

# Cumulative exposure assessment for trace-level polycyclic aromatic hydrocarbons (PAHs) using human blood and plasma analysis

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## Abstract

Humans experience chronic cumulative trace-level exposure to mixtures of volatile, semi-volatile, and non-volatile polycyclic aromatic hydrocarbons (PAHs) present in the environment as by-products of combustion processes. Certain PAHs are known or suspected human carcinogens and so we have developed methodology for measuring their circulating (blood borne) concentrations as a tool to assess internal dose and health risk. We use liquid/liquid extraction and gas chromatography – mass spectrometry and present analytical parameters including dynamic range (0 – 250 ng/ml), linearity (>0.99 for all compounds), and instrument sensitivity (range 2 to 22 pg/ml) for a series of 22 PAHs representing 2-ring through 6-rings. The method is shown to be sufficiently sensitive for estimating PAHs baseline levels (typical median range from 1 to 1,000 pg/ml) in groups of normal control subjects using 1-ml aliquots of human plasma but we note that some individuals have very low background concentrations for 5- and 6-ring compounds that fall below robust quantitation levels.

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## 1. Introduction

The U.S. Environmental Protection Agency (EPA) is conducting environmental exposure research concerned with reducing the reliance on default assumptions for assessing risk to public health and to inform risk mitigation strategies [1]. One of the main features of this research is to assess “cumulative exposures” defined as exposures to groups of compounds over multiple pathways (inhalation, ingestion, dermal contact) [2,3]. Cumulative exposure research has three basic goals related to providing a predictive science for assessing health risk [4,5]; the first is the assessment of absorption, distribution, metabolism, and elimination (ADME) of chemical exposures [6,7], the second is retrospective exposure reconstruction from biomarker measurements [8-12], and the third is determination of preclinical (or early health) effects [13-17]. These areas of investigation all invoke the measurement of biomarker compounds in human biological media; interpretation and use of biomonitoring data have been reviewed by Albertini et al [18] and by Needham et al [19]. Overviews and specific examples of cumulative exposure research at US EPA in the Human Exposure and Atmospheric Sciences Division (HEASD) are available in the literature [20-23].

Currently, we are investigating the polycyclic aromatic hydrocarbons (PAHs) as a group of organic pollutants reported to increase human inflammatory mediated disease and cancer [24-26]. PAHs are a complex set of related organic species produced as by-products of all combustion processes including forest fires, incineration, cooking, and engine exhaust. They are present in air, food, water, dust and soil and so represent a constant low level exposure to humans via inhalation, ingestion and dermal contact. The PAHs as a group, and specific individual compounds such as benzo(a)pyrene, have been identified as human carcinogens associated with human cancers of the skin, lungs and bladder [27]. Additionally, PAHs have been implicated in adverse reproductive outcomes, somatic mutations, and decrease in children’s IQ [28-30]. We have investigated inhalation exposure to PAHs from diesel exhaust and the link with pulmonary cytokine expression [31 - 33].

Body burden of PAHs is generally assessed using hydroxy-PAHs (phase-1 metabolites) in urine; often, only the 1-hydroxy-pyrene compound is used as a surrogate for all PAHs as a class [34-36]. Blood measurements for DNA adducts of PAHs have been employed as markers for future disease [37]. In this paper, we explore the measurement of the original PAHs (before phase-1 metabolism to hydroxy-PAHs) in the circulating blood and plasma. Table 1 lists 22 PAHs analytes selected for this work as representative of the class and also presents U.S. Environmental Protection Agency (EPA) and International Agency for Cancer Research (IARC) classifications for carcinogenicity [38,39]. We also include literature values for toxicity/potency equivalency factors relative to the index compound, benzo(a)pyrene [40-44].

Most published blood measurements of trace environmental species are of aliphatic hydrocarbons, single-ring aromatic compounds, and polycyclic aromatic hydrocarbons using blood headspace analysis and so do not report semi-and non-volatile species [45,46]. There are, however, a few recent studies of PAHs in human blood or

plasma. Naufal, et al. [47] attempted to link internal dose to birth defects in a highly exposed rural Chinese population using sums of seven PAHs. Singh et al. [48] used the sum of 13 PAHs in human whole blood for oxidative stress in highly exposed children living in urban Lucknow, India. They also published speciated results for nine PAHs in a cohort of 56 children [49].

The work presented here is novel in that we extended cumulative exposure assessment to 22 speciated native circulating PAHs and applied this to samples from normal control subjects. We developed a specific method for 2- to 6-ring PAHs using liquid-liquid extraction of blood and plasma samples followed by gas-chromatography – mass spectrometry analysis (GC-MS) and demonstrate sufficient sensitivity for estimating pg/ml PAHs baseline levels in groups of normal control subjects using 1-ml aliquots of human plasma. Furthermore, the methodology is developed using modest benchtop single-quadrupole GC-MS instrumentation and standard wet-chemistry glassware and laboratory equipment to make it accessible to most environmental laboratories.

## **2. Materials and Methods**

Blood and plasma extractions are based on methods developed for pesticides, polychlorinated biphenyl compounds (PCBs), and volatile jet fuel components [50-52]. PAHs analytical methodologies are based on projects for assessing environmental samples from aircraft exhaust [53] and from environmental impact analyses of the September 11, 2001 World Trade Center disaster [54-56].

### *2.1. Biological Specimens*

#### *2.1.1. Frozen blood*

Initial methods development was performed using research blood (Human, Rh negative, type O, in 1-liter bags) purchased from the American National Red Cross (Washington DC USA) under exemption to the common rule 45 CFR 46.101(b)(4) for anonymous biological specimens. The type and Rh factor were arbitrary with respect to the physical nature of these experiments. These were shipped frozen and kept frozen until use. Thawed aliquots of 1 to 5 ml were differentially spiked with PAHs standards and internal standards to assess matrix effects using different extraction solvent systems. These samples were from anonymous donors, and could have been composited from multiple subjects (unknown).

#### *2.1.2. Frozen plasma*

Analyses were performed using human plasma specimens purchased from SeraCare, Life Sciences (Milford MA USA) under exemption to the common rule 45 CFR 46.101(b)(4) for anonymous biological specimens. These samples were shipped frozen in 1.5 ml aliquots, each from different human donors, and in large 500 ml volumes from a single donor (each). These samples were analyzed for PAHs to establish a

nominal baseline for human body burden and to provide quality assurance for spiked and repeat analyses. These will be referred to as “SeraCare” samples.

#### *2.1.3. Human subjects-whole blood*

A series of 50 human whole-blood specimens (10 ml each) representing 10 distinct anonymous University student donors were provided under the auspices of the University of North Carolina Medical School Institutional Review Board (IRB) and the U.S. EPA (Medical IRB Study #95-EPA-66) as methods development test/control samples. These were used in 1-ml aliquots to assess separation and partitioning of PAHs in plasma and whole blood. These will be referred to as “Student subjects” samples.

#### *2.1.4. Human subjects-plasma*

Human plasma samples were provided under the auspices of the University of North Carolina Medical School Institutional Review Board (IRB) and the U.S. EPA (Medical IRB Study #99-EPA-283) representing 10 subjects with uncorrelated repeat samples. Plasma specimens were frozen in 1-ml aliquots from human subjects that were part of a larger study investigating the effects of diesel exhaust exposures. The subjects were nominally healthy, non-smoking, adults with unremarkable recent exposure history; that is, there were no recent occupational exposures, no recent bus or car rides, no recent snacks, drinks, or meals, and they had been observed in the clinical setting for about 1-hr prior to testing. These anonymous specimens served as controls for this study; current results will be incorporated into future work discerning inflammatory response and related health effects. These will be referred to as “Study subjects” samples.

### *2.2. Sample collection, handling and storage*

Human subject specimens were collected by nurse phlebotomists at the EPA Human Studies Facility clinic of in Chapel Hill, NC. Samples were handled using universal precautions in a certified BSL-2/CSL-3 laboratory. Bulk Red Cross blood was stored in a -20°C freezer; standards, internal standards, and prepared extraction fluids were stored under refrigeration at 4°C. All biological specimens and spiked samples (in solvent or biological matrix) were stored at -80°C in a separate freezer.

### *2.3. Solvents, Chemicals, and Materials*

Hexane and dichloromethane (DCM) solvents were purchased from Burdick and Jackson (Muskegan, MI, USA). Internal standards (d8 naphthalene, d10-phenanthrene, d12-benzo(e)pyrene) were purchased from Cambridge Isotope Laboratories, Inc. (Andover MA USA) and external standards of native compounds “PAH Standard Quebec Ministry of Environment PAHs Mixture” were purchased from Accustandard (New Haven CT USA). Laboratory glassware, syringes, septa, and other expendable supplies were periodically ordered from Thermo Fisher Scientific, Inc. (Waltham MA USA) and Agilent Technologies, Inc. (Santa Clara CA USA). Sample extraction vials were glass 20 ml volume with PTFE lined caps (National Scientific Co., Rockwood TN USA).

#### 2.4. *Sample preparation procedures*

Extraction fluid was prepared in 1-liter batches containing 1.0 ng/ml of internal standards (IS). Initially, we prepared both 80/20 mixture of hexane/DCM and neat hexane as candidate extraction fluids. Sample vials were each partially filled with 10 ml extraction to which 1.0 or 1.5 ml blood or plasma aliquots were added. For calibration, a separate sample vial (with 10 ml extraction fluid) was spiked with 15.6 ng/compound of the Quebec PAHs mixture in 100  $\mu$ l hexane. Blank vials contained 10 ml extraction fluid alone. A standard set of 14 vials consisted of 10 actual samples, plus 2 blank and 2 calibration samples. Vials were capped, vortexed for 20 seconds, agitated at 300 rpm for 40 minutes on an orbital shaker, and vortexed again. Vials were centrifuged at 3000 rpm for 5 minutes and frozen at -80°C. For each sample, the solvent layer was then carefully poured off into a new vial leaving behind the frozen layer of aqueous blood or plasma (discarded). The resulting extracts were reduced to  $\leq$ 1 mL under high purity nitrogen gas (National Speciality Gases, Durham, NC USA). The extracts were transferred into conical high-recovery autosampler vials (Agilent, Santa Clara CA USA), and further reduced to approximately 50  $\mu$ L. Samples were sealed with Teflon-lined septum crimp caps and were stored at -20°C prior to analysis by GC-MS. Samples were kept dark as much as possible (under aluminum foil) to avoid PAHs light sensitivity during preparation procedures.

#### 2.5. *Analytical procedures and performance tests*

Two  $\mu$ l of each sample extract were injected into an Agilent 6890N GC via a 7683 autoinjector interfaced with a 5973 MS (Agilent, Santa Clara CA USA). Samples were injected using splitless mode with pulse pressure of 138 kPa (20 psi); the injection port contained a single-gooseneck liner with glass wool (Restek Corp., Bellefonte PA USA) held at 275°C. An RTX-5SILMS (Restek Corp., Bellefonte PA USA) fused silica capillary column (60 m, 0.25 mm-i.d., 0.25- $\mu$ m film thickness) was used with helium as the carrier gas at a flow of 1.0 mL/min. After injection of the sample into the GC, the oven was held at 50°C for three minutes, ramped at a rate of 25°C/min to a temperature of 150°C, and then ramped at a rate of 10°C/min to 300°C where it was held for 24 minutes. The MS transfer line was held at 280°C, the source temperature at 200°C, and the quadrupoles at 100°C. The MS operated with electron impact (EI) at an ionization voltage of 70 electron volts (eV). A selected ion monitoring (SIM) mode program (2 ions per compound) optimized for dwell time per ion to achieve ~2 Hz cycle time [57] was used to acquire data. Analytes, internal standards, ions and retention times are listed in Table 2.

Prior to analyzing human samples, a series of diagnostic and methods assessment tests were performed. Initially, we assessed linear range and sensitivity using analytical standards prepared in the absence of biological matrices and without volume reduction. This was a “best case” scenario as any background contamination in solvents was not concentrated. We assessed the performance contrast between 80/20 hexane/DCM mixture and neat hexane with respect to extraction efficiency of target PAHs from spiked blood samples as well as for extraction of background contamination. We assessed recovery of PAHs in the extraction layer with and without initially freezing the

centrifuged samples. Finally, we assessed the relative performance of the established SIM method for primary and secondary ion quantitation of target PAHs.

Method sensitivity for the GC-MS alone was determined by analyses of a series of 7 low level synthetic samples prepared independently at 0.75 pg/ $\mu$ l. We assigned method level of quantitation (LOQ) values at three times the standard deviation for each compound individually. Linearity was assessed using synthetic standards prepared independently in duplicate at 0.0049, 0.0125, 0.025, 0.05, 0.125, and 0.25 ng/ $\mu$ l. We also assessed practical system LOQs that included sample handling and pre-concentration by evaluating a series of positive control and spiked positive control samples. Replicate analytical precision was assessed using pairs of blank and calibration samples analyzed within one sample batch and assessed for signal to noise (s/n) and individual compounds slope factors.

Instrument slope response calibration for biological specimens was set using positive controls and incrementally spiked positive controls (PC) comprised of 1 ml aliquots of single donor plasma volumes (from SeraCare). Spike range was from 0 to ~0.18 ng/ml similar to the original instrument control calibrations. The 0.18 ng/ml spikes in PC served as the span calibration standards (Cal1 and Cal2) for subsequent specimen batches as described below. Solvent blanks were prepared as well for ongoing quality assurance purposes.

## *2.6. Sample data interpretation*

Sample sets were typically analyzed in batches of 16 comprised of the sequence: blank1, PC1, Cal1, 10 actual samples, PC2, blank2, Cal2. Specifically, all compounds and internal standards were quantified with single ion integration for both ions. To correct for pre-concentration and injection volume variance, each analyte's raw area count value was corrected relative to the internal standard matching in ring number; for 6-ring compounds, the 5-ring IS, benzo(e)pyrene was used. Concentration values for the original blood or plasma samples were calculated based on linear regression of the batch blanks, positive controls and calibration sample results and original (exact) sample volume. Data were post-processed using Excel 2002 sp2 (Microsoft Corporation, Redmond WA USA) and GraphPad Prism 4.03 (Graphpad Software, Inc, San Diego CA USA).

## **3. Results and Discussion**

### *3.1. Method optimization and performance*

#### *3.1.1. Solvent choice and procedures*

In earlier work, we used pure DCM for extracting PAHs from environmental media [55] and an 80/20 mixture of pentane/DCM for extracting pesticides, volatile hydrocarbons, and PCBs from human blood [51,52]. We had found that DCM alone or in a mixture is an excellent broad spectrum extraction solvent for most classes of

environmental organic compounds. Pure DCM is not a pragmatic choice for aqueous media such as blood and plasma as it has greater density than water thus inverting the solvent/sample layer in centrifugation. The pentane/DCM mixture was therefore chosen to retain the solvent as supernatant, and to allow liquid-liquid extraction and subsequent evaporative pre-concentration without losing volatile compounds such as benzene and toluene. Samples extracted in pentane, however, were found to be more difficult to store for longer times due to evaporation. As such, we investigated both hexane and 80/20 hexane/DCM as alternatives, and also used pure DCM as a control. A series of comparison tests showed that extraction efficiency of spiked PAHs was indistinguishable for previously frozen whole blood (American Red Cross) samples and for spiked plasma samples among all three solvent systems. As expected, we found that removing the inverted DCM layer from the extraction vial was a tedious procedure. We observed that DCM, either pure or in a mixture, tended to increase the overall sample background slightly. As such, we chose to use hexane alone for this work as the best alternative for assessing PAHs concentrations.

General procedures for separating solvent from aqueous matrix are to carefully pipette and transfer the solvent layer to another vial, or to employ some configuration of separatory funnel and recovery vials; these methods are time and/or resource consuming and generally leave behind a small amount of sample or transfer a small amount of the cell layer. We tested an alternative to this by freezing the centrifuged composite of solvent and blood/plasma at  $-80^{\circ}\text{C}$  and then simply pouring off the liquid solvent layer into another vial leaving behind the frozen remains of the original sample. A series of paired comparisons of normal and spiked blood or plasma samples showed no bias in PAHs transfer. As such, we opted to incorporate the freezing step into this method to streamline sample handling.

### *3.1.2. Sensitivity, linearity and precision*

GC-MS instrument sensitivity assessment showed that all target PAHs compounds could be distinguished and quantified at or below 1 pg/injection at 99% confidence, with many demonstrating levels of quantitation below 0.1 pg/injection. Regression analysis of the synthetic standards resulted in better than  $r^2 = 0.99$  for all compounds indicating a linear range from 0 to at least 0.50 ng/injection. In terms of actual blood or plasma samples, the upper end corresponds to approximately 12.5 ng/ml concentration. For perspective, the normal span calibration is set at 0.25 ng/ml and many real (control) samples have about 0.001 to 0.2 ng/ml (1 to 200 pg/ml) PAHs compounds. These initial results are shown in Table 2 under the heading entitled “Solvent Matrix Test” and were achieved without benefit of internal standard correction for variance in injection volume. We note that these results are also independent of sample handling and concentration processes, as well as biological matrix effects that would be experienced by routine blood or plasma samples.

Subsequently, we performed a series of quality assurance procedures to assess the instrument performance in the face of perturbation from the biological matrix. For these experiments, we used 1 ml aliquots of plasma from a single donor (purchased from SeraCare) spiked in a similar range as the instrument performance tests in solvent (0.06

to 0.179 ng/ml) to assess linearity where we found  $r^2 > 0.997$  for all compounds. This confirms the ability to extract free PAHs consistently, and also demonstrates the benefit of internal standard correction. Method sensitivity was estimated from positive control samples spiked at the lowest level and found to be about 22 times higher (median value) than the solvent test experiments when constrained to the 99% confidence level. We found, however, that we could consistently estimate levels based on about 3 times signal to noise (s/n) ratio using peak heights of extracted ion chromatograms; this level was subsequently defined as the level of detection (LOD). When such peaks are integrated, we could make reasonable estimates below these values based on area calibration slope factors. Table 2 presents the linearity results ( $r^2$ ), calculations of standard LOQ's, estimates of peak height LOD's, and area count slope factors.

These results are encouraging in that we can assign approximate PAHs concentrations in control human plasma and blood samples based on LOD estimates and low-level peak area integrations. Regrettably, most "real-world control" levels fall below the standard definition of LOQs when applied to actual biological matrices, however, the method demonstrates excellent linearity in both solvent and plasma tests, and instrument LOQ response in solvent matrix is in the pg/ml range. As such, we are reasonably confident that non-zero quantitative estimates are at least relative to each other for purposes of assessing difference among subjects and among compounds. We attribute this loss of sensitivity in biological samples (in contrast to solvent matrix) to disruptive effects in the baseline from lipid fragments and other biological detritus co-extracted with the PAHs. We are hopeful that this issue can be addressed with further development of clean-up procedures.

### 3.2. *Human PAHs biomarkers*

#### 3.2.1. *Partition of PAHs in fresh whole blood and plasma*

Based on comparisons of samples from 10 distinct anonymous subjects, we found that trace PAHs concentrations (PAHs from routine/unremarkable low level exposures) are enriched in the plasma vs. whole blood with a mean of about 1.6/1 for all analytes. When fresh whole blood is spiked with additional PAHs in solvent, we found that subsequent concentrations are about the same in plasma and whole blood. In contrast to the light-yellow color of plasma from unspiked blood, we observed that plasma separated from pre-spiked blood had a distinct pink color indicating that the spiking process disrupted at least a portion of the membranes of the red blood cells. We interpret this to mean that the intact red blood cell partition of the whole blood has a greater volume of distribution than plasma for PAHs as a class. Table 3 shows the partition estimates for all compounds. From these results, we find equivalency in using the whole blood or plasma fraction of human blood but remind the reader that plasma concentrations of PAHs in normal unspiked samples are enriched.

#### 3.2.2. *PAHs concentrations in human samples*

In total, we analyzed 20 plasma samples from control "study subjects" without remarkable (known) PAHs exposure, 10 plasma and blood samples from "student subjects" that may or may not have had exposures, and 30 plasma specimens from

random donors as provided commercially by SeraCare. We found that the within-group concentrations appear lognormally distributed and the SeraCare samples have a slightly higher average outcome than the healthy (student) volunteer groups. The resulting summary statistics (median and ranges) for PAHs concentrations for these groups are shown in Table 3. As expected, the relative biomarker concentrations are inversely correlated to molecular weight as in environmental media measurements (Pleil 2004a, b). As discussed previously, many of our measurements were below strict statistical levels of measurement confidence; the entries in Table 3 represent best effort estimates based on SIM integration and calibrated slope factors. Despite such crude estimation, we find that our results demonstrate unambiguously that PAHs are chronically present in human biological specimens and that they are available for solvent extraction and analysis. As a visual display, we present box and whisker graphs for the three cohorts of normal samples with the dependent axis in base 10 log scale (Figures 1a, b, and c). Typically, mean concentrations of PAHs ranged from a high of about 1,000 pg/ml for naphthalene to about 1 pg/ml or lower for the higher molecular weight compounds. These results confirm the similar trends among uncorrelated cohorts, and also demonstrate the consistent lognormal character of the internal data structure across compounds and specimen groups.

### 3.2.3. Comparison to other studies

Naufal et al. [47] measured plasma concentrations of the sum of seven carcinogenic PAHs: baa, chr, bbf, bkf, bap, ind, and dba (abbreviations identified in Table 1) in a highly exposed Chinese population resulting in a mean value of 13 ng/ml. In comparison, our measurements of normal control subjects in the U.S. re-calculated the same way resulted in mean cohort values ranging from 0.050 to 0.380 ng/ml, (mean = 0.200 ng/ml) which is about 65 times lower. In the Indian children's study by Singh et al. [48], they report the sum of 13 PAHs: nap, acl, flu, phe, ant, flt, pyr, baa, bkf, bbf, bap, dba, and bgp (abbreviations identified in Table 1) as a mean value of 358 ppb in whole blood; we converted this value to an estimate of 430 ng/ml in the absence of a breakdown of speciated values. From our own data of control subjects, we calculate this sum equivalent as 1.99 ng/ml, which is about 216 times lower than the highly exposed cohort of 50 children. The only speciated data we could find was from a cohort of 56 children published for nine PAHs: nap, acl, phe, ant, flu, pyr, bbf, bkf, bap (abbreviations identified in Table 1) also available from Singh et al. [49]. These values are included in Table 3 for comparison. On average, the overlapping PAHs are about 235 times higher in the Indian measurements than for our control subjects. As such, results from our work as shown in Table 3 are both internally consistent and also confirm the expected contrast between unremarkable (control) subjects and subjects studied for their high environmental exposures to PAHs sources.

## 4. Conclusions

This work is important because circulating blood-borne PAHs provide a more direct link as a tool for exposure reconstruction than metabolites or adducts because they are not as affected by variability from inter-subject differences in metabolism and excretion.

However, because PAHs are lipophilic chemicals, they are likely less abundant by volume in circulating blood in contrast to their polar metabolites in urine. Furthermore, any adsorption onto lipids and cellular structures cause the PAHs to demonstrate a higher (apparent) volume of distribution in blood making their quantitation more difficult.

The methods presented here provide a simple yet effective methodology for assessing native PAHs compounds of exposure in circulating human blood and/or plasma despite the limitations of relative abundance. We demonstrate sufficient level of detection (LOD) to estimate background median levels in groups of control subjects and, by inference, for subjects with higher known environmental or occupational exposures. However, we find that signal to noise (s/n) levels should be improved for measurements in biological matrices to reach statistically robust levels of quantitation (LOQ) for all normal control subjects. We hope to achieve this in ongoing experiments by adding clean-up procedures to discriminate against protein and lipid fragments that are presumed to disturb the chromatographic baseline in “real-world” samples.

In future work, we will expand the statistical base to include more control and random subjects and also assess the overall range of internal PAHs body burden in the general population by investigating environmental exposures from smoking, from working with asphalt, and from diesel exhaust. We plan to explore the lipid-bound fraction of PAHs using acid based extraction similar to methods used for cholesterol analysis. We anticipate that measurement of native PAHs will augment exposure modeling efforts that currently rely only on biomarker measurements of phase-I PAHs metabolites in urine (1-naphthol, oh-pyrene, etc.). We expect that native compound assessments will serve to help explain within- and between-subjects ADME variance, and ultimately help identify parameters influencing individual susceptibility to environmental contaminants.

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Table 1. PAHs analytes and toxicity descriptors

PAH analyte	ID	Carcinogen class		Potency/Toxicity			
		EPA [38]	IARC [39]	EPA <sup>1</sup> [44]	Nisbet and LaGoy <sup>2</sup> [41]	Larsen and Larsen <sup>2</sup> [42]	Collins et al. <sup>3</sup> [43]
naphthalene	nap	C	2B	-	0.001	-	-
acenaphthalene	acl	D	-	-	0.001	-	-
acenaphthene	ace	-	3	-	0.001	-	-
fluorene	flu	D	3	-	0.001	-	-
phenanthrene	phe	D	3	-	0.001	0.0005	-
anthracene	ant	D	3	-	0.01	0.0005	-
fluoranthene	flt	D	3	-	0.001	0.05	-
pyrene	pyr	D	3	-	0.001	0.001	-
benzo[c]phenanthrene	bcp	-	2B	-	-	0.023	-
benz[a]anthracene	baa	B2	2B	0.1	0.1	0.005	0.1
chrysene	chr	B2	2B	0.001	0.01	0.03	0.01
7,12-dimethbenz[a]anthracene	dmb	-	-	-	-	-	10
benzo[b]fluoranthrene	bbf	B2	2B	0.1	0.1	0.1	0.1
benzo[j]fluoranthrene	bjf	-	2B	-	-	0.05	0.1
benzo[k]fluoranthrene	bkf	B2	2B	0.01	0.1	0.05	0.1
benzo[e]pyrene	bep	-	3	-	-	0.002	-
benzo[a]pyrene	bap	B2	1	1	1	1	1
3-methylcholanthrene	mca	-	-	-	-	-	1
indeno[1,2,3-cd]pyrene	ind	B2	2B	0.1	0.1	0.1	0.1
benzo[g,h,i]perylene	bgp	D	3	-	0.01	0.02	-
dibenz[a,h]anthracene	dba	B2	2A	1	5	1.1	0.1
dibenzo[ ]pyrene <sup>4</sup>	dbp	-	3-2A	-	-	0.1-1	1-10

<sup>1</sup> Relative Potencies

<sup>2</sup> Toxic Equivalency Factors (TEF)

<sup>3</sup> Potency Equivalency Factors (PEF)

<sup>4</sup> includes dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, and dibenzo[e,l]pyrene

Table 2. Analytical and performance parameters

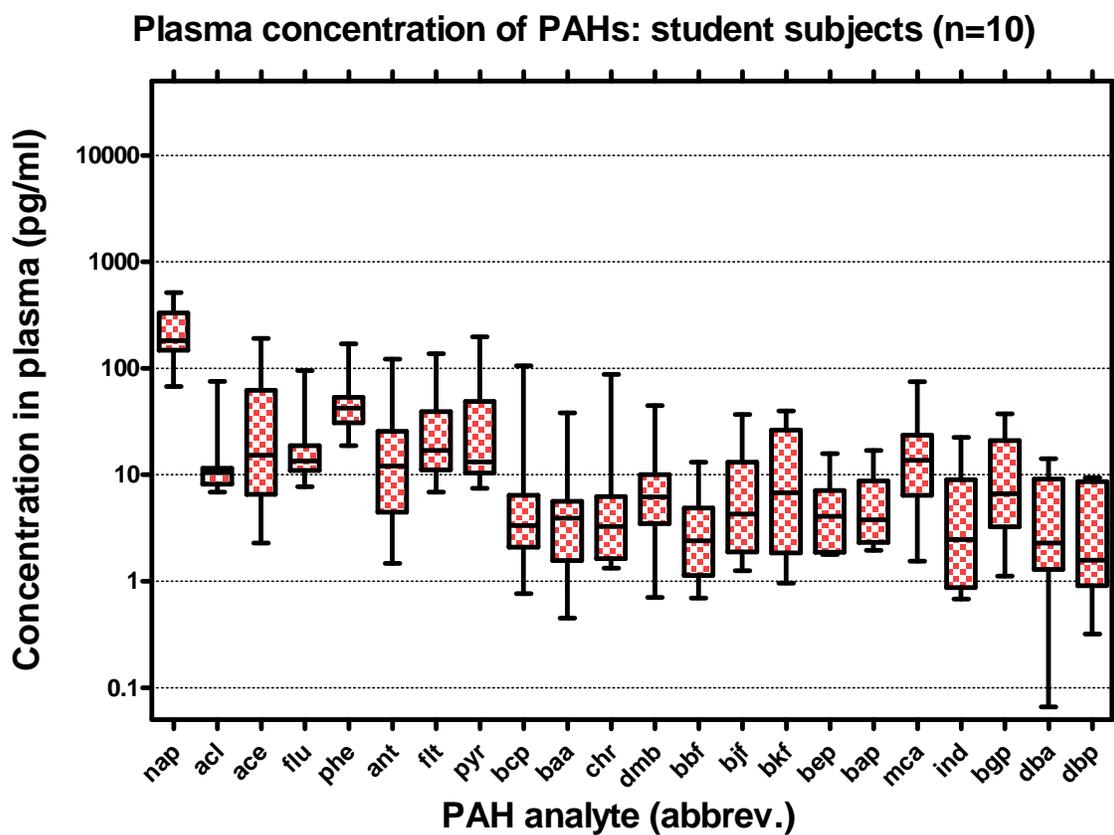
Compound	retention time (min)	Quant. ion (m/z)	Conf ion (m/z)	ion area ratio Conf/Quant	solvent matrix test			plasma matrix test		
					0 - 0.250 ng/ul regression	on column LOQ	in solvent LOQ (est.)	0 - 0.179 ng/ul regression	in plasma LOQ	in plasma LOD (est.)
					r <sup>2</sup>	pg/inj	pg/ml	r <sup>2</sup>	pg/ml	pg/ml
d-naphthalene	12.70	136	135	0.10	na	na	na	na	na	na
naphthalene	12.68	128	127	0.13	0.999	0.07	2	0.999	74	44
acenaphthalene	15.57	152	151	0.19	0.998	0.12	3	0.999	68	11
acenaphthene	15.92	154	153	0.88	0.999	0.13	3	0.999	87	6
fluorene	17.01	166	165	0.86	0.999	0.11	3	0.999	75	13
phenanthrene	19.19	178	176	0.17	0.999	0.08	2	0.999	53	10
d-phenanthrene	19.36	188	184	0.13	na	na	na	na	na	na
d-anthracene	19.27	188	184	0.12	na	na	na	na	na	na
anthracene	19.31	178	176	0.17	0.998	0.10	3	0.999	59	11
fluoranthene	21.99	202	200	0.18	0.999	0.07	2	0.999	50	5
d-pyrene	22.63	212	211	0.21	na	na	na	na	na	na
pyrene	22.55	202	200	0.18	0.999	0.11	3	0.999	61	5
benzo[c]phenanthrene	25.19	228	226	0.47	0.999	0.13	3	0.999	52	13
benz[a]anthracene	25.87	228	226	0.23	0.998	0.12	3	0.997	41	9
chrysene	26.00	228	226	0.26	0.998	0.15	4	0.997	43	10
7,12-dimethbenz[a]anthracene	30.10	256	241	0.46	0.996	0.60	15	0.999	245	32
benzo[b]fluoranthrene	30.19	252	250	0.17	0.996	0.98	25	0.968	656	50
benzo[j]fluoranthrene	30.23	252	250	0.26	0.993	0.53	13	0.992	432	57
benzo[k]fluoranthrene	30.28	252	250	0.24	0.996	0.63	16	0.999	73	49
d-benzo[e]pyrene	31.74	264	260	0.22	na	na	na	na	na	na
benzo[e]pyrene	31.57	252	250	0.26	0.997	0.21	5	0.999	587	37
benzo[a]pyrene	31.85	252	250	0.25	0.994	0.37	9	0.997	210	37
3-methylcholanthrene	33.84	268	252	0.35	0.992	0.90	23	0.998	349	121
indeno[1,2,3-cd]pyrene	39.42	276	277	0.26	0.998	0.38	10	0.999	221	63
benzo[g,h,l]perylene	39.55	278	279	0.23	0.992	0.56	14	0.997	215	90
dibenz[a,h]anthracene	41.70	276	277	0.26	0.994	0.52	13	0.998	211	24
dibenzo[ <i>l</i> ]pyrene	54.95	302	300	0.41	0.993	0.88	22	0.999	97	22

Table 3. Summary statistics for human blood and plasma analyses based on peak areas and slope factor estimates

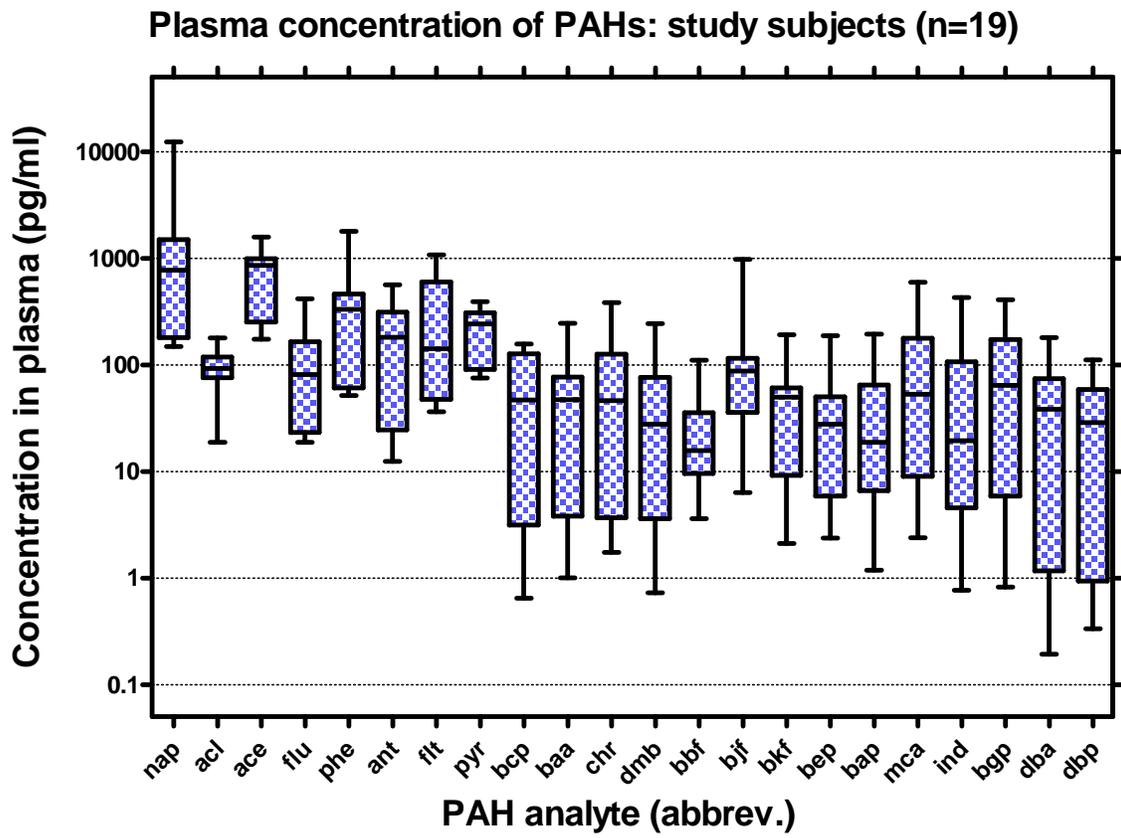
Compound	Student subjects			Student subjects			Study Subjects			SeraCare			Singh 2008*
	Whole blood			Plasma fraction			Plasma fraction			Plasma fraction			blood fraction
	n=10			n=10			n=19			n=30			n=56
	Median	min	max	Median	min	max	Median	min	max	Median	min	max	Median
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
naphthalene	86	47	231	183	68	513	779	149	12292	1459	257	3290	21660
acenaphthalene	5	4	51	10	7	75	93	19	179	21	4	486	7192
acenaphthene	15	-	139	15	-	191	854	174	1578	29	6	207	
fluorene	9	3	28	14	8	95	81	19	416	46	22	261	
phenanthrene	26	15	126	42	19	170	330	52	1793	75	45	245	9567
anthracene	3	-	58	12	-	122	181	12	562	10	-	21	4500
fluoranthene	14	3	58	17	7	137	142	36	1076	27	7	185	7500
pyrene	8	3	96	13	7	197	243	75	392	54	7	376	11439
benzo[c]phenanthrene	-	-	14	3	-	106	47	-	157	3	-	9	
benz[a]anthracene	-	-	9	4	-	38	47	-	246	3	-	9	
chrysene	-	-	4	3	-	88	46	-	386	3	-	10	
7,12-dimethbenz[a]anthracene	6	-	55	6	-	45	28	-	245	30	4	180	
benzo[b]fluoranthrene	3	-	18	-	-	13	16	4	111	11	3	51	5401
benzo[j]fluoranthrene	-	-	21	4	-	37	88	6	979	18	-	50	
benzo[k]fluoranthrene	3	-	25	7	-	40	50	-	192	19	3	78	5144
benzo[e]pyrene	4	-	30	4	-	16	28	-	188	18	3	110	
benzo[a]pyrene	-	-	20	4	-	17	19	-	195	21	5	119	1736
3-methylcholanthrene	4	-	86	14	-	75	53	-	597	57	16	303	
indeno[1,2,3-cd]pyrene	8	-	29	-	-	22	19	-	429	33	5	179	
benzo[g,h,i]perylene	6	-	53	7	-	37	64	-	409	23	7	98	
dibenz[a,h]anthracene	-	-	10	-	-	14	38	-	180	40	10	144	
dibenzo[ ]pyrene	-	-	5	-	-	9	29	-	112	30	-	169	

\* speciated data from Singh et al. [49] estimated from ppb values in blood and presented as pg/ml at 20° C.

A)



B)



C)

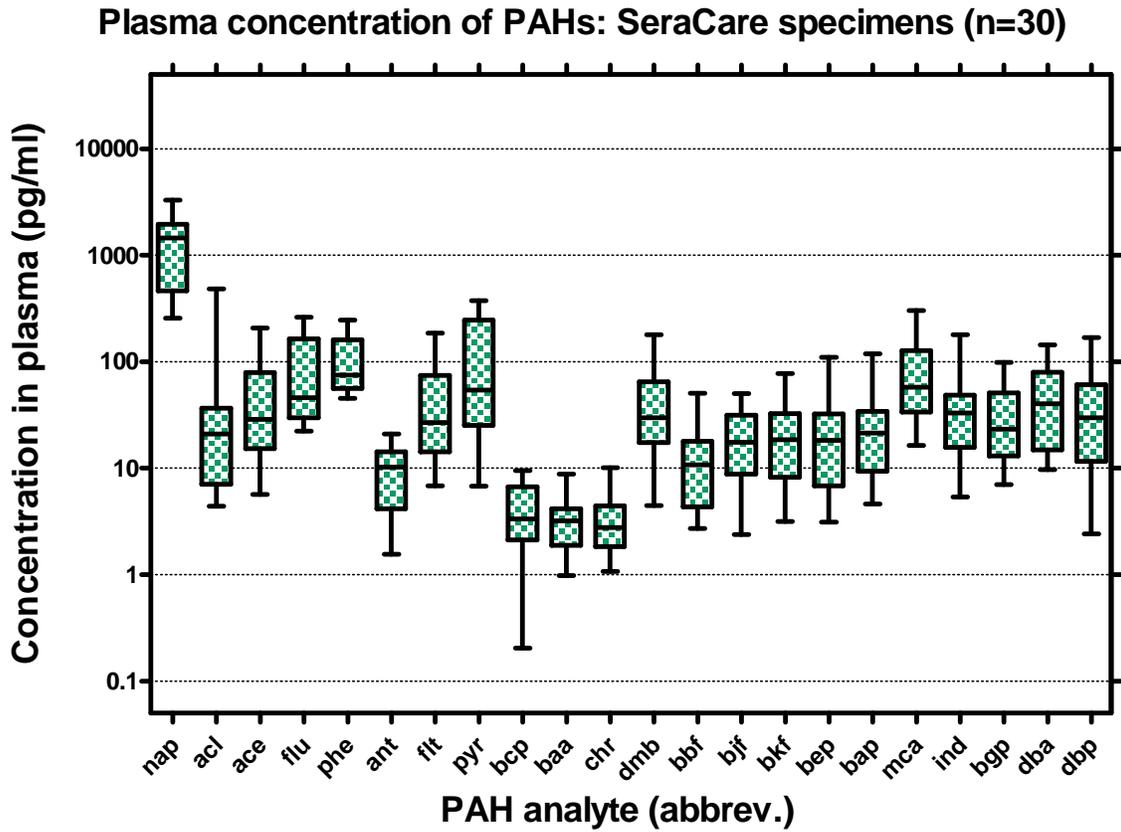


Figure1: Box and whisker graphs of concentration distributions per PAHs compounds for: A) random student subjects, B) random study subjects, and C) random biological specimens from commercial vendor, SeraCare. Three letter abbreviations for analytes are referenced in Table 1.

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