Samplers

The second dermal measure used was the newly developed 5-layer POD sampler. This sampler was tested in the lab prior to field trials; exposure recovery and extraction efficiencies will be detailed in a complimentary article (53).

Extracts from select layers of the POD sampler were analyzed for total mass gained (TP), the dichloromethane soluble fraction of total mass gained (DSF), TOM, PACs, 4–6-ring PACs, asphalt content, and other organic compounds besides asphalt emissions. For asphalt workers, 144 POD samples were collected; and for concrete workers, 24 POD samples were collected. Thirty-six POD background samples and 36 POD field blanks were collected in the study.

Urine Samples

Urine samples were collected as described in a later section. Time of collection was recorded which will allow different work shift durations or variable betimes to be factored into the data analysis. These urine samples were analyzed for cotinine, creatinine, hydroxylated PACs, PACs and their metabolite PACs, and nitrated derivatives of PACs and their metabolites. For HMA workers, 576 urine samples (480 worker plus splits) were collected and for concrete workers, 96 urine samples (80 worker plus splits) were collected.

RAP Samples

Reclaimed asphalt pavement (RAP) previously used for roads was reprocessed and used in the study pavements for all sites by all contractors. Samples were collected of the RAP to assure that it was free of coal-tar.

Height and Weight Data

On the first day of monitoring, height and weight measurements were determined for each study participant to calculate a body mass index (BMI).

METHODS

Air Monitoring

Total Particulates (TP) & Benzene Soluble Fraction (BSF)

TP were determined according to NIOSH Method 5042 (12). The TP samples were collected on preweighed 37-mm poly-tetrafluoroethylene (PTFE) membrane filter laminated to PTFE (2 μm pore size; Cat. No. 225-27-07; SKC, Inc.; Eighty Four, PA) at 2 L/min; the pumps were calibrated pre- and post-shift. Because a sorbent tube was added after the filter cassette, the cellulose support pad was replaced with cellulose o-rings. After sampling, the membrane filter was reweighed, and the difference between the post- and preweight was reported as TP. The membrane filter was then extracted with 3 mL benzene (Cat. No. 270709; Sigma-Aldrich; St. Louis, MO). The extract was filtered using a filter tube (Whatman 6 mL filter tube 1.0 μm pore size made of PTFE;

Cat. No. 09-930-30c; Fisher Scientific; Pittsburgh, PA), and 1.5 mL of the filtered extract was transferred to a preweighed weighing boat and gently evaporated to dryness in a vacuum drying oven. The difference between the post- and pre-weight of the weighing boat was reported as the BSF.

Total Organic Matter (TOM)

After the PTFE membrane filter, a sorbent tube (150 mg XAD-2 followed by 50 mg activated charcoal; Cat. No. CPM032509-001; SKC, Inc.; Eighty Four, PA) was added. The sorbent tube was extracted with 10 mL dichloromethane (HPLC Grade OmniSolv® High Purity; Cat. No. DX0831-1; EMD; Gibbstown, NJ), and 5 mL of the extract was combined with the BSF residue and used for TOM analysis. The hydrocarbons ranging from C6–C42 were defined as the TOM [23] and were determined by gas chromatography/flame ionization detector (GC/FID) using a modification of EPA Method SW846-8015B (54). A Varian model 3400 GC with a 1077 split/splitless injector (set at 250°C) and a flame ionization detector (set at 310°C) were used, along with a 5% phenyl/95% methylpolysiloxane column (30 m × 0.33 mm ID, 0.25 μm film thickness; Restek RTX-5); helium was the carrier gas set at 2 mL/min. Injection volume was 2.0 μL, and the oven temperature program was as follows: 40°C held for 3 min, increased to 120°C at 9°C/min and held for 0.5 min, then ramped to 305°C at 11°C/min, and held for 10.89 min. The instrument was calibrated using a kerosene standard (FU-005N Neat: AccuStandard, Inc.; New Haven, CT), and calibration curves were developed using this standard to determine the concentrations of the TOM samples.

Storage of TP/BSF/TOM Samples

At the end of each sampling day, the samplers were removed from the worker, covered in aluminum foil, transported on ice, and stored at -20°C until analysis.

GC/FID Elution Profiles to Identify Sources of Organic Exposures

Elution profiles of various organic sources (e.g., asphalt, asphalt emissions, diesel oil, biodiesels, biofuels mixed with diesel oil, various lubricants and sources, used motor oil, sunscreen, and other organic samples), operator expertise, and pattern recognition techniques were used to examine the elution profiles collected during the analysis of the TOM samples to identify sources of organic compounds.

Total 4–6 Ring Polycyclic Aromatic Compound Content and Coal Tar in RAP

A fluorescence method similar to NIOSH Method 5800 (13, 14) was used to determine the total 4–6 ring PAC content. By changing the excitation/emission wavelengths, the method became more specific for 4–6 ring PACs (24, 35). The fluorescence analysis was performed on the TOM extracts using a Perkin

Elmer Luminescence Spectrometer model LS50B. The excitation/emission wavelengths were 385/415 nm, respectively, and the results were reported as $\mu g/m^3$ diphenylanthracene.

To determine the presence or absence of coal tar in the RAP samples, full fluorescence spectra were obtained by running emission scans starting at 200 nm and ending at 520 nm with the starting excitation at 200 nm, slit widths at 3 nm, and a scan speed of 1,000 nm/min. With the excitation incremented at 5 nm, 40 scans were produced and combined using the Perkin Elmer software to generate a 3D contour plot. A horizontal cut from the contour plot between 383 and 462 nm at an excitation wavelength of 310 nm provides a distinctive double hump when coal tar is present, with maxima at ~403 nm and ~430 nm (35).

Polycyclic Aromatic Compounds

Thirty-three PACs (see Table 1) were determined using gas chromatography/time-of-flight mass spectrometry (GC/TOFMS) following the guidelines of EPA SW-846 8270C (51) and a published procedure by Kriech et al. (52). A Leco Pegasus II GC/TOFMS (source temperature: 250°C, transfer line temperature: 280°C, mass range: 35–400, 5 spectra/s) with a split/splitless injector (splitless mode, set at 280°C) along with a 5% phenyl/95% dimethylpolysiloxane column (30 m × 0.25 mm ID, 0.25 μm film thickness; Phenomenex ZB-5). Helium was the carrier gas set at 2.0 mL/min. Injection volume was 2.0 μL, and the oven temperature program was 50–260°C at 15°C/min. Three different standards supplied by AccuStandard Inc. (New Haven, CT) were used to prepare a working standard of 33 PACs.

Table 1: List of the 33 PACs determined in air and dermal monitoring samples using gas chromatography/time-of-flight mass spectrometry.

Acenaphthene	Pyrene
Acenaphthylene	Benzo(j)fluoranthene
Anthracene	7,12-Dimethylbenz(a)anthracene
Benz(a)anthracene	Benzo(e)pyrene
Benzo(a)pyrene	3-Methylcholanthrene
Benzo(b)fluoranthene	Dibenz(a,h)acridine
Benzo(ghi)perylene	Dibenz(a,j)acridine
5-Methylchrysene	7H-Dibenzo(c,g)carbazole
1-Nitropyrene	Dibenzo(a,e)fluoranthene
Benzo(k)fluoranthene	Dibenzo(a,e)pyrene
Chrysene	Benzo(rst)pentaphene
Dibenz(a,h)anthracene	Dibenzo(a,h)pyrene
Fluoranthene	Dibenzo(a,l)pyrene
Fluorene	Benzo(b)naptho(2,3-d)thiophene
Indeno(1,2,3-cd)pyrene	Cyclopenta(cd)pyrene
Naphthalene	Triphenylene
Phenanthrene	

(The working standard actually contains 34 PACs; however, we did not determine benzo[c]phenanthene in the samples.) The three standards included a standard of 24 PACs (Cat. No. H-QME-01), a custom order standard of nine PACs (Cat. No. S-13911-R1), and dibenzo[a,e]fluoranthene (Cat. No. H-247S); the first two standards were combined and diluted with dichloromethane to form a 50 μg/mL working standard of 32 PACs. To this working standard the remaining standard was added and diluted with dichloromethane to form calibration standards. To each 100 μL of calibration standard, 10 μL of a semivolatile internal standard (Cat. No. 4-8902; Supelco; St. Louis, MO) was added.

Dermal Monitoring

Passive Organic Dermal (POD) Sampler & Hand Washing Method

A multilayer (five sorbent layers) passive organic dermal (POD) sampler was developed for use in this investigation. The outer layer (top layer) is preweighed to allow total particulate measurements. All five layers are placed on a sheet of aluminum foil, centered onto a 40 mm diameter opening (sampling area of 12.6 cm^2). After sealing the layers in place with the foil, the PODs are heat-sealed in a thin muslin fabric. Flexible and easily adhered to various surfaces, it is stored in a Mylar® bag before and after use. Each worker wore a POD on each monitoring day. The POD samplers were attached to the outer forearm of each worker's dominant hand. On cold days these were placed over the clothing except during the dermal protection experiments in which case they were placed underneath the clothing next to the skin. Details relating to sampler design, construction, method of extraction, and validation were presented at the 22 ISPAC by Olsen et al. (53, 55) and will be published separately. Theoretically, this sampler should collect emissions from workplace exposures including that from smoking and diesel exhaust, although these parameters were not separately tested.

Background POD samplers were collected each day, placed next to the background air collection system positioned upwind of the paving operation. Descriptive information was collected on potential confounders from the site, e.g., vehicle exhaust, construction dust and any other background interferences. A POD field blank was also collected on each day of sampling for each crew (36 blanks for paving workers, 6 blanks for control group workers). Before and after sample collection, all POD samplers were stored in Mylar® bags. When it was time to be analyzed, the outer layer (polypropylene) of the POD sampler was handled and extracted as described in the next section.

The next two layers (PUF/C-18 SPE disk) were extracted in 20 mL dichloromethane, and the extract was filtered with a syringe filter (non-sterile syringe filter, PTFE, 0.45 μm pore size, 25−mm diameter; Cat. No. 09-719-7;

Fisher Scientific; Pittsburgh, PA). Five mL of the extract were gently evaporated, reconstituted in 500 μL dichloromethane and analyzed for asphalt. From the remaining 15 mL of filtered extract, 2 mL were gently evaporated, reconstituted in 200 μL dichloromethane and aliquoted for TOM and PAC analysis.

The bottom two layers (ethylene tetrafluoroethylene/100% carbon cloth) were extracted with 3 mL dichloromethane and the extract was filtered using a Whatman autovial 5−mL syringeless filter with a PTFE membrane, 0.45 μm pore size; Cat. No. 09-921-18; Fisher Scientific; Pittsburgh, PA. This filtered extract was aliquoted for TOM and PAC analysis.

An existing hand washing method was used to wash the hands and wrists using sunflower oil as previously described by Jongeneelen et al. (56) and Väänänen et al. (40). Three mL of sunflower oil was applied to the palm, and the workers rubbed their hands together for 1 min. Each worker wiped the sunflower oil from their hands (care was taken to wipe the front and back of their hands and between the fingers.) using a piece of 8.25″ × 8.25″ crepe material (50:50 wood pulp and polyester; DuPont™ Sontara®; the wipes as used were creped by the Micrex Corporation). The crepe material was placed in a glass vial and stored in a cooler before transport. Crepe materials were extracted with 25 mL dichloromethane; this extract was aliquoted for TOM, PAC, and asphalt analysis.

POD Samples Analyzed for Total Mass Gained and Dichloromethane Soluble Fraction

Total mass was determined from the difference of post- and preweight of the outer layer (polypropylene) of the POD sampler. Once the post-weight was determined, the outer layer of the POD sampler was extracted with 3 mL dichloromethane. The extract was filtered using a syringe filter (non-sterile syringe filter, PTFE, 0.45 μm pore size, 25−mm diameter; Cat. No. 09-719-7; Fisher Scientific; Pittsburgh, PA), and 1 mL of the extract was transferred to a preweighed weighing boat and gently evaporated to dryness in a chemical fume hood. The difference between the post- and pre-weight of the weighing boat was reported as the dichloromethane soluble matter. The solubles residue was reconstituted in 1 mL dichloromethane, concentrated down, and analyzed for asphalt. The remaining filtered extract was concentrated to 300 μL and aliquoted for TOM and PAC analysis. The amount that was concentrated varied due to the filtration step, but averaged 1.31 mL for these samples.

POD and Hand Wash Samples Analyzed for TOM

Because the sunflower oil used for hand washing contributed to the TOM signal, the sunflower oil used for hand washing was removed using a cyanopropyl solid-phase extraction (CN-SPE) column (Sep-Pak Vac 12 cc (2g); Cat. No. WAT054645; Waters; Milford, MA). Once the samples were prepared

for analysis, the same analysis procedure used on the TOM air-monitoring samples also was used on the TOM dermal-monitoring samples.

POD and Hand Wash Samples Analyzed Using GC/FID to Collect Elution Profiles to Identify Sources of Organic Exposures

The same procedure used to evaluate TOM elution profiles on the air-monitoring samples also was used on the TOM dermal-monitoring samples (see responding section under air monitoring for instrumentation and experimental conditions used).

POD and Hand Wash Samples Analyzed for Polycyclic Aromatic Compounds

Once the samples were prepared for analysis, the same analysis procedure used on PAC air-monitoring samples also was used on the PAC dermal-monitoring samples; however, because of interferences from the sunflower oil used for hand washing, the GC inlet liner had to be replaced daily. Instrumentation and experimental conditions used are given in the PAC section under air monitoring.

POD Samples Analyzed for Asphalt

Once the samples were prepared for analysis, a high temperature GC/FID (HTGC/FID) was used to distinguish between dermal exposure fractions, i.e., asphalt particles versus asphalt emissions or other organic sources. For this investigation, hydrocarbons > C-25 (401°C) were defined as being from the asphalt (bitumen) using ASTM Method D 6352-04 (57). This is based on the temperature at which the baseline first starts to raise for the asphalt calibration standards. An Agilent 7890A GC with an FID (detector set at 400°C) was configured with a Gerstel MS2 autosampler. A programmed temperature vaporizer inlet and liquid nitrogen cryogenics (Agilent 7890A) in the splitless mode (conditions: 37.2 mL/min at −40°C for 2 min, 15°C/min to 390°C for 8 min), and data analysis system (Agilent Chemstation E.02.00) were used. A methyl silicone polarity-phase column (5 m × 530 μm ID, 0.1 μm film thickness; Restek MXT-1HT Sim Dist column—Cat. No. 70100) was used with a helium flow rate of 5.7 mL/min. The injection volume was 3.0 μL, and the oven temperature program was as follows: −40°C held for 2 min, increased to 390°C at 15°C/min and held at 390°C for 31 min. A C30-C120 Polywax Standard (Cat. No. 36227: Restek; Bellefonte, PA) was used to optimize the instrument. Calibration curves were developed using standards prepared in the laboratory from a PG 64-22 asphalt at five different concentrations, ranging from 100 mg/L to 1000 mg/L in dichloromethane; these curves were used to determine asphalt concentrations in the samples by Nguyen et al. (58).

Hand Wash Samples Analyzed for Asphalt

While the HTGC/FID procedure was useful for analyzing for asphalt from the POD samplers, the sunflower oil used for hand washing interfered in the analysis, and a new method had to be developed to analyze these samples. The key for solving the interference was exchanging the samples from dichloromethane to hexane, because it made a significant difference in preventing an interfering response from the sunflower oil. Known amounts (20–100 μL) of the dichloromethane extract used for the analyses described previously were gently taken to dryness and reconstituted in 1.0 mL of hexane. This hexane solution was analyzed for asphalt using an Agilent 1100 series high performance liquid chromatography (HPLC) with fluorescence detection (G 1321A FLD) set with excitation and emission wavelengths of 325 and 392 nm, respectively. These conditions were empirically selected to maximize the response of the asphalt and minimize the response of the sunflower oil. To accommodate the normal phase conditions used, special pump seals were employed (Agilent P/N 0905-1420). The mobile phase was 90% hexanes (Optima Fisher Scientific; Cat. No. H3034: Pittsburgh, PA) and 10% dichloromethane (HPLC Grade OmniSolv® High Purity; Cat. No. DX0831-1: EMD; Gibbstown, NJ) and was pumped isocratically at 1.0 mL/min through the column. The column was a Betasil CN 250 mm $\times$ 4.6 mm ID, 5 μm. (Cat. No. 70805-254630: Thermo Scientific) and was held at 23.5°C. Although the asphalt peak elutes within 5 min under these conditions, each sample injection of 10 μL required a 20-min run time to allow for elution of other compounds in the samples. The five asphalt calibration standards were prepared from a PG 64-22 grade asphalt and ranged from 2.0–100 mg/L in hexane, with a linear response showing a correlation of 0.999. An instrument detection limit of 8.4 ng on column was achieved. Based on the hand washing surface area of 820 cm^2 (59) and the sample preparation process, a sample detection limit of 0.13 $\mu g/cm^2$ was achieved, although the practical quantification limit based on field blanks was between 3.9 and 5.1 $\mu g/cm^2$.

Nicotine by GC/TOFMS

Suggested as an option after completion of all Phase II analyses, the presence of nicotine [2-(1-methyl-2-pyrrolidinyl)-pyridine] was investigated by examining masses 84 and 133 using the data files from the GC/TOFMS PAC analysis. Although air, POD and hand wipe samples were initially screened using samples expected to contain high levels, only the hand wipe samples consistently contained nicotine. Subsequently, all hand wipe samples from Phase II were evaluated and concentrations were estimated by performing a post-analysis 5-point internal standard calibration using 2-(1-methyl-2-pyrrolidinyl)-pyridine (AS-E0519 AccuStandard, Inc.; New Haven, CT).

Results were reported as ng/wipe and an estimated practical quantitation limit of 12.5 ng/wipe was achieved.

Urine Samples

Urine Collection and Storage

A single void urine specimen was collected from each worker: pre- and post-shift, and at bedtime for each exposure scenario. An additional pre-shift sample was collected the morning after the third day of sampling. Time of collection was recorded.

Except for the bedtime samples, all samples were aliquoted after they were taken; the bedtime samples were stored on ice packs or refrigerated and aliquoted the next workday. Each urine sample was assigned a random number to limit possible bias in the order of sample analysis. Each urine sample was aliquoted as follows: 5 mL of urine was added to a 8-mL polypropylene tube for the Ames mutagenicity assay. 10 mL of urine was added to each of two 15-mL glass tubes for either the enzyme linked immunosorbant assay (ELISA) or hydroxylated (OH-) PAC analysis. Another 15-mL glass tube for the PAC-ELISA was prepared containing 3 mL urine and 1 mL methanol (HPLC grade; Fisher Scientific; Fair Lawn, NJ) added; this was done in the field to minimize adsorption of the PACs to the walls of the tube as recommended by the ELISA kit manufacturer (RaPID Assay PAH Test kit Product (A00156/A00157); Strategic Diagnostics; Newark, DE). 1.8 mL urine was added to a 2-mL polypropylene vial for the creatinine analysis. 1.25 mL urine was added to a 1.5 mL cryovial for the cotinine analysis; the remaining urine sample was transferred to a 50-mL polypropylene centrifuge tube. The aliquots were stored on ice in the field and transferred to -20°C freezer at the end of each workday. Except for the urine sample for the Ames mutagenicity assay, at the end of each sampling week, samples were transported on either ice or dry ice depending on travel time to a NIOSH laboratory in Cincinnati, OH; upon arrival, all urine samples in glass tubes were stored in a freezer at -20°C freezer and all other urine samples were stored in a freezer at -50°C. The urine samples for ELISA, cotinine and creatinine analysis were analyzed at the NIOSH laboratory whereas the urine samples for OH-PAC analysis were shipped by overnight express on dry ice to the CDC laboratory in Atlanta, GA. The urine samples for Ames mutagenicity assay were shipped by overnight delivery on ice to PetroLabs Inc., Ivyland, PA.

Hydroxylated (OH-) PACs

At least 24 OH-PACs (metabolites of PACs) can be determined using gas chromatography/isotope dilution high-resolution mass spectrometry (GC/IDHRMS). However, only 10 OH-PACs are predominately excreted in

Table 2: List of the 10 hydroxylated polycyclic aromatic compounds (OH-PACs, Metabolites of PACs) monitored in urine using gas chromatography isotope dilution high resolution mass spectrometry

Hydroxylated Polycyclic Aromatic Compounds	
1-OH-naphthalene	1-OH-phenanthrene
2-OH-naphthalene	2-OH-phenanthrene
9-OH-fluorene	3-OH-phenanthrene
2-OH-fluorene	4-OH-phenanthrene
3-OH-fluorene	1-OH-pyrene

urine; hence, only these OH-PACs were determined (60) (see Table 2). Enzymatic deconjugation of the glucuronide and/or sulfate conjugates was used to free the OH-PACs. The free OH-PACs were isolated with automated liquid-liquid extraction into pentane using a Gilson 215 Liquid Handler. Once the pentane evaporated, the sample was reconstituted in toluene and derivatized to the trimethylsiloxane derivatives. Analysis was performed using GC/IDHRMS (MAT95XP; ThermoFinnigan MAT). Additional details about the procedure, instrumentation, and experimental conditions were reported by Li et al. (46).

Cotinine

Cotinine (Nicotine-N-oxide), a metabolite of nicotine, was used to assess a worker's exposure to nicotine in tobacco products. Cotinine was determined using the Immulite® 2000 (Siemens Medical Diagnosis) analytical platform (61, 62) that uses solid-phase competitive chemiluminescent immunoassay. Beads coated with polyclonal rabbit anti-cotinine antibody, 20 μL of sample or standard, and alkaline phosphatase conjugated to cotinine were incubated for 30 min (61)]. The beads were then washed, and the chemiluminescent substrate was added. The light produced was measured by a photometer, and the intensity was indirectly proportional to cotinine concentration. For the assay, the limit of detection and limit of quantitation were 5 ng/mL and 10 ng/mL, respectively. Other assay conditions were the same as those recommended by the manufacturer.

Creatinine

Creatinine can be used to normalize the urinary metabolite results. Creatinine was determined using the Vitros Autoanalyzer (Ortho Clinical Diagnosis) (63)). The specialized VITROS CREA slide is a multilayered polyester support containing all the analytical reagents needed. Creatinine diffuses to the

reagent layer where it is hydrolyzed and eventually oxidized to a colored product. Reflection density was measured at two time points, and the difference is proportional to creatinine concentration.

Immunochemical Method for PACs and their Metabolites

An ELISA was developed for total PACs including the metabolites of PACs using an existing ELISA kit (RaPID Assay PAH Test kit Product (A00156/A00157) Strategic Diagnostics; Newark, DE) and phenanthrene standards. Additional details relating to the ELISA kit and validation for use in the investigation were presented at the 22 ISPAC (64) and will be published separately.

Ames Mutagenicity Assay of Nitrated Derivatives of Urinary PACs and Their Metabolites

This assay is a variation of that reported in Blackburn et al. (25, 26). The method is a sensitive and selective detector of PACs and PAC metabolites. PACs are easily nitrated under the conditions used (8 N HNO_3, 80°C, 30 min). This unique property confers part of the selectivity of the assay. The nitrated derivatives produced are extremely potent mutagens in the Ames *Salmonella* mutagenicity assay without metabolic activation (65). This property confers the extreme sensitivity of the method and also contributes to its selectivity. Because the assay measures aggregate levels of nitratable compounds, it is most useful for relative comparison of urine samples from workers with closely related exposures. The relative nature of the measurements also means that the assay cannot provide speciation information on the nitratable compounds present in the test samples.

Height and Weight

Height was measured using a stadiometer SECA 220. Weight was measured using a scale (SECA 770). These instruments were calibrated against a laboratory balance and length measurement.

SUMMARY

The Health Effects of Occupational Exposure to Emissions from Asphalt Symposium in Dresden, Germany (2) in 2006 attracted scientists from all over the world. One of the data gaps discussed at that meeting related to better understanding the source, nature, pathway and biological relevance of PAC exposures in asphalt workers. To fill this data gap, a partnership was formed by researchers from a variety of disciplines. The study that resulted from that partnership required developing new methods or improving existing methods for monitoring the relative importance of the inhalation and dermal routes of

exposure, as well as the relationship between exposure and the absorbed dose of PACs as measured by the excretion of urinary PAC metabolites. It also required better strategies for assessing the different sources of PACs contributing to the exposures, as well as techniques for identifying and quantifying their presence by different routes of exposure. The external scientific advisory committee performed an essential role in reviewing study design and identifying both potential problems and opportunities for improvement prior to conducting this complicated field study.

The present publication serves to document the study design and methods utilized for this research and to guide other researchers who wish to perform similar investigations. The actual results of the study are being prepared for future publications.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health. Mention of company names and/or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

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