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# Picogram Measurement of Volatile *n*-Alkanes (*n*-Hexane through *n*-Dodecane) in Blood Using Solid-Phase Microextraction To Assess Nonoccupational Petroleum-Based Fuel Exposure

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**We describe here a new method for the analysis of alkanes (*n*-hexane, *n*-heptane, *n*-octane, *n*-nonane, *n*-decane, *n*-undecane, and *n*-dodecane) in blood using headspace solid-phase microextraction gas chromatography/mass spectrometry. This method is used to measure picogram per milliliter levels of *n*-alkanes in blood that may result from nonoccupational exposure to alkanes and other volatile nonpolar compounds from common sources such as petroleum-based fuel. This alkane signature is useful in distinguishing typical fuel biomarkers (e.g., benzene and toluene) from other confounding exposure sources such as cigarette smoke. Development of this method required special attention to sample handling as alkanes are not highly soluble in aqueous matrixes and exist as ubiquitous compounds found in many laboratory materials and the environment. In particular, significant *n*-hexane contamination (~0.4 ng/mL) occurred from collecting blood samples in vacutainers. This residue was removed by boiling the vacutainer stoppers in methanol followed by vacuum baking. For all the alkanes, the calculated accuracy demonstrated for the water-based standards ranged from 3.3% to 17% as deduced from the difference of the lowest and middle standards from the curve fit. Quality control data among runs over a 10 month period were found to vary from 14% to −29%, with a few exceptions. The resulting quantification limits for *n*-hexane through *n*-decane ranged from 0.069 to 0.132 ng/mL. In the analysis of 1200 blood samples from people with no known occupational exposure, median blood levels for all *n*-alkanes were below these quantification limits. *n*-Hexane levels above the method detection limit were, however, found in 1.3% of the samples.**

Fuel and fuel emissions exist as a widespread source of nonoccupational exposure to the general population. In the United States approximately 110 million people refuel vehicles regularly, and approximately 200 million live near urban centers where fuel emissions persist.[1] Various petroleum-based fuels, as well as a number of their most toxic and carcinogenic constituents, have been studied for their effect on animal and human health. Unfortunately, a recent comprehensive review concludes that despite improved scientific approaches more accurate information is needed to clarify current uncertainty (e.g., confounding) regarding overall risk from fuel exposure.[2]

Nevertheless, the International Agency for Research on Cancer (IARC) identifies diesel exhaust as a group 2A carcinogen and gasoline, gasoline exhaust, and marine diesel as group 2B carcinogens. Kerosene is listed as only slightly toxic. Unfortunately, these definitions do not help simplify risk assessment as fuels are complex with formulations that vary. One approach to study health effects associated with fuels has been to isolate the effects of individual constituents. Although this approach is useful in identifying harmful components, their association with health effects can be complicated if an individual compound has several exposure sources. For example, benzene, a proven carcinogen, is identified as a biomarker for both fuel[3] and cigarette smoke exposure.[4] Benzene is also a common contaminant found in butyl rubber,[5] which is used in many laboratory supplies. Thus, reliance on a chemical signature containing numerous compounds is sometimes necessary when identifying the source of chemical exposure.

Determining an important, representative, and unique signature for fuel is difficult, especially when a fuel can contains over 500 chemicals of various aromatics, straight-chain, branched, and cyclic hydrocarbons. Overlap among different fuel types potentially makes bulk composition hard to distinguish. For example, hydrocarbon composition for gasoline and diesel typically ranges from C4−C12 and C10−C22, respectively, and aviation kerosene from C8−C15 and wide-cut from C5−C15. Nevertheless, alkanes make up the majority of these formulations (e.g., >65% on average for gasoline) and serve as good signature candidates to compli-

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(1) Wixtrom, R. N.; Brown, S. L. *J. Exposure Anal. Environ. Epidemiol.* **1992**, *2*, 23–78.
(2) Caprino, L.; Togna, G. I. *Environ. Health Perspect.* **1998**, *106*, 115–125.
(3) Dement, J. M.; Hensley, L.; Gitelman, A. *Ann. N.Y. Acad. Sci.* **1997**, *837*, 53–76.
(4) Darrall, K. G.; Figgins, J. A.; Brown, R. D.; Phillips, G. F. *Analyst* **1998**, *123*, 1095–1101.
(5) Chambers, D. M.; McElprang, D. O.; Waterhouse, M. G.; Blount, B. C. *Anal. Chem.* **2006**, *78*, 5375–5383.

ment toxicologically important compounds such as aromatics and fuel oxygenates.

Different approaches for monitoring fuel biomarkers have been reviewed,[6] the most common of which involves analysis of urine,[7–9] breath,[10] and blood.[11] Polar urinary metabolites, which are easy to collect and handle, are often diverse, nonspecific, and provide only an indirect measure of exposure that can be subject to metabolic inhibition[12] and individual excretion. Although collection of blood is more invasive, it is preferred for this application because exposure intensity has been shown to closely correlate with blood levels[9] that are the concentrations likely encountered by important tissues. Method development is also less complicated with blood, which can be directly measured for a complex array of compounds.[11] Furthermore, given the ability for hermetic blood collection in vacutainers, sample collection and storage are also well suited for routine, remote, and uncomplicated collection. One noteworthy limitation in blood analysis is the potential for volatilization loss of nonpolar compounds during handling. However, it is this nonpolar nature that makes these compounds well suited for headspace collection and analysis by gas chromatography/mass spectrometry (GC/MS).

A few groups have demonstrated analysis of fuel exposure using GC/MS that encompasses monoaromatic and *n*-alkane compounds.[13,14] Although methods for the analysis of certain volatile hydrocarbons have been established,[5,11] the solubility and volatility limitations of alkanes make their analysis more challenging. Recent experiments targeting alkane analysis occurred at high concentrations using spiked samples to mimic exposure.[13,14] These studies demonstrate the analysis of higher molecular weight alkanes (C9−C14); however, these compounds pose a lower exposure threat as they are poorly absorbed and inhaled because of their low volatility and nonpolar character.[15,16]

The purpose of this work is to develop a method for the analysis of unbranched alkanes (C6 through C12) in blood for the purpose of identifying nonoccupational exposure to fuels using headspace solid-phase microextraction (SPME) cryotrap GC/MS. This method addresses a number of challenges as these alkanes are volatile, not highly soluble in aqueous phases, and exist as ubiquitous compounds found in many laboratory materials as well as the environment. In this discussion we will identify the fundamental requirements of this method, approaches to minimize contamination or loss, and characterization of the method in long-term biomonitoring.


(6) Gregg, S. D.; Fisher, J. W.; Bartlett, M. G. *Biomed. Chromatogr.* **2006**, *20*, 492–507.
(7) Hamelin, G.; Truchon, G.; Tardif, R. *Int. Arch. Occup. Environ. Health* **2004**, *77*, 264–270.
(8) Serdar, B.; Egeghy, P. P.; Gibson, R.; Rappaport, S. M. *Am. J. Ind. Med.* **2004**, *46*, 234–244.
(9) Ikeda, M. *Toxicol. Lett.* **1999**, *108*, 99–106.
(10) Lindstrom, A. B.; Pleil, J. D. *Biomarkers* **2002**, *7*, 189–208.
(11) Blount, B. C.; Kobelski, R. J.; McElprang, D. O.; Ashley, D. L.; Morrow, J. C.; Chambers, D. M.; Cardinali, F. L. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2006**, *832*, 292–301.
(12) Yu, X. Z.; Johanson, G.; Ichihara, G.; Shibata, E.; Kamijima, M.; Ono, Y.; Takeuchi, Y. *J. Occup. Health* **1998**, *40*, 293–301.
(13) Liu, J. T.; Hara, K.; Kashimura, S.; Kashiwagi, M.; Hamanaka, T.; Miyoshi, A.; Kageura, M. *J. Chromatogr., B* **2000**, *748*, 401–406.
(14) Liu, S. M.; Pleil, J. D. *J. Chromatogr., B* **2001**, *752*, 159–171.
(15) McDougal, J. N.; Pollard, D. L.; Weisman, W.; Garrett, C. M.; Miller, T. E. *Toxicol. Sci.* **2000**, *55*, 247–255.


**Table 1. GC/MS Parameters Used for the Analysis of *n*-Alkanes, Monoaromatics, and MTBE**

| elution order | analyte | ISTD | native retention time (min) (SIM group) | primary/confirmation/ISTD ions (*m/z*) (dwell time in ms) |
|---|---|---|---|---|
| 1 | MTBE | $^{2}H_{12}$ | 10.47 (1) | 73 (20)/57 (20)/57 (15) |
| 2 | *n*-hexane | $^{13}C_{1}$ | 11.32 (1) | 86 (20)/41 (20)/66 (15) |
| 3 | benzene | $^{13}C_{6}$ | 13.66 (2) | 78 (50)/77 (50)/84 (40) |
| 4 | *n*-heptane | $^{2}H_{14}$ | 14.61 (3) | 71 (20)/43 (20)/66 (15) |
| 5 | toluene | $^{13}C_{7}$ | 17.13 (4) | 91 (20)/92 (20)/98 (15) |
| 6 | *n*-octane | $^{2}H_{16}$ | 17.74 (4) | 57 (20)/43 (20)/66 (15) |
| 7 | ethylbenzene | $^{13}C_{6}$ | 19.94 (5) | 91 (20)/106 (20)/97 (15) |
| 8 | *m/p*-xylene | $^{13}C_{6}$ | 20.31 (5) | 91 (20)/106 (20)/97 (15) |
| 9 | *n*-nonane | $^{2}H_{18}$ | 20.64 (5) | 57 (20)/43 (20)/66 (15) |
| 10 | styrene | $^{13}C_{6}$ | 20.88 (6) | 104 (20)/103 (20)/110 (15) |
| 11 | *o*-xylene | $^{2}H_{6}$ | 21.01 (6) | 91 (20)/106 (20)/112 (15) |
| 12 | *n*-decane | $^{2}H_{20}$ | 22.87 (7) | 57 (20)/43 (20)/66 (15) |
| 13 | *n*-undecane | $^{2}H_{22}$ | 24.08 (7) | 57 (20)/43 (20)/66 (15) |
| 14 | *n*-dodecane | $^{2}H_{24}$ | 24.95 (7) | 57 (20)/43 (20)/66 (15) |

## EXPERIMENTAL SECTION

**Reagents and Materials.** Standards for *n*-alkanes (C6−C12), benzene, toluene, ethylbenzene, *m/p*-xylene, *o*-xylene styrene, and methyl-*tert*-butyl ether (MTBE) used in this work were of ≥99% purity and were purchased from Sigma-Aldrich Corp. (Milwaukee, WI). Corresponding internal standards that are described in Table 1 were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Reagents used included high-performance liquid chromatography (HPLC)-grade water from Mallinckrodt Baker, Inc. (Phillipsburg, NJ) and purge and trap (P&T)-grade methanol from Honeywell Burdick & Jackson (Muskegon, MI).

Commercially available HPLC-grade water often contains unacceptable levels of volatile organic contaminants. Water used here was obtained from o2si (Charleston, SC) who removed contaminants from HPLC-grade water by nitrogen purging and distillation. The cleaning procedure, which is described in a previous work,[17] involved sparging the water for 24−30 h with ultrahigh-purity nitrogen (i.e., 99.999%) at 60 °C followed by 4 h of boiling before the water was removed through a dip tube for ampulization. The precleaned water was transferred at no higher than 80 °C to permit ease of ampulization. Water ampoules were flame-sealed using a water torch, an oxyhydrogen torch whose gas supply is generated on demand by water electrolysis.

Standard crimp-top 10 mL headspace vials (PN 10751 produced by Worldwide Glass) were purchased from Lab Depot, Inc. (Dawsonville, GA). The 20 mm headspace vial septa with bimetal cap were also purchased from Lab Depot, Inc. (PN 020-03-8127 [level 4] produced by Integrated Liner Technologies). The septa were nominally 20 mm diameter and 3 mm thick and were composed of silicone with a poly(tetrafluoroethylene) (PTFE) barrier layer between 0.10 and 0.15 mm thick. The headspace vial septa were cleaned by the producer to meet our minimal specifications equivalent to 17 h at 100−110 °C under vacuum below 1.3 kPa or nitrogen purging. Before use, the septa were then reprocessed in-house for 17 h at 100 °C under vacuum below 1.3 kPa to remove any residue or postprocess contaminants from packaging, shipping, and storage.[25]


(16) Kim, D.; Andersen, M. E.; Nylander-French, L. A. *Toxicol. Lett.* **2006**, *165*, 11–21.
(17) Cardinali, F. L.; Mccraw, J. M.; Ashley, D. L.; Bonin, M. A. *J. Chromatogr. Sci.* **1994**, *32* (Jan), 41–45.

**Blood Sample Collection.** Whole blood samples were collected in accordance with protocols approved by the institutional review board (IRB) of the Centers for Disease Control. Blood samples were collected by venipuncture in precleaned vacutainers described below containing a mixture of potassium oxalate and sodium fluoride. Vacutainers were filled completely to minimize headspace. To avoid chemical contamination from isopropyl alcohol used to disinfect the venipuncture site, the cleaned area was then dried with a sterile gauze and allowed to air for 5−10 s. After samples were collected, they were mixed thoroughly for 3 min to completely distribute the anticoagulant. If a mixer was not available, the vacutainer was inverted by hand approximately 30 times. In addition, samples that were shipped from a remote site were shipped overnight and were packed with enough cold or gel packs to ensure that the samples remained cool throughout the shipment. Samples were not frozen at any time and were stored in a 4 °C refrigerator. Samples were typically analyzed within 2 weeks of collection but not more than 5 weeks.

**Sample Preparation.** An intermediate internal standard mixture was prepared from neat labeled isotopes, which were diluted with P&T-grade methanol, flame-sealed in glass ampoules, and stored at −70 °C. A working internal standard solution was prepared weekly by diluting the intermediate stock solution with methanol by 125:1 and then storing at −20 °C in the 25 mL volumetric flask in which it was prepared. Before analysis, 40 μL of the internal standard working solution was added to all unknowns, blanks, standards, and quality control specimens. Final concentrations achieved for the internal standards ranged between 0.34 and 0.39 ng/mL. All glassware was rinsed three times with methanol and baked at 150 °C for at least 17 h prior to use.

The native standard solution set, consisting of seven calibration concentrations, was prepared in a manner similar to the internal standard using intermediate solutions previously prepared with P&T-grade methanol and stored at −70 °C in flame-sealed glass ampoules. Aqueous calibration standards were prepared fresh weekly by transferring 40 μL of each intermediate standard solution into 25 mL of precleaned water. Final concentrations given below differed by analyte and ranged 3 orders of magnitude. A standard blank ($B_S$) was prepared along with calibration standards to assess contamination during standard prep. Following addition of internal standard, standard solutions were delivered into headspace vials with a gastight glass-barreled multipipette, crimp-sealed, and stored at 4 °C until analysis.

Performance standards formulated at four different concentrations were obtained from a third-party vendor (o2si, Charleston, SC). The vendor prepared these samples from neat material to intermediate concentrations using P&T-grade methanol and then flame-sealed the samples in ampules with a water torch to minimize VOC contamination from natural gas. For analysis, the samples were prepared in-house by diluting 40 μL of the intermediate sample in 25 mL of precleaned water which was then transferred to headspace vials as 3 mL aliquots.

Quality control (QC) samples were used to verify performance of the analytical assay. These samples were prepared by adding standard to fetal bovine serum (Hyclone Laboratories, Logan, UT) that first underwent a cleaning process to reduce chemical background levels. This cleaning process involved heating approximately 2 L of serum in a 4 L round-bottom flask to 40 °C while purging for 48−72 h with ultrahigh-purity nitrogen (>99.999%), which was filtered with a carbon trap. The purpose of this treatment was to remove a broad range of VOCs, including *n*-alkanes, without contaminating or foaming the serum. Following cleaning, the mixture was cooled to approximately 10 °C, spiked with standard, thoroughly mixed for approximately 15 min, and transferred to a low-headspace glass container. Material was then repipetted to ampules for flame sealing with a water torch. QC serum samples were prepared at two different concentrations. The low-level QC samples were prepared at a targeted concentration that was 6.5 times the lowest standard, and the high-level QC samples were prepared at a targeted concentration that was 30 times the lowest standard. After preparation QC samples were stored at −70 °C. On the day of use, an ampule of QC serum was thawed, mixed, and sampled as though it were an unknown. Mean concentrations of analytes in each QC pool were measured by 20 separate determinations. Blind QC samples were evaluated by an independent QC officer using Westgard QC rules.[18] If the results from analysis of a QC sample did not meet Westgard rules for an analyte, then all results for the analyte in that run were rejected.

Blood samples were collected in 10 mL vacutainers that were reprocessed to minimize residue contamination.[19] This cleaning procedure was modified to improve extraction efficiency of *n*-alkanes and aromatic compounds by adding a sequential liquid extraction step of the vacutainer stoppers by either sonicating or boiling in P&T-grade methanol. Following the third extraction the stoppers were vacuum baked for 21 days under 1.3 kPa vacuum at 110 °C. Vacutainers that underwent this procedure were evaluated for cleanliness using the precleaned water. For this evaluation the vacutainers were stored horizontally (water in contact with the stopper) for 6 days at 4 °C. If the background levels found in the water were higher than the lowest standard, the vacutainer stoppers were vacuum baked for a longer time.

To prepare the blood samples for analysis, we placed the samples on a rotating mixer in a level 1 (type II class A/B3) biological safety cabinet at room temperature for a minimum of 15 min. Blood was removed from the vacutainers with a 5 mL glass-barrel gastight syringe (Hamilton). Once 3 mL of blood was drawn into the syringe, the blood was dispensed into a tared 10 mL headspace vial. The internal standard working solution (40 μL) was added to the vial which was then capped and weighed. Sample weights were used to correct the final concentrations. Quality control fetal bovine serum samples and precleaned water blanks were prepared using the same technique as for the blood samples.

The cleanliness of the method reagents and materials was verified using the same sample preparation and analysis approach as for QC and blood samples. Reagents were analyzed in a 10 mL headspace vial as a neat solution or diluted with precleaned water to yield 3 mL and then spiked with internal standard. Analyte concentrations in the vacutainer stoppers and headspace vial septa were measured by performing consecutive 5 mL methanol extractions of the material. Each extraction was performed in a sealed 10 mL headspace vial that was placed for 15 min in an ultrasonic bath. For analysis, 40 μL of the methanol extract and

(18) Westgard, J. O.; Barry, P. L.; Hunt, M. R.; Groth, T. *Clin. Chem.* **1981**, *27*, 493–501.
(19) Cardinali, F. L.; Mccraw, J. M.; Ashley, D. L.; Bonin, M.; Wooten, J. *J. Chromatogr. Sci* **1995**, *33* (Oct), 557–560.

**Table 2. Comparison of Water Solubility and Partitioning Properties**

| compound | lowest standard (ng/mL) | highest standard (ng/mL) | water solubility (ng/mL) (25 °C, 760 Torr) | calcd $K_{WA}$ (25 °C, 760 Torr) | blood/air partition coefficients[a] |
|---|---|---|---|---|---|
| benzene | 0.012 | 12 | 1 780 000 | 5.23779 | 7.79[b] |
| toluene | 0.012 | 12 | 515 000 | 4.66819 | 17.01[b] |
| *n*-hexane | 0.025 | 25 | 9500 | 0.01476 | 0.80[b] |
| *n*-heptane | 0.025 | 25 | 2930 | 0.01021 | 1.9[b] |
| *n*-octane | 0.025 | 25 | 660 | 0.00707 | 3.13[c] |
| *n*-nonane | 0.025 | 25 | 220 | 0.00524 | 5.80[c] |
| *n*-decane | 0.037 | 37 | 52[d] | 0.00362 | 8.13[c] |
| *n*-undecane | 0.096 | 96 | 4.0[d] | 0.00251 | 20.41[c] |
| *n*-dodecane | 0.24 | 24 | 3.7[d] | 0.00173 | 24.57[c] |

[a] Refs 22–24. [b] Human blood. [c] Rat blood. [d] Below concentration of highest standard.

40 μL of the internal standard working solution were added to 3 mL of precleaned water.

**Instrumentation.** The headspace SPME/cryotrap GC/MS instrumentation used for these analyses have been described in more detail previously.[5] For this application, headspace collection was performed with a 75 μm Carboxen−PDMS fiber (Supleco, Bellefonte, PA) for 15 min at 40 °C. In using this approach, which has been previously established for the analysis of a number of other VOCs including benzene, toluene, and MTBE, we are able to combine the analysis of *n*-alkanes with other important compounds.[11] Before collection, samples remained in a Peltier-cooled tray at 15 °C. The cryotrap GC was configured with a 40 m × 0.18 mm i.d. × 1 μm film DB-VRX column (Agilent, Palo Alto, CA). Volatiles were focused at a cryotrap temperature of −100 °C using a 50 psi pulsed splitless injection. Separation occurred at a 1 mL/min He flow as the cryotrap was heated to 225 °C (∼13 °C/s) and the column was ramped (0 °C held for 1.5 min, 7 °C/min to 140 °C, 40 °C/min to 220 °C, and held for 4.5 min). Electron impact ionization MS response was measured in selected ion monitoring mode using a primary quantification ion, a confirmation ion, and an internal standard ion as listed in Table 1 where dwell time is given in parentheses.

**Quantification.** The quantification approach used for this work was the same as previously described.[5] The water-based standards consisted of seven levels ranging in concentration from 0.025 to 25 ng/mL for *n*-hexane, *n*-heptane, *n*-octane, and *n*-nonane, 0.037−37 ng/mL for *n*-decane, 0.096−96 ng/mL for *n*-undecane, 0.24−24 ng/mL for *n*-dodecane, 0.012−12 ng/mL for benzene, toluene, ethylbenzene, 0.016−16 ng/mL for *m*/*p*-xylene, 0.006−6 ng/mL for *o*-xylene, and 0.025−25 ng/mL for styrene. Blood samples with response ratios above that of the highest calibrator were diluted with precleaned water and reanalyzed. Water-based calibration standards were used instead of serum or blood because of the broad variation of VOC levels found in biological samples. A weighted, $1/x$, least-squares model was fitted to the calibration data, where $x$ is the standard concentration. Compound identification was confirmed from the retention time (within 2 s) and agreement between the primary and confirmation ion quantification results (within 20%). Each peak was visually inspected to ensure that width and symmetry were similar to that achieved using the standards. Samples with any *n*-alkane level above 0.1 ng/mL were repeated to confirm quantification, providing sufficient sample quantity. Because *n*-alkanes proved to be stable in blood, repeat analysis was not expedited and occurred between 1−3 months after the initial analysis. Analyte levels in reanalyzed blood samples were typically within the precision demonstrated for the QC samples. If a sample was more than two standard deviations outside this range, it was marked as suspect and excluded.

Instrument response was calibrated with every sample batch analyzed within a maximum 24 h period. Samples were analyzed beginning with the four lowest concentration standards in order of increasing concentration, then a low-concentration QC, a high-concentration QC, the unknowns, a second low-concentration QC, a second high-concentration QC, and ending with the remaining three standards in order of increasing concentration.

## RESULTS AND DISCUSSION

**Method Development.** The main reason for using headspace SPME is to permit analysis of *n*-alkanes in combination with other fuel-based compounds of interest (e.g., monoaromatics and MTBE).[11] The Carboxen−PDMS-coated fiber was selected to achieve efficient collection of monoaromatics and MTBE as well as collection of other volatile hydrocarbons capable of van der Waals interactions such as the *n*-alkanes. Carboxen−PDMS contains a substantial percentage of PDMS. The Carboxen, a porous carbon-based absorbent, is the material primarily responsible for intermolecular bonding and trapping the analyte. Published values demonstrate collection efficiency for *n*-hexane to be a factor of 200 greater for Carboxen−PDMS as compared with 100 μm PDMS.[20] Although higher *n*-alkanes might be less efficiently absorbed by this fiber, their poor skin and inhalation absorption also makes them less suitable candidates for biomonitoring.[15,16]

Because of their low solubility *n*-alkanes are inherently difficult to measure in aqueous phases. In addition, these compounds are widely dispersed through the use of petroleum-based fuel and materials, thus posing a contamination threat. Together, these attributes present a challenge to sample preparation and handling in minimizing loss as well as identifying and eliminating contamination encountered in sample collection, storage, and analysis.

In contrast, as a result of the lipophilic nature of blood, the solubility of *n*-alkanes in blood is better than that in water. These differences are compared in Table 2, which gives the water solubility and the water and blood partition coefficient data for different *n*-alkanes. Data for benzene and toluene have been included only for point of reference. The water data was calculated assuming a temperature of 25 °C using the online SPARC

(20) *Applications of Solid Phase Microextraction*; Royal Society of Chemistry: Cambridge, U.K., 1999.

modeling system (http://ibmlc2.chem.uga.edu/sparc/).[21] Understanding water solubility limits is important because standards are prepared in water, which can be obtained as a clean and cost-effective matrix.

Water solubility for the *n*-alkanes is low. *n*-Hexane, the most water soluble compound in the series, is soluble in water between 1 and 3 orders of magnitude lower than that for benzene and toluene. In fact, the solubilities for *n*-undecane and *n*-dodecane fall below the highest intended standard. Similarly, the water−air partition coefficient ($K_{WA}$), which is related to water solubility, is about 1−3 orders of magnitude greater for benzene and toluene than *n*-hexane. These differences indicate that it will be more difficult to minimize diffusion loss while preparing samples with the *n*-alkanes than with benzene and toluene; however, headspace sampling will be more efficient as the *n*-alkanes will prefer the gas phase.

Although blood solubility and partition data are difficult to characterize and are not readily available, the literature values given in Table 2 include a combination of data on human and rat blood and serve as approximations. From this data it is apparent that the *n*-alkane partition coefficients for blood/air are significantly higher than for water and are on the same order as benzene and toluene. Given the differences seen among the water and blood partition coefficients, limiting the diffusion loss of *n*-alkanes during preparation of the water standards will be substantially more difficult than when preparing blood samples. These properties particularly limit analysis of the larger alkanes at high concentrations. Moreover, as detection limits increase with chain length, the calibration range narrows, making this method less practical for larger *n*-alkanes. Nevertheless, overcoming this limitation is not vital because skin absorption and inhalation studies show that larger *n*-alkanes (>C12) are poorly absorbed and inhaled.[15,16]

Most of the bias from diffusion loss occurs either during sample preparation or during the time (<24 h) before analysis that the samples are queued in the autosampler. Although the internal standard can be used to compensate for the latter, it does not correct for handling losses before the internal standard has been added or for contamination gain. The sample collection and handling process remains hermetic up until the point when samples are transferred from the vacutainer into the SPME headspace vial and spiked with internal standard. This nonhermetic handling of the standards, QCs, and blood samples is unavoidable as samples need to be added to flasks and vials. In fact, low-boiling-point compounds that have low water solubility are more susceptible to diffusion loss than to contamination. Once all material contamination sources were identified and controlled, general contamination did not present a problem for this method. Absence of contamination was verified with each run using water-control, QC, and even blood sample data.

Handling bias was found to be somewhat problematic for *n*-hexane, which has a relatively low boiling point of 69 °C. To help differentiate among the loss mechanisms that could bias the data, we evaluated absolute response as a function of sample preparation and run-sequence order. Specifically, samples were analyzed in the order in which they were prepared and compared to samples analyzed in reversed order of the sample preparation. Data from this experiment can be seen in Figure 1 for *n*-hexane where normalized internal standard response is plotted against analysis order. The cycle time was approximately 48 min; thus, the maximum standing time for these samples was 20 h. During this time, the absolute internal standard response decreased approximately 43% when the samples were analyzed in the same order in which they were prepared. However, when the analysis order was reversed the absolute internal standard response decreased only about 3%. This near-zero value indicates that the loss experienced from the samples standing on the analysis tray was similar to that which occurred during sample preparation. With this result, analyte loss during sample preparation ($x$) can be calculated as 20% ± 26% (i.e., $x + (x - 3 \pm 8\%) = 43\% \pm 25\%$).

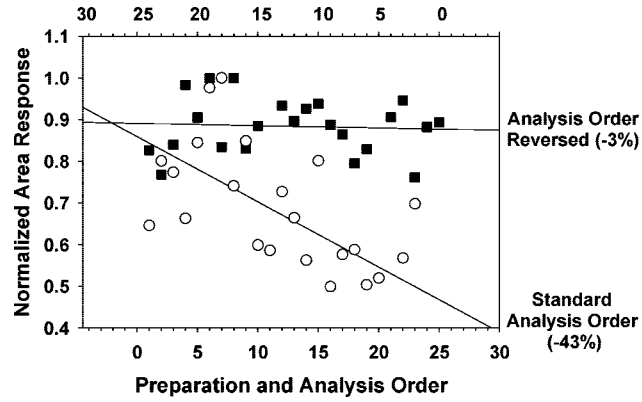


**Figure 1.** Comparison of drift in absolute ISTD response for *n*-hexane resulting from loss during sample preparation and analysis.

The major source of internal standard loss during sample preparation occurs from volatilization of the internal standard stock solution as the samples are prepared and cannot be avoided. However, this loss is not statistically significant compared to the sample-to-sample variation of the absolute internal standard response that can occur. This large standard deviation is most likely caused by headspace vial seal integrity. Other causes of variation can include matrix differences and variation in instrument response resulting from such things as air entrainment when the GC septum is punctured. Fortunately, the use of an internal standard corrects for these variations. Losses that occur during sample analysis mainly result from diffusion through the glass/PTFE interface barrier on the headspace vial. These losses are minimized by using a beveled rim vial and a thick (i.e., 3 mm) silicone/PTFE barrier septa with ample compression to keep the seal firm. Loss during sample preparation occurs either as loss of the native from the sample before the internal standard is added or loss of internal standard from the storage and handling of the stock solution.

Contamination gain from the laboratory environment, the reagents, and most of the materials was negligible for the *n*-alkanes, although in our previous work we have found these same materials to be significant sources of other compounds of

(21) Hilal, S. H.; Karickhoff, S. W.; Carreira, L. A. *QSAR Comb. Sci.* **2003**, *22*, 565–574.
(22) Fisher, J.; Mahle, D.; Bankston, L.; Greene, R.; Gearhart, J. *Am. Ind. Hyg. Assoc. J.* **1997**, *58*, 425–431.
(23) Perbellini, L.; Brugnone, F.; Caretta, D.; Maranelli, G. *Br. J. Ind. Med.* **1985**, *42*, 162–167.
(24) Smith, A. Q.; Campbell, J. L.; Keys, D. A.; Fisher, J. W. *Int. J. Toxicol.* **2005**, *24*, 35–41.

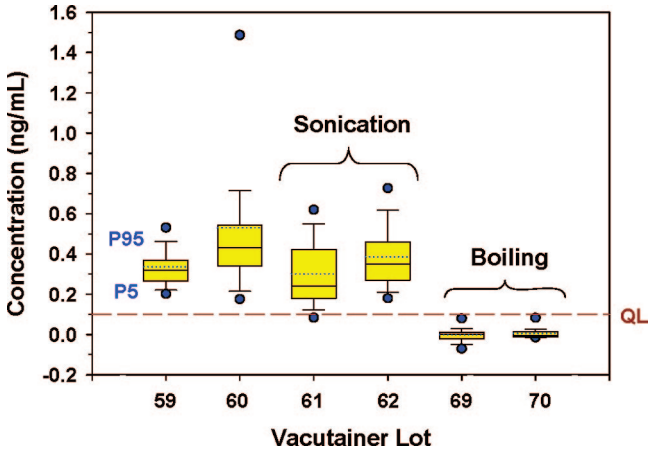


**Figure 2.** Comparison of *n*-hexane levels in blood collected in vacutainers prepared using different processes.

interest.[5,25] The only exception to this was *n*-alkane residue found in butyl rubber materials, such as the vacutainer stoppers. This contaminating residue might originate with the butyl rubber monomer (i.e., isobutylene) that is collected from petroleum cracking streams. Furthermore, alkanes are often used in material processing because of their ability to dissolve butyl rubber. As a result, butyl rubber has high levels of aromatics, alkanes, and olefins. Because of presence of high levels of *n*-alkane residue, all butyl rubber materials were eliminated from the method with the exception of the vacutainer stoppers, which could not be substituted. Two solvent extraction approaches were attempted to improve removal of the interfering residue—one involved sonication and the other involved boiling of the stoppers in methanol. Following either solvent extraction approach the stoppers were then baked at 110 °C for 3 weeks as described above. Shown in Figure 2 are *n*-hexane blood levels from individuals with no known occupational exposure to alkanes. Vacutainer lots 59 and 60 were cleaned with only vacuum oven baking. Lots 61 and 62 were cleaned with first sonication in methanol followed by vacuum oven processing. The boiling approach proved more successful in producing lowest sample contamination, as can be seen with lots 69 and 70. In addition to *n*-hexane this approach successfully reduced other compounds of interest below the detection limits including *n*-heptane, benzene, toluene, xylenes, styrene, chlorobenzene, MTBE, bromoform, and dibromomethane.

Sporadic contamination is difficult to identify. Contamination from a particular vacutainer stopper can be identified by performing a second analysis following several weeks of storage to determine whether the contamination increases with time. The only other significant source of sporadic contamination identified was found in reagents, standards, and QC materials that were flame-sealed using natural gas. Flame sealing in glass ampoules is necessary to ensure hermetic storage of these materials. We have found natural gas to contain significant levels of alkanes and other hydrocarbons. If these species are not consumed in the torch, they can partition into the sample. For this reason, all flame sealing was performed with a water torch. Flame sealing with natural gas also presents a problem when procuring third-party standards.

(25) Chambers, D. M.; McElprang, D. O.; Mauldin, J. P.; Hughes, T. M.; Blount, B. C. *Anal. Chem.* **2005**, *77*, 2912–2919.

**Table 3. Calibration Curve Linear Regression Correlation and Corresponding Calculated Accuracy and Standard Deviation of the Lowest and Middle Standards among Runs ($n = 20$)**

| | mean $R^{2a}$ | mean accuracy ± SD[b] for lowest standard (1) relative to curve fit (%) | mean accuracy ± SD[b] for middle standard (4) relative to curve fit (%) |
|---|---|---|---|
| *n*-hexane | 0.9938 | 17 ± 8.2 | 12 ± 8.2 |
| *n*-heptane | 0.9939 | 12 ± 7.7 | 12 ± 7.6 |
| *n*-octane | 0.9962 | 9.5 ± 3.3 | 8.6 ± 5.0 |
| *n*-nonane | 0.9839 | 8.9 ± 6.3 | 16 ± 13 |
| *n*-decane | 0.9885 | 9.3 ± 8.4 | 11 ± 9.2 |
| *n*-undecane | 0.9962 | 12 ± 12 | 10 ± 9.6 |
| *n*-dodecane | 0.9982 | 9.2 ± 8.3 | 3.3 ± 2.3 |

[a] $R^2$ = correlation coefficient. [b] SD = standard deviation.

**Method Characterization.** Once fundamental parameters of the method approach have been identified and limitations have been overcome, the next step in method development is establishment of method figures of merit. The most important of these in this application include accuracy, precision, and detection range. When working with lower concentrations, poor accuracy and precision begin to affect the method detection limit more than they affect sensitivity. Examples would include handling losses, background biases, or coeluting spectral interferences, which can increase the method detection limit above the signal-to-noise ratio demonstrated within a single run.

**Accuracy and Precision.** Accuracy and precision within and among runs were characterized using standard, third-party standard, QC, and water-blank data. In Table 3 the accuracy and precision within the standard set is demonstrated for 20 separate runs using mean standard curve correlation (i.e., mean $R^2$) and the mean of the calculated accuracy (= measured value/calculated value) for the first and middle standards (measured values) in relation to the curve fit (calculated value). From this data an average correlation of 0.99 is achieved for all the *n*-alkanes with the exception of *n*-nonane, which was 0.98. These correlations correspond to calculated accuracy ranging from 3.3% ± 2.2% to 17% ± 8.2% as demonstrated with the lowest and middle standards. Despite previously described challenges of formulating *n*-alkane standards in water, we were able to achieve reasonably good $1/x$-weighted linear fits of the standard curves.

Standard-set accuracy was verified by evaluating performance standards prepared by a third party. Table 4 shows the relative recovery, which we define as the ratio of measured concentration divided by the formulation concentration for the third-party standard. This test is performed on a single PT standard set ($n = 1$) and is intended to capture method accuracy similar to that encountered by an unknown sample, which is typically analyzed only once. Any statistically significant difference between the measured and calculated results may be due to inaccuracy of the standard set or the third-party pool or both. If the relative recovery is less than the calculated accuracy that was demonstrated among the standard sets, then there would be cause to verify the standard set or the third-party standard concentrations. In this case, the relative recovery seen for the third-party standards is within the calculated accuracy demonstrated among the standards with the exception of *n*-dodecane. The apparent bias seen for *n*-dodecane averaged 32%, suggesting either a positive error in the

**Table 4. Accuracy Verification Using Third-Party Standard ($n = 1$)**

| | calcd concn (ng/mL) | measured result (ng/mL) | relative recovery (%) |
|---|---|---|---|
| *n*-hexane | 0.244 | 0.220 | 90.2 |
| | 0.488 | 0.372 | 76.2 |
| | 0.732 | 0.735 | 100.4 |
| | 0.977 | 0.954 | 97.6 |
| *average* | | | *91.1* |
| *n*-heptane | 0.267 | 0.281 | 105.2 |
| | 0.533 | 0.460 | 86.3 |
| | 0.800 | 0.954 | 119.3 |
| | 1.066 | 1.268 | 118.9 |
| *average* | | | *107.4* |
| *n*-octane | 0.272 | 0.289 | 106.3 |
| | 0.543 | 0.535 | 98.5 |
| | 0.815 | 1.004 | 123.2 |
| | 1.086 | 1.292 | 119.0 |
| *average* | | | *111.8* |
| *n*-nonane | 0.270 | 0.253 | 93.7 |
| | 0.540 | 0.545 | 100.9 |
| | 0.810 | 1.017 | 125.6 |
| | 1.090 | 0.915 | 83.9 |
| *average* | | | *101.0* |
| *n*-decane | 0.299 | 0.341 | 114.0 |
| | 0.598 | 0.631 | 105.5 |
| | 0.897 | 1.143 | 127.4 |
| | 1.196 | 1.472 | 123.1 |
| *average* | | | *117.5* |
| *n*-undecane | 0.286 | 0.331 | 115.7 |
| | 0.583 | 0.570 | 97.8 |
| | 0.874 | 0.985 | 112.7 |
| | 1.166 | 1.346 | 115.4 |
| *average* | | | *110.40* |
| *n*-dodecane | 0.291 | 0.415 | 142.6 |
| | 0.583 | 0.706 | 121.2 |
| | 0.874 | 1.153 | 131.9 |
| | 1.166 | 1.552 | 133.1 |
| *average* | | | *132.2* |

**Table 5. QC Data Summary Collected over a 10 Month Period**

| compound | concn level | mean for all runs (ng/mL) | % RSD[a] | drift % difference |
|---|---|---|---|---|
| *n*-hexane | low | 0.074 ± 0.035 | 47 | 14 |
| | high | 0.272 ± 0.102 | 37 | 28 |
| *n*-heptane | low | 0.264 ± 0.110 | 41 | −79 |
| | high | 0.620 ± 0.169 | 31 | −26 |
| *n*-octane | low | 0.338 ± 0.057 | 21 | −2.6 |
| | high | 0.825 ± 0.213 | 17 | 9.3 |
| *n*-nonane | low | 0.263 ± 0.089 | 34 | 0.11 |
| | high | 1.197 ± 0.213 | 19 | 14 |
| *n*-decane | low | 0.678 ± 0.087 | 13 | −23 |
| | high | 1.620 ± 0.210 | 13 | −6.6 |
| *n*-undecane | low | 0.747 ± 0.299 | 40 | −52 |
| | high | 2.765 ± 0.336 | 12 | −29 |
| *n*-dodecane | low | 1.518 ± 0.355 | 21 | −13 |
| | high | 5.634 ± 1.203 | 23 | −14 |

[a] RSD = relative standard deviation.

concentrations of the third-party standards or a negative bias of the standard set. Even so, because of its limited dynamic range, solubility limitations, and low dose risk for dermal and inhalation exposure, no attempt was made to recalibrate this compound.

Imprecision among runs can be characterized using QC and water-blank data. Table 5 contains the long-term precision and drift data for QC samples at two concentrations analyzed over a 10 month period. Drift refers to the change observed across the QC pool over time and can be due to sample decomposition, contamination, or instrument bias. In this case, drift is mainly the result of volatilization losses that occurred while aliquoting the QC sample lot. This phenomenon was identified by comparing samples across the preparation lot after the lot was made. Although extensive efforts were made to minimize this drift before the samples were flame-sealed in glass ampules (e.g., preparation speed, cooling), diffusion loss is difficult to eliminate. Relative standard deviation and drift were greatest for *n*-hexane (≤47% and ≤28%, respectively) and *n*-heptane (≤41% and ≤−79%, respectively), which likely results from diffusion losses during handling of either the standards or the internal standard. A negative drift is probably due to diffusion loss of the analyte from the QC material during preparation of the QC batch. Even though the positive bias seen for *n*-hexane was not statistically significant, a positive bias would be indicative of diffusion loss of analyte across the production of the water-based standard pool. The relative standard deviations for the low-concentration QCs for *n*-nonane and *n*-undecane were relatively high because of coeluting interferences that were present in the bovine serum. High-concentration QCs for these analytes were, however, less affected.

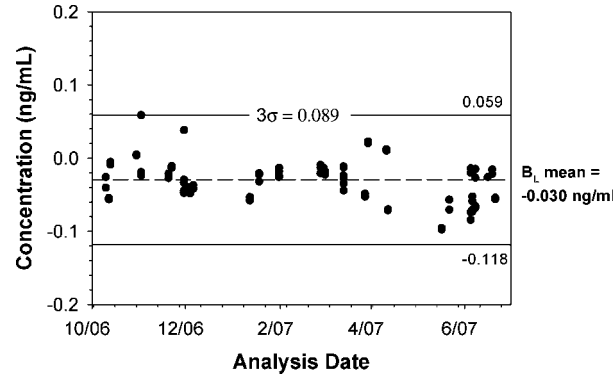


**Figure 3.** Water-blank ($B_L$) data for *n*-hexane collected over 8 months used in the determination of the method detection limit.

Figure 3 shows the precision among runs achieved for *n*-hexane using laboratory water-control blanks ($B_L$) collected over an 8 month period. Blanks were prepared at the same time as the unknown samples using internal standard prepared and stored at −4 °C up to 5 days earlier with the standard set. Three standard deviations from the mean corresponds to approximately ±0.1 ng/mL and is banded with control lines. The relatively small negative bias of 0.030 ng/mL is likely due to contamination of the standard set during storage. The use of an internal standard only compensates for diffusion loss—not for contamination gain. As a result, contamination of the standards will negatively bias the water-control blanks. Positively biased $B_L$ values can result from spectral interferences or contamination of the internal standard. When combined, these biases were found to have a greater influence on the method detection limit than on the sensitivity limitations as described below.

**Quantification Range Parameters.** Typically, we calculate the method detection limit using the "$3S_o$" approach as described by Taylor wherein the standard deviation at zero concentration, $S_o$, is determined through extrapolation.[26] Unfortunately, this approach predicted lower method detection limits than practical.

(26) Taylor, J. K. *Quality Assurance of Chemical Measurements*; Lewis Publishers: New York, 1987.

**Table 6. Method Concentration Ranges and Method Detection Limits**

| compound | lowest standard (ng/mL) | highest standard (ng/mL) | method detection limit (ng/mL) |
|---|---|---|---|
| *n*-hexane | 0.025 | 25 | 0.089 |
| *n*-heptane | 0.025 | 25 | 0.084 |
| *n*-octane | 0.025 | 25 | 0.069 |
| *n*-nonane | 0.025 | 25 | 0.071 |
| *n*-decane | 0.037 | 37 (sol. lim. 25 °C = 52) | 0.13 |
| *n*-undecane | 0.096 | 96 (sol. lim. 25 °C = 4.0) | 0.20 |
| *n*-dodecane | 0.240 | 242 (sol. lim. 25 °C = 3.7) | 0.25 |

The primary reasons for this are that the $3S_o$ approach extrapolates the quantification limit based on the precision at different concentrations above the detection limit. Therefore, the extrapolated $S_o$ can be biased lower by higher concentration standards that tend to have relatively low standard deviation and may not accurately reflect the situation approaching zero concentration. Also, the $3S_o$ approach does not account for a bias that can occur from differences in analytical conditions between the standard set and the unknown samples (e.g., interferences). Consequently, we used a modified Taylor approach where $S_o$ was determined from the standard deviation of the water-control blanks among runs.[26] The method detection limit is defined as three times the standard deviation of the $B_L$ ($3B_L$), which is demonstrated in Figure 3 for *n*-hexane to be 0.089 ng/mL. Note that for *n*-hexane, this definition of detection limit was above the low-concentration QC, which was 0.074 ± 0.035 ng/mL. Although the characterization of the low-concentration QC is statistically valid, it would not be included in a formal study.

Table 6 compares the lowest standard with method detection limits for all of the *n*-alkanes using the $3B_L$ approach. The method detection limits were around 0.1 ng/mL for *n*-hexane through *n*-nonane but progressively increased to 0.25 ng/mL for *n*-dodecane. The quantification range of *n*-undecane and *n*-dodecane are limited to less than 2 orders of magnitude because of their relatively high method detection limits and low water solubilities.

**Method Application.** The ability to analyze *n*-alkanes provides additional selectivity with which to identify petroleum-based fuel exposure historically associated with benzene, toluene, ethylbenzene, xylenes, styrene (BTEXS) and fuel oxygenates. However, because elevated BTEXS blood levels also result from other types of exposure, analysis of alkane components in fuel may become increasingly important as distinct fuel oxygenates such as MTBE are replaced with common solvents such as methanol and ethanol.

Shown in Figure 4 is an example of relevant compounds found in a blood sample collected within an hour after an individual briefly handled fuel in a nonoccupational activity. Although conditions of the exposure were unknown as the sample was anonymous, this data demonstrates the detection of *n*-alkanes in blood following consumer handling of fuel. It is presumed that exposure was primarily by inhalation; however, dermal exposure may have also occurred. Nevertheless, substantial increases in MTBE and BTEXS levels were accompanied by elevated *n*-alkane levels. Levels of *n*-alkanes from *n*-hexane to *n*-octane are well above preexposure blood levels. Yet, the levels of *n*-nonane and higher molecular weight *n*-alkanes were less pronounced.

This method was further applied to analysis of 1200 anonymous blood samples from individuals living in the United States. Most

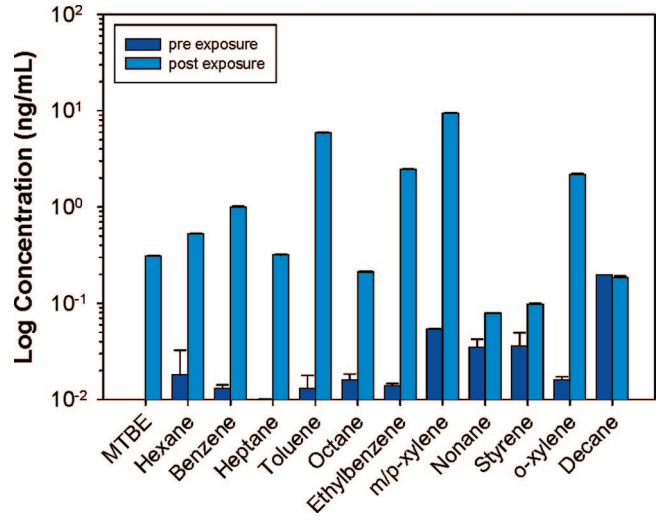


**Figure 4.** Demonstration of petroleum-based fuel exposure signature found in blood resulting from nonoccupational inhalation exposure.

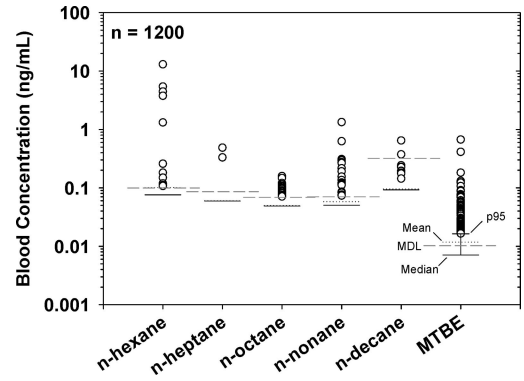


**Figure 5.** Nonoccupational *n*-alkane and MTBE blood reference levels found in 1200 blood samples.

of these samples did not have detectable levels of *n*-alkanes as shown in Figure 5. Specifically, only 1.3% of the samples had *n*-hexane levels above the quantification limit of 0.089 ng/mL. Overall, the mean (solid line), median (dotted line), and 95th percentile ($p95$) (whisker cap) blood levels were found to fall below the method detection limits (dashed line) for all *n*-alkanes.

The correlation among the alkanes and fuel related biomarker responses was evaluated using a two variable correlation approach wherein maximum correlation occurs at 1, no correlation at 0, and maximum inverse correlation at −1. Responses below the method detection limit were estimated to be $1/\sqrt{2}$ times the method detection limit. By using 2,5-dimethylfuran as a biomarker for smoking,[27] we identified 125 smokers (i.e., 15% of the population above) and excluded these individuals because tobacco smoke is a significant source of benzene and other monoaromatics. Only 13 of the "nonsmoker" samples had *n*-hexane levels above the method detection limit. Among these samples there was poor positive correlation with the other *n*-alkanes, monoaromatics, and MTBE (<0.22); however, *n*-hexane did correlate fairly well with benzene (0.91) and styrene (0.58). The only strong correlation seen among the *n*-alkanes was between *n*-nonane and *n*-decane, which was 0.90. Differences between these *n*-hexane

(27) Ashley, D. L.; Bonin, M. A.; Hamar, B.; McGeehin, M. *Int. Arch. Occup. Environ. Health* **1996**, *68*, 183–187.

correlation results and that shown in Figure 4 for a known petroleum-based fuel exposure suggest confounding from different exposure sources or from endogenous processes. A plausible explanation for these correlation results would warrant further research, which is outside the scope of this paper. Nevertheless, these results emphasize the importance of correlating multiple compounds across different compound classes, when possible, to minimize confounding such as that which may have occurred here.

**Comparison with Previously Published Results.** Only one previous study reports blood reference levels for an *n*-alkane in the general population.[28] This study reported mean *n*-hexane blood levels for clerks (assumed to have no occupational hexane exposure) at 0.411 ng/mL. These results are significantly higher than those found in this work and can be attributed to either method bias or to differences in the populations. Because the previously reported nonoccupational levels were similar to those found here from vacutainer stopper contamination (~0.400 ng/mL) and exceeded levels demonstrated in this work for diffusion loss (20% ± 26%), we suspect contamination bias on the part of the earlier study.

## CONCLUSIONS

The nonpolar nature of alkanes and their presence in many common laboratory materials pose a number of challenges in handling and analysis in biomonitoring applications. Yet, these and other highly nonpolar compounds can be measured in blood and other aqueous matrixes with minimal bias provided that samples are handled hermetically and contamination is removed from laboratory reagents and materials. For most of the *n*-alkanes, handling loss that can occur during nonhermetic preparation steps of the standards and samples was found to be below 20%. Fortunately, the most significant loss of ~20% demonstrated for *n*-hexane occurs after samples are prepared and is compensated for by the use of an internal standard. Still, this tendency for handling loss lowers precision and increases the method detection limits to the 0.1 ng/mL range.

In addition to diffusion loss, there is also the possibility for contamination bias from common laboratory materials. The most significant source was found to be butyl rubber materials. As a result, this material was avoided with the exception of that used for vacutainers needed to collect blood samples. In this case, the vacutainer stoppers were found to contaminate samples in the high nanogram per milliliter range prompting the need for precleaning. Of the limited manufacturers and lots tested, three methanol boiling treatments followed by 3 weeks of vacuum baking at 110 °C at 10 mTorr was found sufficient to bring contamination levels below the lowest standards of 0.025 ng/mL.

Though the blood VOC signature for a known exposure contained high levels of a combination of *n*-alkanes, monoaromatics, and MTBE, the *n*-alkane blood levels measured in 1200 anonymous samples taken from the general population were typically below method detection limits, which ranged from 0.069 to 0.25 ng/mL. For example, only a small percentage (1.3%) of these samples had *n*-hexane levels above the method detection limit of 0.089 ng/mL. Despite the fact that this detection limit is more than 2 orders of magnitude lower than recent methods for the detection of its urinary metabolite,[29] the incidence of detection for *n*-hexane and the other *n*-alkanes remains low. Enhanced analytical sensitivity would likely increase the prevalence of detection; however, our finding that few individuals have blood *n*-alkane levels around the picogram per milliliter level is useful information for establishing an upper bound on background exposure levels. The most plausible explanation of the low detection rate is that recent exposure to *n*-alkanes is minimal in the population evaluated. The low detection rate is likely due to a combination of no recent acute exposure, a relatively short physiological half-life (~2−3 h[30] for *n*-hexane), and minimal chronic exposure.

Nevertheless, in most of the samples with *n*-hexane levels above the method detection limit, no significant correlation was found with the other *n*-alkanes and MTBE. Positive correlation of these samples was seen with benzene. These correlation results were unexpected because known exposure demonstrated in Figure 4 showed an association among these compounds. These results suggest either an unanticipated exposure source for *n*-hexane different from that demonstrated for petroleum-based fuel exposure or that there is an endogenous process influencing alkane blood levels. Nevertheless, this work underscores the importance of using chemical signatures rather than individual chemicals in assessing chemical exposure and its relation to health effects.

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(28) Brugnone, F.; Maranelli, G.; Romeo, L.; Giuliari, C.; Gobbi, M.; Malesani, F.; Bassi, G.; Alexopoulos, C. *Int. Arch. Occup. Environ. Health* **1991**, *63*, 157–160.

(29) Strassnig, S.; Gfrerer, M.; Lankmayr, E. P. *J. Chromatogr., B* **2004**, *813*, 151–158.

(30) Perbellini, L.; Mozzo, P.; Brugnone, F.; Zedde, A. *Br. J. Ind. Med.* **1986**, *43*, 760–768.