Vol. 11(1), pp. 1-8, January 2019
DOI: 10.5897/JTEHS2018.0424
Article Number: 364147C59885
ISSN: 2006-9820

http://www.academicjournals.org/JTEHS

ACADEMIC JOURNALS
expand your knowledge

Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Determination of polycyclic aromatic hydrocarbons in blood plasma of neurology patients

Olabanji Iyabo Oluremi[1*], Asubiojo Olabode Idowu[1], Komolafe Morenikeji Adedoyin[2], Akintomide Anthony[2] and Adeniji Ayodeji Oluwole[1]

[1]Department of Chemistry, Faculty of Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Medicine, Faculty of Health Sciences Obafemi Awolowo University, Ile-Ife, Nigeria.

Received 20 November, 2018; Accepted 12 December, 2018

The etiology of many neurological cases cannot be easily delineated, making the investigation, and treatment to be challenging. This study aims to screen the blood of neurology patients presenting for the first time in the hospital for Polycyclic Aromatic Hydrocarbons (PAHs) using Flame Ionization Detector – Gas Chromatography. Fourteen PAHs were detected in the samples. The results showed that flourene and phenanthrene were common to all the patients in the range (1.37 to 8.08 and 1.66 to 8.34 ng/mL respectively), but were not detected in the control samples. Pyrene, fluoranthene and acenaphthene were present in 80, 75 and 70% in the blood plasma of the patients at the range of 2.96 to 236.86 ng/mL, 1.96 to 11.55 ng/mL and 1.08 to 1.81 ng/mL respectively. These were not found in the control samples. The body burden of these congeners was much higher in neurology patients than controls and literature values of similar study. The concentrations detected were statistically significant, and could be possible causative agents. This can also become one of the investigative tools for these diseases.

Key words: Polycyclic aromatic hydrocarbons, blood plasma, neurology patients.

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of ubiquitous and environmentally persistent organic compounds. They are formed by thermal decomposition such as in burning of coal, exhaust engines, garbage, wood, or other organic substances such as tobacco and smoked fish or meat (ATSDR, 1995; Pleil et al., 2010). They are present in air, water, food, dust, soot and so represent a constant flow level of exposure to human via skin contact, inhalation and ingestion (Farhadian et al., 2011).

They are chemically related contaminants that are of various structures and varied toxicity. They exist as volatile, semi-volatile and non- volatile organic compounds. They are ranked number nine in the priority

*Corresponding author. E-mail: ioolabanji@yahoo.com.

list of hazardous substances. Over the years Polycyclic Aromatic Hydrocarbons (PAHs) have received much attention because they are suspected to be carcinogenic and they also induce human inflammatory mediated diseases (CDC, 2009; Sexton et al., 2007; Lewtas, 2007; Schober et al., 2007). They have been implicated in adverse reproductive outcomes, somatic mutations and decrease in children's IQ (Sram et al., 1999; Perera et al., 2002). Inhalation of PAHs from diesel engine exhaust has been linked with pulmonary cytokine expression (Sobus et al., 2008; Swanson et al., 2009). Due to their carcinogenic activity, PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists.

The body burden of PAHs had been assessed in urine (Hansen et al. 2008). The analysis for PAHs as potential biomarker and measurement of aliphatic hydrocarbon, single ring aromatic compound as well as analysis of PAHs in blood headspace had been reported (Chambers et al., 2008; Kim et al., 2006; Gyorffy et al., 2008). Pleil et al. (2010) analyzed blood and plasma of healthy people to determine PAHs and found them at varying concentrations.

Neurology disease is a disease that affects the nervous system, structural, biochemical or electrical abnormalities in the brain, spinal cord and nerves. Symptoms may include paralysis, seizures, confusion, loss of sensation and many others. Since PAHs had been implicated of reducing children's IQ (Perera et al., 2002) and neurology diseases affect the brain and the nervous system, it is pertinent to investigate whether there is a link between the pollutant and the disease.

In this study, blood plasma of twenty neurology patients and 12 plasma of healthy subjects were analyzed for PAHs to determine how the concentration levels compared with literature values of healthy subjects.

The concentration levels will give an assessment of the PAHs toxicity levels in the patients compared to literature values of healthy subjects and the healthy subjects used as controls.

MATERIALS AND METHODS

The out patients neurology clinic of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria was used for sampling twenty neurology patients experiencing it for the first time

Sampling

Ten milliliters of whole blood was collected via the vein using syringe and needles in heparinized bottle and transferred to the laboratory and centrifuged. The plasma was immediately transferred to 20 mL capacity amber bottles to prevent sample degradation, PAHs being UV light – sensitive. These were stored in the refrigerator at 4°C prior to extraction.

Sample extraction and clean up

Liquid - liquid extraction technique was employed using ratio 4:1 n-hexane and Dichloromethane (DCM). The extractant (10 mL) and plasma samples (2 mL) were added in the vials, capped and vortex for 20 s at 300 rpm. The organic layer was sucked out using pipette attached with pipette filler into clean and thermally treated amber bottles. The extracts were cleaned up in a column (1 cm x 15 cm, internal diameter and length) with slurry of silica gel as stationary phase, preconditioned by distilled water and hexane/DCM before the samples were eluted, collected and concentrated in a stream of nitrogen gas.

Sample analysis

Samples were analyzed after reconstitution with hexane/DCM at Nigerian Institute of Oceanography and Marine Research (NIOMR) Lagos. The standards of the congeners were supplied and analyzed and used to identify and quantify the PAHs in the samples. The corresponding relative retention time with respect to each congener was used to identify each PAH by Gas Chromatography Flame Ionization Detector (GC- FID) using Agilent Model 7890A (Agilent Technologies, Delaware, MD. USA). Separation was carried out in a GC column HP-5 (Agilent; 30 m × 320 µm × 0.25 µm film thickness) using a temperature programmed GC for elution of analytes. The column temperature was set initially at 60°C and held for 1 min. This was ramped to 210°C at 12°C/min and increased to 320°C at 8°C/min held for 5 min. The total run time was 32.25 min and the detector temperature was 325°C. The flow rate was 1.2 ml/min with injection volume of 1 µL. The inlet mode was splitless with temperature of 270°C.

RESULTS AND DISCUSSION

PAHs concentrations

All twenty patients sampled were found positive to PAHs and exhibited wide variations in concentrations. The United States Environmental Protection Agency (USEPA) classified 16 PAHs as priority pollutants because they are common in environmental media, toxic and they can easily be absorbed by humans (EPA, 2010). Fourteen out of these sixteen on EPA priority list were detected in the neurology patients' plasma and only six PAHs were detected in the plasma of control subjects. Out of the twelve healthy subjects, six were positive to pyrene, two to chrysene, two to Benzo[b]fluoranthrene, six to Benzo[k] fluoranthrene and three were positive to Benzo(g,h,i)perylene which was not detected in the patients. Table 1 shows the mean concentrations of various congeners in the samples. The result shows that, 18 of the patients (80%) had pyrene at varying concentrations in the plasma, 75% had fluoranthrene and 70% had acenaphthene. Fluorene and phenanthrene were present in all the patients screened. With the exception of acenaphthene, the above four analytes had

Table 1. Statistical comparison of the concentrations of PAHs in plasma of neurology patients with the normal subjects in the literature.

PHAs	This study				Pleila et al. 2010 (EPA)									Singh 2008
	Patient Plasma, n=20,		Control Plasma, n= 12		Plasma fraction, n=19			Plasma fraction, n=10			Plasma fraction, n=30			Blood Fraction, n=56
	Patient subjects		Control Subjects		Study subjects			Student subjects			Sera care (Reference)			
	ng/mL (ng/mL=10^3 pg/mL)		ng/mL(ng/mL= 10^{3}pg/mL)		pg/mL			pg/mL			Pg/mL			Pg/mL
	Mean	Median	C-mean	C-median	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median
Naphthalene	6.17	6.08	-	-	779	12292	149	183	513	68	1459	3290	257	21660
Acenaphthalene	0.45	-	-	-	93	179	19	10	75	7	21	486	4	7192
Acenaphthene	0.84	1.15	-	-	854	1578	174	15	191	-	29	207	6	-
Fluorene	3.49	2.82	-	-	81	416	19	14	95	8	46	261	22	-
Anthracene	2.49	-	-	-	181	562	12	12	122	-	10	21	-	4500
Phenanthrene	3.92	3.39	-	-	330	1793	52	42	170	19	75	245	45	9567
Fluoranthene	2.57	2.29	-	-	142	1076	36	17	137	7	27	185	7	7500
Pyrene	24.72	8.98	2.36	2.01	243	392	75	13	197	7	54	376	7	11439
benz[a]anthracene	1.64	-	2.79	2.34	47	246	-	4	38	-	3	9	-	-
Chrysene	3.46	-	0.222	0.222	46	386	-	3	88	-	3	10	-	-
benzo[b]fluoranthrene	4.32	-	4.43	3.65	16	111	4	-	13	-	11	51	3	5401
benzo[k]fluoranthrene	3.95	-	2.32	2.14	50	192	-	7	40	-	19	78	3	5144
benzo[a]pyrene	31.71	31.71	-	-	19	195	-	4	17	-	18	119	5	1736
dibenz[a,h]anthracene	13.79	13.97	-	-	39	180	-	-	14	-	40	144	7	-
Benzo(g,h,i)perylene	-	-	10.34	10.077	-	-	-	-	-	-	-	-	-	-

been classified by EPA (2010) as group D of carcinogens and International Agency for Research on Cancer (IARC) (2009) classified them as number 3 potential carcinogens but they were not present in the control subjects.

But two patients had Benzo(a)pyrene in the plasma with concentration above the acceptable limit of 0.1 µg/L Toxicity Equivalent Factor as recommended by EPA (2010). It is a potent carcinogen and generally used as an environmental indicator for PAHs.

Skupinska et al. (2004) stated that size influences the fate of the gaseous PAHs mixtures. Those that are more than four rings are heavier, because they are particulates and tend to adsorb on the environmental media, while the PAHs that are less than four rings tend to remain in the gaseous state until removed via precipitation. The majority of PAHs analyzed in this study are less than or equal to four rings (about ten), hence, inhalation may likely be one major route of absorption by the patients due to PAHs emission while drinking of shallow well water might be a minor source because of their low solubility in water. Leaching of bitumen used in road construction to surface water could also be an additional source.

The mean concentrations of the PAHs in all the patients are summarized in Figure 1. Pyrene concentration constitutes 43% of all the PAHs concentrations in all the patients, being found in 18 patients. The geometric mean of plasma concentrations of pyrene in this study was 11.33 ng/mL with maximum concentration of 236.86 ng/mL. Its urinary metabolite, 1-hydroxypyrene, had been used as an indicator of exposure to PAHs chemicals (Popp, 1997; CDC, 2005). This metabolite was recommended for measurement as end-of-work-week urine samples by The American Conference of Governmental Industrial Hygienists (ACGIH) as biological exposure index (BEI) for assessment of exposure to mixtures

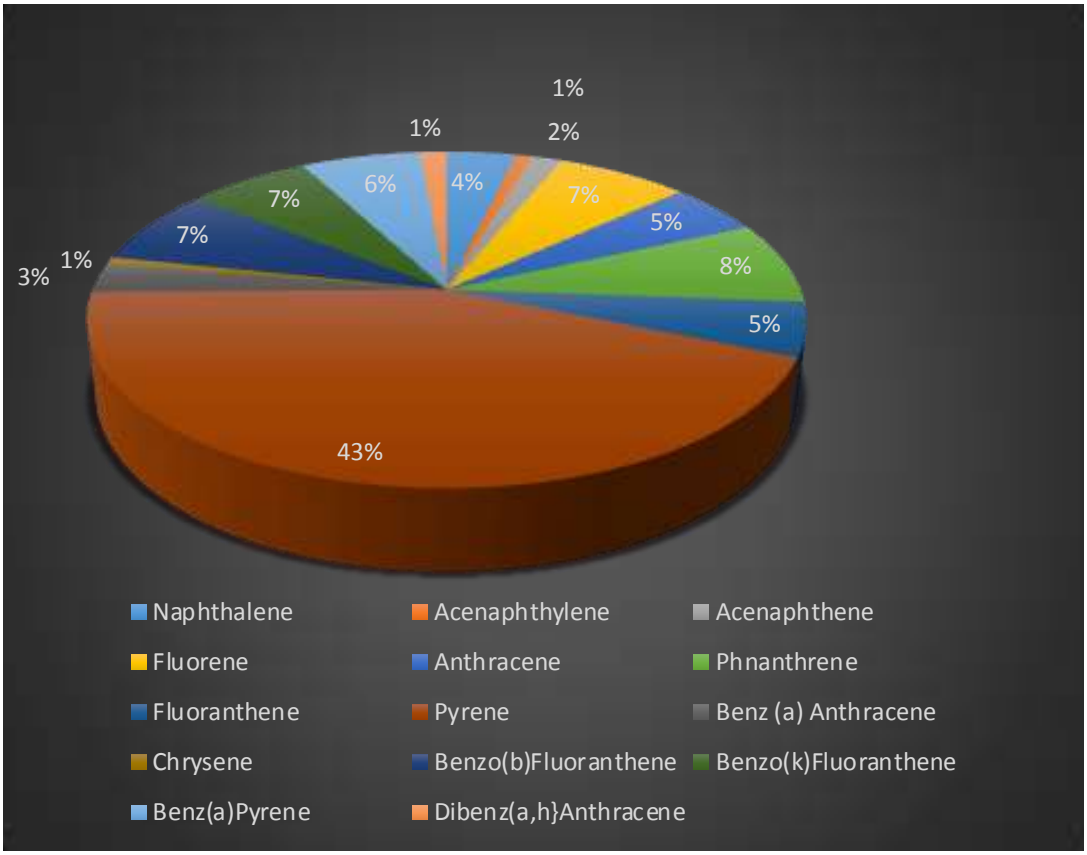

Figure 1. Percentage composition of PAHS in blood of neurology patients.

containing PAHs (Heikkila et al., 1995; ACGIH, 2005). Since the relative amounts of PAH congeners in mixtures vary from place-to place, this does not provide direct information on the relationship between exposure to pyrene and urinary 1-hydroxypyrene concentrations (Alghamdi et al., 2015). Like most PAHs, pyrene is used to make dyes, plastics and pesticides. It has also been used to make another PAH called Benzo(a)pyrene (ATSDR, 1990; Faust, 1993). Pyrene as a common chemical in materials that are daily been used could make people to be more exposed, lifestyle of the patients and occupation could also play a major role in high percentage constitution.

As shown in this study, the statistical concentrations of PAH's found in the patients, and an earlier study of regular subjects by Pleila et al. (2010) and Seracare (the reference blood sample)', it is evident that the body burden of PAH's in the blood of the neurology patients is higher. The individual value in this study measured at ng/mL as compared with the literature values in (Table 1) measured in pg/mL showed the high concentration at which the PAHs were present in the neurology patients. Figure 2 shows the levels of the difference, what was found in the neurology patients (Blue colour) were higher than the literature values. The bio accumulative effect of the volatile and particulate forms of these PAHs may be capable of neurological damage.

Correlation of PAHs in the samples

Rappaport et al. (2005), in their study, proposed Naphthalene as an alternate biomarker for the determination of PAHs in the body and the metabolites 1- and 2-hydroxynapthalene in urine as an alternative to 1-hydroxypyrene. Since naphthalene (two ring PAHs) sublimes into gaseous state, it will be a suitable biomarker for industrial exposure to airborne PAHs mixtures. AIOH (2016) suggested that where elevated levels of lower ring number PAHs may be present, the monitoring and assessment could be done using Naphthalene. A proximate matrix of the mixture of PAH was done using the mean concentration of the PAHs, naphthalene correlated with Phenanthrene (0.647),

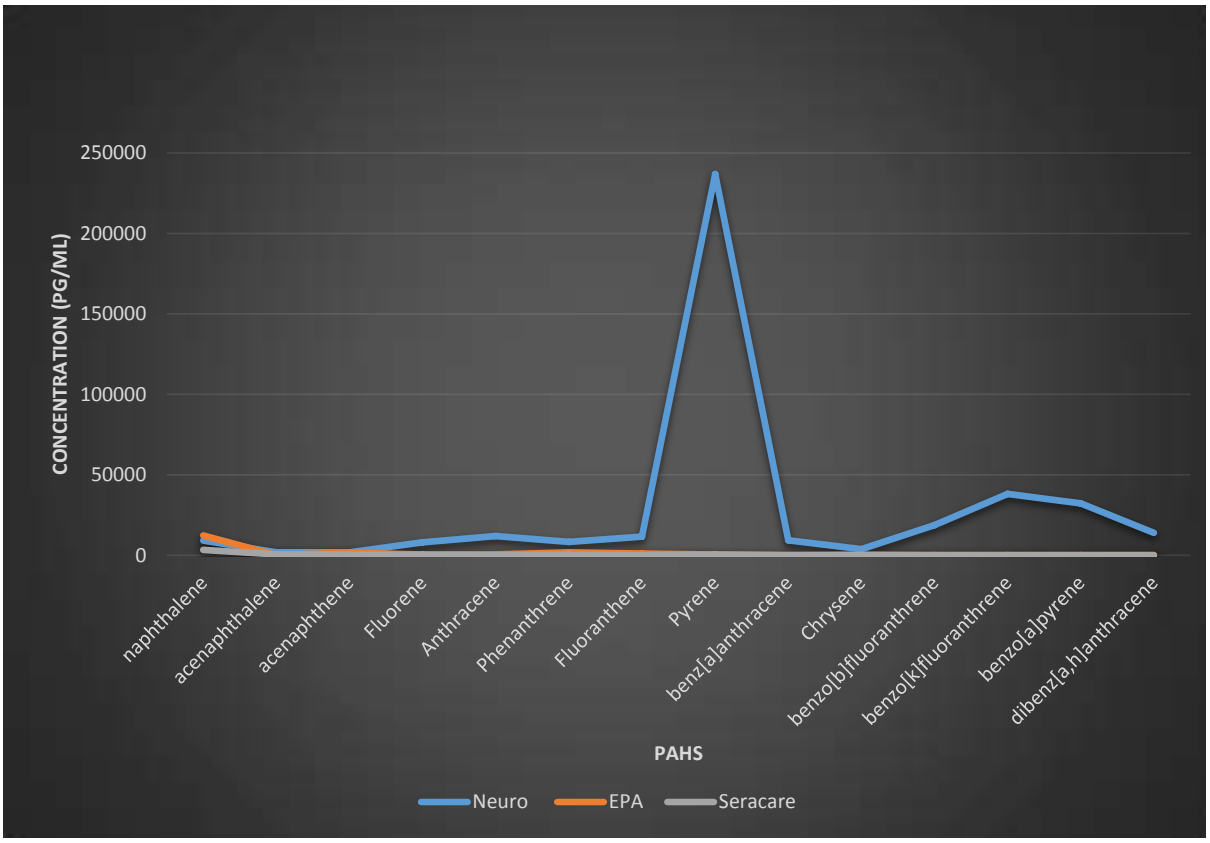

Figure 2. Maximum Concentrations of PAH's in Neurology Patients and Pleila et al. (2010) study.

Pyrene (0.595) and Benz(a)Pyrene(0.466) (Table 2). This may be a pointer that the source of absorption is not predominantly through inhalation, but may be dermal, food and air borne. According to Rappaport et al. (2004) where there is a mixture of dermal and airborne exposure, an alternative marker correlating better with the higher number ring compounds could be more suitable as biomarker. Thus, any of the following three could be used as biomarker; Phenanthrene, Pyrene and Benz (a)Pyrene as corroborated by Sobus et al. (2008). Sobus et al. (2009) in their study concluded that levels of naphthalene and phenanthrene in urine reflect airborne exposures to these compounds and are promising surrogates for occupational exposures to PAH mixtures. The control samples used in this study were blood plasma of undergraduate students (majorly, clinical students, >20 ≥30 years); all the three proposed biomarkers were absent in the control samples. This suggests that the occupation of subjects may play major role in the exposure and concentrations of these congeners in the body. In this study, blood plasma was used similar to urine in the sense that, they are both body fluid that represent the current state or on- going activities in the body. Seidel et al. (2008) determined metabolites of phenanthrene in post shift urine of occupational exposed worker (and concluded that phenanthrene was also a reliable biomarker for PAH exposure).The higher member ring, especially, B[a]pyrene; a potential carcinogen correlates with 5 other PAHs (Naphthalene, Pyrene, Benzo (a) Anthracene, Chrysene and benzo[k]fluoranthrene), of which Benzo (a) Anthracene, Chrysene and benzo[k]fluoranthrene were classified as possible carcinogens (IARC, 2010). This suggests that they have common possible source. The high values of these PAHs give course for concern as they have been implicated as being carcinogenic.

Risk assessment

The Toxicity Equivalency Factor (TEF) methodology was developed by the U.S. Environmental Protection Agency (EPA) to evaluate the toxicity and assess the risks of a mixture of structurally related chemicals with a common mechanism of action. A TEF is an estimate of the relative toxicity of a chemical compared to a reference chemical

Table 2. Proximity Matrix of Mixture of PAHs in Neurology Patients.

	Nap	Aclene-	Acthene	Fluo	Anthra	Phnant	Fluoran.	Pyre	B (a)A	Chrys	B(b)F	B(k)F	B(a)P	DB(a,h)A.
Nap	10.000													
Aclene-	-0.098	10.000												
Acthene.	060	-0.108	10.000											
Fluo	0.122	0.058	0.220	10.000										
Anthra	-0.234	0.290	0.404	-0.178	10.000									
Phnant	**0.647**	-0.245	0.261	**0.451**	-0.479	10.000								
Fluoran.	138	-0.013	-0.386	-0.035	-0.025	-0.251	10.000							
Pyre	**0.595**	-0.119	-0.352	-0.027	-0.209	0.007	0.447	10.000						
B (a) A	0.212	0.121	0.016	-0.125	0.117	-0.293	**0.403**	**0.645**	10.000					
Chrys	-0.015	0.248	0.171	-0.285	**0.655**	-0.266	-0.049	-0.125	-0.178	10.000				
B(b)F	-0.216	**0.707**	0.164	-0.292	**0.569**	-0.432	-0.028	-0.209	0.232	0.481	10.000			
B(k)F	0.000	**0.559**	0.236	-0.207	**0.717**	-0.237	-0.013	-0.124	0.083	**0.676**	**0.688**	10.000		
B(a)P	**0.466**	0.218	-0.120	-0.180	0.306	-0.129	0.349	**0.635**	**0.410**	**0.503**	0.290	**0.624**	**1**0.000	
DB(a,h)A.	098	0.434	0.132	-0.178	0.557	-0.113	-0.020	-0.082	-0.123	0.755	0.525	0.928	0.701	10.000

Correlation at 95% confident level.

Table 3. Risk Assessment Table of PAHs in ng/mL.

PAH analyte	ID	Carcinogen class		Potency/Toxicity Equivalent Factor			
		EPA (2006)	IARC (2010)	WDNR (2015)	Nisbet and LaGoy(1992)	BaP_n Ratio	$BaP_{eq} = C_nTEF_n$
Naphthalene	Nap	C	2B	-	0.001	0.195	0.00617
Acenaphthalene	Acl	D	-	-	0.001	0.014	0.00045
Acenaphthene	Ace	-	3	-	0.001	0.027	0.00084
Fluorene	Flu	D	3	-	0.001	0.110	0.00349
Phenanthrene	Phe	D	3	0.001	0.001	0.124	0.00249
Anthracene	Ant	D	3	-	0.001	0.079	0.00392
Fluoranthene	Flt	D	3	0.001	0.001	0.081	0.00257
Pyrene	Pyr	D	3	0.001	0.001	0.78	0.02472
Benz[a]anthracene	Baa	B2	2B	0.1	0.1	0.052	0.1640
Chrysene	Chr	B2	2B	0.001	0.01	0.109	0.00346
Benzo(b)fluoranthene	Bbf	B2	2B	0.1	0.1	0.136	0.4320
Benzo(k)fluoranthene	Bkf	B2	2B	0.01	0.1	0.125	0.0395
Benzo[a]pyrene	Bap	B2	1	1	1	1	31.71
Dibenzo(a,h)anthracene	Dba	B2	2A	1	5	0.435	13.79

EPA, Environmental Protection Agency; IARC, International Agency for Research on Cancer; WDNR, Wisconsin Department of Natural Resources; –, Not Available

(Table 3). For mixtures of PAHs, the reference chemical is benzo(a)pyrene, the Toxicity Equivalency Factor for each PAH is an estimate of the relative toxicity of the PAH compound compared to benzo(a)pyrene (AIOH 2016).

The Toxicity Equivalence Factors (TEF) of PAHs together with the WHO Quantitative Risk Assessment Model (Ramírez et al., 2011; WHO, 2000) was used to estimate the Excess Lifetime Neurology Risk due to PAH exposures. Benzo (a) pyrene equivalents (BaP_{eq}) were determined by multiplying individual PAH concentrations with the corresponding TEF developed by US EPA, 2010 and Nekhavhambe (2014) based on the toxicity equivalent factor of individual PAH.

Thus:

$$BaP_{eq} = (Cn \times TEFn)$$

Total Toxicity Equivalent factor = $\sum_{n=1}^{k} CnXTEFn$

Where, Cn = individual PAH concentration in the complex mixture, TEF*n* = toxic equivalent factor of individual PAH, and k = total number of PAH compounds

The total toxicity of the congeners was calculated on the basis of the method for calculating the concentration of 10 group of PAH to reflect a toxicity equivalent factor (TEF) based on benzo(a)pyrene in order to normalize the toxicity. Adding the concentrations of the 10 congeners together directly will lead to over estimation of the toxicity (WDNR, 2015), thus the use of toxicity equivalent factor.

The 10 PAHs include Benzo(a)anthracene, Benzo(b) fluoranthene, Benzo(g,h,i) perylene, Benzo(k) fluoranthene, Chrysene, Dibenzo(a,h)anthracene, fluoranthene, indeno(1,2,3-cd)pyrene, phenanthrene and pyrene whose BaP_{eq} should not be more than 0.1 µg/L. Only Benzo (g,h,i)perylene and indeno(1,2,3-cd) pyrene were not detected in this study. Although the PAHs mixture were more than 10 congeners in this study, only 8 of the congeners were used in the calculation and the concentrations totaled 14.459 ng/mL or 14.459 µg/L which is well above the expected value of 0.1 µg/L. The mixture also reflected all classes of classification of PAHs ranging from carcinogenic – probable carcinogen – not classifiable. Napthalene has a maximum Concentration limit of 70 µg/L in water (since there is no limit set for PAHs in the blood by any standard organization), but the mean value in this study was 6.17 µg/L. Maximum Contamination Level(MCL) of some PAHs in water given by USEPA (1984) were 0.2 ng/mL for Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene and chrysene while Benz[a]anthracene and Dibenzo(a,h) anthracene were given MCL of 0.1 ng/mL and 0.3 ng/mL respectively. The mean values for these PAHs were found to be higher in the plasma of the neurology patients. The non-classifiable member of PAHs is capable of causing problem(s) at a high concentration which may not be known for now.

Conclusion

The study shows that the concentration levels of the PAHs detected in the plasma samples of the neurology patients are significantly higher at 95 % level than the control samples. The MCL values provided for water and the literature values of the similar research were lower in concentration than what was found in the neurology patients. Volatile PAHs are most prevalent in the samples and there was high correlation in the concentrations of the volatile congeners, thus, they could be used as biomarkers in the blood/plasma analysis. This result represents a veritable baseline study of PAHs in Neurology Patients in the country. Further work could be done in assessing the level of these PAHs in the same group of patients as they continue their treatment, and also to further establish the actual roles of these congeners in the causation of these neurological diseases. This can further open up a new vista in therapeutic approaches to treatment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (1990). "Public Health Statement, Polycyclic Aromatic Hydrocarbons". Atlanta, GA: U.S. Department of Health and Human Services. www.atsdr.cdc.gov/

Alghamdi MA, Alam MS, Stark C, Mohammed N, Harrison RM, Shamy M, Khoder MI, Shabbaj II, Göen T (2015). "Urinary metabolites of polycyclic aromatic hydrocarbons in Saudi Arabia School children in relation to sources of exposure". *Environmental Research* 140:495-501.

American Conference of Governmental Industrial Hygienists ([ACGIH) (2005). "Polycyclic aromatic hydrocarbons (PAHs) biologic exposure indices (BEI) Cincinnati, OH". American Conference of Governmental Industrial Hygienists.

Australian Institute of Occupational Hygienists (AIOH) (2016). "Polycyclic Aromatic Hydrocarbons (PAHs) and Occupational Health Issues"; Position Paper prepared by AIOH Exposure Standard Committee and Authorised by AIOH Council, pages I-17 htpp://www.aioh.org.au.

Centers for Disease Control (CDC) (2009). Polycyclic Aromatic Hydrocarbons (PAHs) https://www.epa.gov/sites/production/files/2014-03/documents/pahs_factsheet_cdc_2013.pdf

Centers for Disease Control and Prevention(CDC) (2005). "Third National Report on Human Exposure to Environmental Chemicals". Atlanta GA [accessed 2009 June]. Available from: http://www.cdc.gov/ExposureReport/pdf/thirdreport.pdf.

Chambers DM, Blount BC, McElprang DO, Waterhouse MG, Morrow JC (2008). "Picogram measurement of volatile n-alkanes (n-hexane through n-dodecane) in blood using solid-phase microextraction to assess nonoccupational petroleum-based fuel exposure". Analytical Chemistry 80(12):4666-4674.

Farhadian A, Jinap S, Hanifah HN, Zaidul IS (2011). "Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat". Food Chemistry 124(1): 141–146.

Faust RA (1993). Chemical Hazard Evaluation Grou. Biomedical and Environmental Information Analysis section, Health Sciences Research Division. Oak Ridge National Laboratory. Oak Ridge, Tennessee. https: //rais.ornl.gov/tox/profiles/pyrene_f_V1.html

Gyorffy E, Anna L, Kovacs K, Rudnai P, Schoket B (2008). "Correlation between biomarkers of human exposure to genotoxins with focus on carcinogen-DNA adducts. Mutagenesis adducts". Mutagenesis 23(1):1-18.

Hansen AM, Mathiesen L, Pedersen M, Knudsen LE (2008). "Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies-a review". International Journal of Hygiene and Environmental Health 211:471e503.

Heikkila P, Luotamo M, Pyy L, Riihimaki V (1995). “Urinary 1-naphthol and 1-pyrenol as indicators of exposure to coal tar products”. *International Archives of Occupational and Environmental Health* 67(3):211-217.

International Agency for Research on Cancer ([IARC) (2009). Lyon, France, http://monographs.iarc.fr/ENG/Classification/index.php.

Lewtas J (2007). “Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive and cardiovascular effects”. Mutation Research/Review in Mutatation Research 636(1–3):95-133. https://doi.org/10.1016/j.mrrev.2007.08.003

Nekhavhambe TJ, Van Ree T, Fatoki OS (2014). Determination and distribution of polycyclic aromatic hydrocarbons in rivers, surface runoff, and sediments in and around Thohoyandou, Limpopo Province, South Africa. Water SA 40:415-424. http://dx.doi.org/10.4314/wsa.v40i3.4

Nisbet IC, LaGoy PK (1992). “Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs)”. Regulatory Toxicolology and Pharmacology 16:290-300. https:// www.ncbi.nlm.nih.gov/pubmed/1293646

Perera F, Karl H, Wieslaw J, Robin W, Ulka C, Yanzhi H, Regina S, Richard A, James PO (2002). “*In Utero* DNA Damage from Environmental Pollution Is Associated with Somatic Gene Mutation in Newborns”. Cancer Epidemiology, Biomarkers and Prevention 11:1134-1137. http://cebp.aacrjournals.org/content/11/10/1134.

Pleila JD, Stiegelb MA, Sobusa JR, Tabucchic S, Ghiod AJ, Maddend MC (2010). “Cumulative exposure assessment for trace-level polycyclic aromatic hydrocarbons (PAHs) using human blood and plasma analysis”. Human Exposure and Atmospheric Sciences Division, NERL/ORD, U.S. Environmental Protection Agency, Research Triangle Park, NC, United State.

Popp W, Vahrenholz C, Schell C, Grimmer G, Dettbarn G, Kraus R, Brauksiepe A, Schmeling B, Gutzeit T, Bulow JV, Norpoth K (1997). “DNA single strand breakage, DNA adducts, and sister chromatid exchange in lymphocytes and phenanthrene and pyrene metabolites in urine of coke oven workers”. Occupational and Environmental Medicine 54:176-183. http://oem.bmj.com/

Ramírez N, Cuadras A, Rovira E, Marcé RM, Borrull F (2011). “Risk assessment related to atmospheric polycyclic aromatic hydrocarbons in gas and particle phases near industrial sites”. *Environmental Health Perspectives*, 119(8): 1110-1116. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3237345/PDF/EHP.1002855.pdf

Rappaport SM, Kupper LL, Lin YS (2004). “On the importance of exposure variability to the doses of volatile organic compounds”. Toxicological Sciences 83(2):224-236. Epub 2004 Nov 17. https://doi.org/10.1093/toxsci/kfi039

Rappaport SM, Kupper LL, Lin YS (2005). “On the importance of exposure variability to the doses of volatile organic compounds”. Toxicological Sciences 83(2):224-36.https://doi.org/10.1093/toxsci/kfi039

Schober W, Lubitz S, Belloni B, Gebauer G, Lintelmann J, Matuschek G, Weichenmeier I, Eberlein-König B, Buters J, Behrendt H (2007). “Environmental polycyclic aromatic hydrocarbons (PAHs) enhance allergic inflammation by acting on human basophils”.Inhalation Toxicology 19 Suppl 1:151.

Seidel A, Spickenheuer A, Straif K, Hans-Peter R , Marczynski B, Scherenberg MG, Dettbarn G, Angerer J, Wilhelm M, Brüning T, Jacob J, Pesch B (2008). “New Biomarkers of Occupational Exposure to Polycyclic Aromatic Hydrocarbons”. Journal of Toxicology and Environmental Health, Part A 71, (11-12): 734-745. https://doi.org/10.1080/15287390801985265

Sexton KD, Hattis K, Sexton D, Hattis D (2007). “Assessing Cumulative Health Risks from Exposure to Environmental Mixtures—Three Fundamental Questions”. *Environmental Health Perspectives* 115:825-832.

https://uthealth.influuent.utsystem.edu/en/publications/assessing-cumulative-health-risks-from-exposure-to-environmental-

Skupinska K, Misiewicz I, Kasprzycka-Guttman T (2004). “Polycyclic aromatic hydrocarbons: physiochemical properties, environmental appearance and impact on living organisms”. Acta Poloniae Pharmaceutica 61(3):233-240. Skupinska: Polycyclic aromatic hydrocarbons: physicochemi- Google Scholar http:ptfarm.pl/pub/file/Acta_poloniae/2004/3/233.pdfpl

Sobus JR, Pleil JD, Madden MC, Funk WE, Hubbard HF, Rappaport SM (2008). “Identification of surrogate measures of diesel exhaust exposure in a controlled chamber study”. *Environmental Science Technology* 42(23):8822-8828.

Sobus JR, McClean MD, Herrick RF, Waidyanatha S, Onyemauwa F, Kupper LL, Rappaport SM (2009). “Investigation of PAH biomarkers in the urine of workers exposed to hot asphalt”. Annals of Occupational Hygienes 53:551. DOI:10.1093/annhyg/mep041

Sram RJ, Binkova B, Rossner P, Rubes J, Topinka J, Dejmek J (1999). “Adverse reproductive outcomes from exposure to environmental mutagens”. Mutagenesis 428(1-2):203-215. https://doi.org/10.1016/S1383-5742(99)00048-4

Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs) (ATSDR) (1995). U.S. Department of Health and Human Services, U.S. Public Health Service, Agency for Toxic Substances and Disease Registry: Atlanta, GA, USA. P 271.

United State Environmental Protection Agency (USEPA) (1984). “Health effects assessment for polycyclic aromatic hydrocarbons (PAH)”. Cincinnati, OH: Environmental Protection Agency. EPA Report No. 540/1–86–013. nepis.epa.gov

US EPA (2010). “Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures”: EPA/635/R-08/012A; US EPA: Washington, DC, USA.

Wisconsin Department of Natural Resources (WDNR) (2015). Bureau of Water Quality Program Guidance WPDES. “PAH Group of 10 Calculation of Concentration Using Toxicity Equivalent Factors”. May 2015 Guidance Number: 3400-2015-01. https://dnr.wi.gov/topic/wastewater/documents/46531fs.pdf.

World Health Organization (WHO) (2000). Regional Office for Europe. Chapter 5.9 PAHs. In WHO Regional Office for Europe. “Air Quality Guidelines” 2nd ed. Denmark: WHO Regional Office for Europe. www.euro.who.int