Health Risk Assessment of Polycyclic Aromatic Hydrocarbons: A Review

Shashi Nandar Kumar¹, Pankaj Verma², Banajit Bastia¹ and Arun Kumar Jain¹

¹Environmental Toxicology Lab, National Institute of Pathology (ICMR), Safdarjung Hospital Campus, New Delhi-110029, India.
²Department of Pharmacology, PDM College of Pharmacy, Bahadurgarh, Haryana -124507, India.

*Corresponding Author: Dr. Arun Kumar Jain
Email: drakjain@gmail.com
Received: 24/10/2014 Revised: 27/12/2014 Accepted: 29/12/2014

Abstract
Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous environmental contaminants and found almost everywhere. Most of PAHs are formed during the incomplete combustion of organic matter such as wood, petroleum product, coal and tar. These chemicals are biologically active and pose a risk for human health because of their potentially toxicity such as carcinogenicity, teratogenicity, mutagenicity and genotoxicity. The aim of this paper is to give current information on PAHs exposure, worldwide spread distribution and health risk associated with their exposure.

Key words: Polycyclic aromatic hydrocarbons, Biological effects, Toxicities.

1. Introduction
Polycyclic aromatic hydrocarbon (PAHs) are persistent organic pollutant that belong to such as low molecular weight compounds consisting of fewer than four rings and high molecular weight compounds of four or more rings (Kim et al., 2013). There are several hundred PAHs, Which usually occur as complex mixtures rather than as individual compounds, which are ubiquitous environmental contaminants that are mainly formed during incomplete combustion of fuel and other organic materials (Guo et al., 2004). Cautreels and Van Cauwenberge (1978) reported that PAH are primarily associated with ultrafine particulate matter. They accumulate in the bronchial epithelium, while volatile PAHs are readily absorbed through the alveolar epithelium (Ramirez et al., 2011). PAHs are known to be mutagenic, genotoxic and include some of strongest known carcinogens. PAHs cause acute and chronic toxicity. Therefore PAHs may have a potential health impact on human. Earlier research indicates that non-smoking individuals, ingestion via food and unintentional consumption of house hold dust (of particular concern for young children) is main supplier to PAHs exposure (Ramesh et al., 2004). According to EPA, seven non-substituted PAHs are considered possible carcinogen (group 2 B) and 16 PAHs are listed as main concern pollutants because of their prevalence in urban and suburban air (EPA, 2012). Prenatal exposure to PAHs, which occurs primarily through tobacco smoke, occupational exposure, and air pollution, could increase the risk of cancer during childhood (Cordieri et al., 2004). Several studies indicate that PAHs exposure, due to environmental air pollutions are associated with reduced fetal growth (Choi et al., 2008), neural tube defect (Ren et al., 2011) and various other morbidities in human pregnancy (Cnattingius, 2004; Vishnevetsky et al., 2015). The primary aim of this paper is to review current information on the risk associated with the presence of these chemicals in the environment including its mechanism, ubiquitous sources of PAH, routes of exposure and toxicological studies.

2. Chemical Properties
PAHs are weakly volatile and lipophilic in nature. PAH are also chemically inactive but binds to particulate matter (Table 1). When adsorbed at surface of dust, PAHs become highly active thermally and photosensitive. They can be cracked at high temperature (50°C). PAHs have very characteristic UV absorbance spectra. As each ring structure has a unique UV spectrum, each isomer exhibits a unique UV absorbance spectrum. This is especially useful in identification of PAHs. Although the health effects of individual PAHs differ, some PAHs have been identified to be of greatest alarm due to deleterious effects on humans. PAHs are hydrophobic compounds and their persistence in the environment is mainly due to their low water solubility and electrochemical stability (Kim et al., 2013).

3. Biodegradation
PAHs do not decay under normal atmospheric conditions. Microbial degradation is one of the major -
Table 1: Properties of PAHs in reference to Molecular Formula (MF), Molecular Weight (MW), Melting Point (MP), Boiling Point (BP) and Solubility in solvent

<table>
<thead>
<tr>
<th>Compound</th>
<th>MF</th>
<th>Mol. Wt.</th>
<th>MP</th>
<th>BP</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>C_{12}H_{10}</td>
<td>154.21</td>
<td>93.4° C</td>
<td>279° C</td>
<td>Benzene, Chloroform, Toluene, Anhydrous acid and Petroleum ether</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>C_{12}H_{8}</td>
<td>152.19</td>
<td>78-82° C</td>
<td>280° C</td>
<td>乙醇 (EtOH), Diethylether, Benzene and Chloroform</td>
</tr>
<tr>
<td>Anthracene</td>
<td>C_{14}H_{10}</td>
<td>178.23</td>
<td>218° C</td>
<td>340° C</td>
<td>MeOH and Hexane</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>C_{18}H_{12}</td>
<td>228.28</td>
<td>158° C</td>
<td>438° C</td>
<td>Diethyl ether, Acetone and 0.00001g/100 ml in water</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>C_{20}H_{12}</td>
<td>252.31</td>
<td>179° C</td>
<td>495° C</td>
<td>Benzene, Toluene, Xylene and sparingly soluble in alcohol</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>C_{20}H_{12}</td>
<td>252.309</td>
<td>168°</td>
<td>228.6°</td>
<td>Benzene, Acetone and Alcohol</td>
</tr>
<tr>
<td>Benzo(g,h,i)perylene</td>
<td>C_{22}H_{12}</td>
<td>276.34</td>
<td>278°</td>
<td>500°</td>
<td>Acetone, Dichloromethane and 1,4 dioxane</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>C_{20}H_{12}</td>
<td>252.31</td>
<td>217°</td>
<td>228.6°</td>
<td>Benzene</td>
</tr>
<tr>
<td>Chrysene</td>
<td>C_{18}H_{12}</td>
<td>228.28</td>
<td>254°</td>
<td>448°</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Dibenz(a,h)anthracene</td>
<td>C_{22}H_{14}</td>
<td>278.34</td>
<td>262°</td>
<td>524°</td>
<td>Petroleum, Ether, Benzene, Toluene, Xylene and slightly soluble in alcohol and ether</td>
</tr>
<tr>
<td>Fluranthene</td>
<td>C_{16}H_{10}</td>
<td>202.26</td>
<td>375°</td>
<td>110.8°</td>
<td>Benzene, Ethyl ether, Ethanol, Acetic acid and hot alcohol</td>
</tr>
<tr>
<td>Fluorene</td>
<td>C_{13}H_{10}</td>
<td>166.223</td>
<td>295°</td>
<td>116-117°</td>
<td>Benzene and Ether</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>C_{22}H_{12}</td>
<td>276.337</td>
<td>162-164°</td>
<td>497.101°</td>
<td>Benzene</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>C_{10}H_{8}</td>
<td>128.17</td>
<td>218°</td>
<td>80.26°</td>
<td>Very soluble in ether, chloroform, carbon disulfide, less soluble in ethanol/methanol and insoluble in water. Approximately 30 mg/L in water.</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>C_{14}H_{10}</td>
<td>178.23</td>
<td>101°</td>
<td>332°</td>
<td>Insoluble in water but is soluble in most organic solvents such as toluene, carbon tetrachloride, ether, chloroform, acetic acid and benzene. 1.6 mg/L soluble in water.</td>
</tr>
<tr>
<td>Pyrene</td>
<td>C_{16}H_{10}</td>
<td>202.25</td>
<td>145-148°</td>
<td>404°</td>
<td>Soluble in ethanol, ether and benzene</td>
</tr>
</tbody>
</table>

degradation processes. Algae, fungi and bacteria are the main micro-organism involved in PAHs degradation. An aerobic and anaerobic condition favours biodegradation of PAHs. However, anaerobic biodegradation is a slow process and its biochemical mechanism has not yet been elucidated. PAHs get bound to soil for a long time which shows reduced bioavailability towards biodegradation. Particles are not easily accessible to solution phase and are partially immobilized. These particles pose lesser threat to surroundings and human health. Mycobacterium possess lipophilic surface which makes them prone to bind with pollutants from soil particles and hence widely used in biotransformation of aged contaminated sites (Haritash et al., 2009; Bacosa et al., 2015). UV mediated photolysis is also one of the major process for PAHs degradation. PAHs absorb sunlight readily in UV (280-400 nm) and visible regions (400-760 nm). PAHs toxicities is directly proportional to extent of UV exposure i.e., toxicity increase in dose-dependent fashion with increased exposure to UV radiation.

4. Sources

4.1 Natural Sources

4.1.1 Fires

Forest fires and agricultural fires contributes huge amount of PAHs in environment. Amount of PAHs generated and particulates emitted during the
fires depends on type of organic material burned, nature of blaze (flaming vs smouldering), intensity of fire and type of fire (backing fire or heading fire) (Maliszewska-Kordybach, 1999; Khaiwal et al., 2006; 2008).

4.1.2 Fossil Fuels
Fossil fuels contribute very low levels of PAHs in atmosphere which accounts for a reason that oil deposits are formed beneath layers of rocks, so there is less risk of PAHs to emit from same environment. PAHs which occur near the surface (e.g. sands) are easily capable of being exposed in the environment. (Maliszewska-Kordybach, 1999; Khaiwal et al., 2006; 2008).

4.2 Anthropogenic Sources

4.2.1 Industries
Iron, steel foundries, aluminium production, coal gasification and coke production are the main sources of PAHs. Petroleum refining and thermal power plants emit the large amount of PAHs in environment to make air polluted (Knawel et al., 2008).

4.2.2 Automobile
Motor vehicle emissions (especially diesel vehicles) make a considerable contribution to PAH concentration in air due to burning and incomplete combustion of diesel and gasoline. Air craft engine as a source of PAH in atmosphere has also been identified. (Khaiwal et al., 2008)

4.2.3 Domestic
Cooking (fuel burning) and waste refuse incineration (WHO, 2002).

4.2.4 Human Habitats
Smoking cigarettes, cigar and tobacco (Ding et al., 2007).

4.2.5 Other Sources
Involves forest and prairie fires, rural and urban sewage sludge, municipal wastewater discharge runoff, river borne pollution, accidental spills from oil tankers and other ships. In the Indian urban environment, cooking fuel combustion is a likely source of PAH. High concentrations of PAHs have been measured in smoke from solid-fuel stoves, burning wood, coal and dried cattle manure (Raiyani et al., 1993a) and kerosene stoves (Saksena et al., 1996) used as the primary cooking device by urban slum residents. Agarwal et al. (2009) reported 2 to 5 times higher PAH values in urban soil as compared to rural sites in Delhi.

4.3 PAH in Ecosystem

4.3.1 Atmosphere
PAHs in ambient air exist in vapour phase or absorb into airborne particulate matter depending on atmospheric conditions (ambient temperature, relative humidity, etc.), the nature (i.e. origin and properties) of the aerosol, and the properties of individual PAH (Khaiwal et al., 2008; Zhang and Tao, 2009; Wang et al., 2013). Light chained PAHs reacts with other environmental pollutants like ozone, nitrogen oxides, and sulphur dioxide and form diones, nitro- and dinitro-PAHs, and sulphuric acids. Levels of PAHs in gaseous phase are found very high during summer or in tropical regions and particulate phase, PAHs show dominance during winter or in arctic regions. The most dominant source of PAHs in atmospheric aerosol has been found to be vehicular emissions (Khaiwal et al., 2008) and the highest concentrations of atmospheric PAHs are commonly originated in the urban environment due to increasing vehicular traffic and the dispersion of the atmospheric pollutants. India is a developing nation which has experienced an increase in the population and industrial expansion that has been accompanied by drastic increase in vehicular transportation. In rapidly growing urban agglomerations like the megacity Delhi, transport sector is a major source of emissions of air pollutants including PAHs (Kumari et al., 2011). Due to high density of population in metropolitan cities such as Delhi NCR, the risk associated with the human exposure to atmospheric PAHs is the highest (Kumar et al., 2014). Sharma et al. (2007) evaluated the atmospheric concentrations of PAHs in Delhi at three different locations; Okhla, Dhaulakuan and Daryaganj and found the annual average concentration of total PAHs at these sites to be 668 ± 399 and 672 ± 388 ng/m² in the years 2002 and 2003, respectively. Kumari et al. (2011) estimated PAH emissions from mobile sources in Delhi during 1999 to 2006 and reported that total annual emissions of Σ23–PAHs from road transport has increased approximately 4 times amongst which naphthalene emissions emerged as the most prominent (8 times). Further a two fold increase was seen for the carcinogen benzo[a]pyrene emissions.

Recent studies were performed from ambient air samples from 7 sites of Delhi during winter season (ITO, Janakpuri, Nizamuddin, Pitampura, Shahzabad, Siri Fort, Shahdra) which suggested presence of average concentration of Benzo(a)pyrene ranging from 0.27 to 17.86 ng/m³ as well as total PAH content was found to be ranging from 4.61 to 52.96 ng/m³ (Sharma et al., 2014). These data also suggested seasonality dependence of PAHs concentration in atmosphere. Another study performed in India suggested higher
concentration of PAHs in summer (18.17 ug g⁻¹) and autumn (16.38 ug g⁻¹) season as compared to winter season (4.04 ug g⁻¹) (Kumar et al., 2014).

### 4.3.2 Plants

Many plants have greater surface area covered with waxes which facilitates the accumulation of hydrophobic chemicals. Leaf features such as surface, hairs, cuticular waxes, number of stomata plays an important role in PAH uptake and accumulation (Jouraeva et al., 2002). PAHs are transferred to plants by particle phase deposition on waxy leaf cuticle or by uptake in the gas phase through stomata (Kipopolov et al., 1999; Lehndoerff and Schwark, 2004). Seasonal variations in concentration of PAHs with higher values in winter than in summer are found in Azalea leaves (Nakajima et al., 1995).

### 4.3.3 Food

Humans may be exposed to PAHs by eating grilled or charred meats, contaminated cereals, flour, bread and vegetables (Grova et al., 2006). Dhananjayan et al. (2012) reported that estimated intake of PAHs among general population consuming fish ranges from 1.77 – 10.70 ng/kg body weight/day. PAHs are also occurred in substantial quantities in some food, depending on mode of cooking, preservation and storage. Largest contribution to daily PAH intake came from sugar and sweets, cereals, oils, fats and nuts. In vivo studies suggest a transfer in intestinal epithelium by diffusion, which appears extensively governed by physic-chemical properties of PAHs particularly lipophilicity. However, other mechanisms such as metabolism are considered to intervene (Cavret and Feidt, 2005; Phillips, 1999). In addition, Phillips reported that cereals and vegetables are also major dietary sources of PAHs except for high consumption of meat cooked over an open flame (Phillips, 1999). Grova et al. (2006) reported the impact of oral exposure of PAHs to lactating goats for 28 days led to an excretion of PAHs into milk under native forms.

### 4.3.4 Water

PAHs enter surface waters mainly via atmospheric fallout, urban run-off, municipal effluents, industrial effluents and oil spillage or leakage. Water contamination also occurs from industrial effluents and accidental spills during oil shipment at sea. PAHs are semi volatile organic compounds, exist in both gaseous and particulate phase in air and are subjected to both vapour and particle washout from the atmosphere during precipitation. Higher concentration of PAHs in urban run-off were found during autumn and winter due to higher incidence of vehicles in streets, coupled with use of heating system. It has been found that disinfection technique of chlorination of drinking water may lead to formation of oxygenated and chlorinated PAHs i.e compounds that are more toxic than parent PAHs (Manoli and Samara, 1999). The presence of PAHs in drinking water may be due to surface or ground water used as raw water sources, or to the use of coal tar coated pipes in public water supply (Srogi et al., 2007).

### 4.3.5 Soils

Accumulation of PAHs in soils may lead to further potential contamination of vegetables and food chains (Kipopolov et al., 1999; Mueller and Shann, 2006), and then cause direct or indirect exposure to human. Moreover, leaching, evaporation and migration are possible PAHs sources of atmospheric or groundwater contamination.

### 4.3.6 Sediments

Sediments are deposited materials consisting of organic matter in various stages of decomposition, particulate mineral matter and inorganic material of biogenic origin. They have been proven an efficient tool to identify environmental impacts (Evans et al., 1990). Sediments constitute a pollutant trap and are an important factor to establish the assimilative capacity of the environment. Sediments may contain a high level of pollutants ready to pass on to the food chain or be mobilised by anthropogenic or natural means. Sediment retention capacity may be related to physico-chemical properties such as grain size and organic matter. Changes in PAHs accumulation in sediments take place depending on direct influence of anthropogenic activities, mainly direct influence of anthropogenic activities, mainly combustion processes of traffic and industrial activities.

### 4.3.7 Toxicokinetics

PAH compounds enter the human body through different mode of exposure such as inhalation of ambient and indoor polluted air, direct inhalation of tobacco smoke or breathing smoke from open fireplaces, ingestion of contaminated and processed food, water and dermal contact (Ramesh et al., 2004).

#### 4.3.7.1 Absorption

PAHs are highly lipid soluble and are absorbed from lung, gut and skin of mammals. Studies on lung retention of microcrystalline PAHs or PAHs in solution after intra-tracheal instillation in female rats have indicated that they are rapidly cleared from respiratory tract. However, inhaled PAHs are predominantly adsorbed on soot particles. After deposition in airways, the particles can be eliminated by bronchial clearance. PAHs might be partly removed from particles during transport on ciliated mucosa and...
may penetrate into bronchial epithelium cells where metabolism takes place. BaP and other PAHs are readily adsorbed from gastrointestinal tract when present as solutes in various dietary lipids. The absorption is facilitated by presence of bile salts in the intestinal lumen.

4.3.7.2 Distribution: Once absorbed, PAHs enter the lymph, circulate in the blood and are metabolised primarily in liver and kidneys. PAHs differ with respect to distribution patterns and lipophilic properties (Busbee et al., 1990). Because of their lipophilic nature, PAHs can accumulate in breast milk and adipose tissue. However, biliary and urinary excretion of PAHs is relatively efficient because of wide distribution of enzymes that transform PAHs into polar metabolite.

4.3.7.3 Metabolism, Activation and Excretion: The enzyme systems that metabolise PAHs are widely distributed in cells and tissues of humans and animals. The highest metabolising capacity is present in the liver, followed by lung, intestinal mucosa, skin and kidneys; but metabolism may also take place in nasal tissue, mammary glands, placenta, uterus, platelets, erythrocytes, spleen, hair follicles, brain and leukocytes. The enzyme system primarily responsible for PAH metabolism is the microsomal mixed function oxidase system, which converts the non-polar PAHs into polar hydroxy and epoxy derivatives. Epoxides are the major intermediates in oxidative metabolism of aromatic double bonds. The metabolism and activation of all PAHs is done by phase I and phase II reactions.

a. Phase I Reactions: There are three main pathways of PAHs activation; formation of neutral PAH radical cation in a metabolic oxidation process involving cytochrome p450 peroxidase, formation of PAH o-quinones by dihydrodiol dehydrogenase – catalysed oxidation and finally the creation of dihydrodiol epoxides, catalysed by cytochrome p450 enzymes (Guengerich, 2000). Most common mechanism of metabolic activation of PAHs, such as benzo[a]pyrene, is via the formation of bay-region dihydrodiol epoxides. The most important enzymes in the metabolism of PAHs are CYP 1A1, 1A2, 1B1 and 3A4. CYP1A1 is highly inducible by PAHs (Kim et al., 1998). CYP1A2 and 1B2 are also inducible by exposure to PAHs. In fact, these enzymes share the same mechanism with which PAH molecules interact with, aryl hydrocarbon receptor (AhR). The AhR is present in cytoplasm as a complex with other proteins such as heat shock protein 90 (Hsp90), p23 and AhR interacting protein. After forming a complex with PAHs, the HSP90 is released and the AhR-PAH complex translocates to the nucleus. Here, it creates a heterodimer with ARNT (Ah Receptor Nuclear Translocator) and afterwards binds to DNA via the xenobiotic response element (XRE) situated in promoter region of CYP1A and CYP1B genes (Shimada et al., 2006).

PAH are activated by a pathway that involves both CYP enzymes and epoxide hydrolase. Other phase I enzymes related to PAHs metabolism are the aldoketo reductases. These enzymes oxidize PAH trans-dihydrodiols to reactive and redox active o-quinones in vitro (Quinn and Penning, 2006). Specifically, AKR1C1, and members of AKR dihydrodiol/hydroxysteroid dehydrogenase subfamily, AKR1C1-AKR1C4 are involved in metabolic activation of PAH trans-dihydrodiol. Production of o-quinone metabolites by those enzymes has been shown in vitro and in cell lines to amplify ROS and oxidative damage to DNA bases to form the highly mutagenic lesion 8-oxo-dGuo and render damaged and carcinogenic DNA (Quinn et al., 2008).

b. Phase II Reactions: It involves conjugation of metabolites from phase I with small molecules catalysed by specific enzymes such as sulfotransferases (SULTs), UDP-glucuronyl transferases (UGTs) or glutathione s-transferases (GSTs). SULTs have been shown to activate some metabolites of PAH such as 7,12-dimethylbenz[a]anthracene and its methyl-hydroxylated derivatives, in different tissues (Chou et al., 1998). Polymorphism of SULT1A1 have been associated with PAH-DNA adduct levels (Tang et al., 2003). Like sulfation, glucuronidation produces polar conjugates that are readily excreted. Oxygenated benzo[al]pyrene derivatives are common substrates of UDP-glucuronyl transferases (Bansal et al., 1981), the resulting metabolite, 1-hydroxyurene glucuronide, and the parenteral 1-hydroxyurene glucuronide are used as a biomarker of PAH exposure (Strickland et al., 1994). The importance of GSTs involvement in conjugation of PAH derivatives has been demonstrated in vitro using corresponding diol epoxides of dibenzo[a,l]pyrene and benzo[a]pyrene as substrates for these enzymes (Sundnerg et al., 1998, 2001, 2002).

5. Mechanism of Toxicity
Metabolic activation of PAHs leads to formation of diol-epoxides which binds covalently to DNA. Thereafter, they form adducts (PAH-DNA adduct) or induce oxidative stress which results in mutations. Adduct formation may affect DNA repair mechanism.
which further causes carcinogenesis due to accumulation of mutations in DNA. Previous studies indicate that the degree of PAH exposure is related with number of adducts formed. However, other factors involve genetic profile as well as concentration of PAHs as well as life stage of organism exposed to PAHs (Bologninessi et al., 1991).

5.1 Adduct Formation
Metabolism of PAHs generates reactive diol epoxide enantiomers. These enantiomers forms DNA adduct with different structures, motifs and biological activities. DNA adduct of diverse conformations are excised by DNA repair enzymes at different rates, PAH diol epoxides (PAHDEs) bind covalently to exocyclic amino groups of guanine and adenine, forming stable adducts within DNA (Lin et al., 2001). Geacintov and colleagues (1997) have described several structural motifs by nuclear magnetic resonance analysis. These structure types are divided into (a) minor groove, when the PAH is partially accessible to solvent, (b) classical intercalation, when PAH is protected from environment and forms a “sandwich structure” and (c) base-displaced intercalation, when PAHs substitute the healthy base (Buterin et al., 2000; Geacintov et al., 1997). Molecular studies have revealed that, adducts in DNA block polymerase replication activity, contributing to increase DNA damage by reducing repair activity (Hsu et al., 2005).

5.2 Oxidative Stress
Reports have suggested the capacity of Benzo[a]pyrene derivatives to enter redox cycles and induce production of reactive oxygen species (ROS), thereby causing oxidative stress (An et al., 2011). 6-OH-BaP is the precursors for BaP radical cations. Auto-oxidation may result in formation of BaP quinines such as 6,12,1,6- and 3,6-BaP diione (Briede, et al., 2004). These metabolites undergo redox-cycling to their corresponding BaP diols and produce superoxide ROS which are then converted to hydroxyl radicals by Haber-Weiss reaction. The OGG1 genes codes for a DNA glycosylase involved in base excision repair of 8-oxo-dG that arises from ROS species. When this system fails, there is an increase in mutation rate (Bonnet et al., 2005).

Balance between generation of ROS species and scavenging of these molecules is fundamental in repairing DNA damage. More DNA damage will result, if rate of ROS generation is greater than their removal. PAHs may absorb light energy in UV region (200-800 nm) and may induce DNA damage by production of ROS for e.g Chrysene induces apoptosis and DNA damage in human keratinocytes by generating ROS in response to UV radiation (Ali et al., 2011).

6. Biological Effects

6.1 Short Term Health Effects
The acute effects of PAHs on human health mainly depends on the extent of exposure (e.g., length of time), the concentration of PAHs during exposure, the toxicity of the PAHs and the route of exposure e.g., via inhalation, ingestion, or skin contact. Other factors like age and pre-existing health conditions also affect health. Occupational exposure to high levels of pollutant mixtures containing PAHs are known to result in symptoms such as eye irritation, nausea, vomiting, diarrhea etc. Anthracene, benzo(a)pyrene and naphthalene are direct skin irritants while anthracene and benzo(a)pyrene are known to be skin sensitizers, i.e. as cause of an allergic skin response in animals and humans (IPCS, 2010).

6.2 Chronic or Long Term Health Effects
Labourers and workers who often gets exposed to mixtures of PAHs are more vulnerable to a series of health problems such as increased risk of skin, lung, bladder and gastrointestinal cancers (Bach et al., 2003). Exposure to PAHs may also result in cataracts, kidney and liver damage and jaundice (ATSDR, 1995). Repeated dermal exposure to naphthalene may result in redness and inflammation of the skin. Breathing or swallowing large amounts may result in break down of red blood cells (Srogi, 2007). Moreover, as PAHs have the potential to interfere with hormonal systems, they can exert harmful effects on reproduction and immune function. Long term exposure to PAHs is suspected to raise the risks of cell damage via gene mutation and cardiopulmonary mortality (Kuo et al., 2003).

6.3 Carcinogenicity
Reactive metabolites (e.g., epoxides and dihydrodiols) of some PAHs have become one of the major health concerns because of their potential to bind to cellular proteins and DNA with toxic effects, despite the presence of some unmetabolized PAHs (Armstrong et al., 2004). The resulting biochemical disruption and cell damage can lead to mutations, tumours and cancer (Bach et al., 2003). Siddens et al. (2012) showed exposure of PAHs resulting primarily in Papilomas which is followed by Squamous cell carcinoma as well as carcinoma in situ. According to the U.S Environmental Protection Agency (USEPA, 2008), seven PAHs have been classified as poten carcinogens: benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene,
dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene. Association between human exposure and carcinogenesis have been acquire from occupational exposure of workers to PAHs e.g., during coke production, roofing using bituminous products, oil refining and coal gasification (Bach et al., 2003; Zhang and Tao, 2009). Earlier, chimney sweeps and workers using tar were dermally exposed to large amount of PAHs. Increased incidence of lung cancer was also shown in epidemiological studies of gas workers (Armstrong et al., 2004). Correlations exists between increase in lung cancer cases with the duration of time exposed to top of ovens, where the average BaP concentration was about 30 mg m\(^{-3}\) (Moolgavkar et al., 1998). Epidemiological studies have also suggested small increased risk of cancer in workers exposed to diesel exhaust (Boers et al., 2005; Clapp et al., 2008). However, these working environments may include other potent pollutants than PAHs which may be carcinogenic. Animal studies showed effects of certain PAHs on haematopoietic and immune systems producing reprotoxic, neurologic and developmental effects (Jong et al., 1999; Latif et al., 2010).

### 6.4 Immunotoxicity

PAHs are also immunosuppressant as well as immunotoxic. Various hypotheses were elucidated to understand the mechanistic approach behind immunotoxicity caused by PAHs. Exposure to PAHs results in decreasing immune response towards various antigens. Two possible approaches involves either by reaction of Ah receptor with PAHs or by modifying the intra cellular calcium concentration among immune cells. As a result, there is significant alteration between antigen and mitogen receptor signaling pathways, further leading to proliferation and/or death (apoptosis) of immune cells (SCF, 2002). However, impacts of PAHs on both specific and non-specific immunity are contradictory which depends on dose, mode of exposure as well as species studied. Among innate and acquired immunity, macrophages as well as lymphocytes appear to be sensitive towards exposure to polycyclic aromatic hydrocarbons (Reyna et al., 2006). Several immunotoxic effects reported during exposure to benzo(a)pyrene in rats and these effects involves decreased B cells in spleen, alterations in lymph nodes weight and thymus, altered white and red blood cells (De Jong et al., 1999). Oostingh et al. (2011) also reported immunological responses on human cell lines exposed to PAHs.

### 6.5 Developmental Toxicity

Studies on mice have demonstrated ingestion of high levels of benzo(a)pyrene during pregnancy which result in birth defects and decreased body weight in the offspring. It was concluded that synergistic effect of lead and Benzo(a)pyrene results in impairment of fertility (Kristensen et al., 1995). Another study showed gradual Dose – response relationship between PAHs and Neural tube defects. About 4.52 times fold PAHs concentration were associated with Neural tube defects and 3.71 times fold PAHs concentration was associated with occurrence of Spina bifida and Anencephaly (Ren et al., 2011). Langlois et al. (2013) showed risk of Oral Cleft-affected pregnancies with PAHs exposure. PAHs may act directly on the embryo to negatively affect its growth, however, PAH effects on fetal growth could differ depending on the timing of exposure (Dejmek et al., 2000) and exposure to BaP may pose serious risks to foetal development since the conversion of BaP to carcinogenic metabolites can be mediated by placental and foetal enzymes. US’s Centre for Children’s Environmental Health (CCEH) conducted various studies which conclude that PAH exposure during pregnancy may lead to adverse birth outcomes including premature delivery, low birth weight and delayed child development (Perera et al., 2005). Prenatal exposure to PAHs is also associated with low IQ at ages 6 to 8 and childhood asthma (Edwards et al., 2010; Perera and Herbstman, 2011). PAH exposure has also been associated with developmental impairments, including reduced birth length and head circumference in children of women exposed to PAHs during pregnancy (Perera et al., 1998; Choi et al., 2006; Polańska et al., 2010). Earlier a study on mice fed high levels of benzo[a]pyrene during pregnancy reported difficulty in reproduction in not only experimental mice but also in their offspring. The offspring of pregnant mice fed benzo[a]pyrene also showed other harmful effects, such as birth defects and decreased body weight (ATSDR, 1995). Moreover, Singh et al. (2008) observed higher placental PAHs levels in preterm delivery cases and hypothesized possible association between PAH and preterm delivery in women of Lucknow. Due to lipophilic nature of PAH, penetration among cellular membranes (including placenta) is high. Enzymatic activity during PAH metabolism may result in formation of covalent bonds among reactive intermediates and DNA (Wells et al., 2010). Langlois et al. (2013) demonstrated an association among maternal periconceptional occupational exposure to PAHs with risk of oral clefts in offsprings. According to experimental model systems, congenital heart disease may result due to exposure from PAHs. In china, newborns with neural tube defects or PAH levels in placenta of 80 fetuses was also investigated by Ren et al. (2011). Reports concluded 4-5 times greater risk of defect when lipids contains above average (597 ng/g) levels of PAHs. In recent, Jayasundara et al. (2015) reported that exposure...
to PAH mixture during embryo development of zebrafish consisting of an AHR agonist (benzo(a)pyrene-BaP) with fluoranthene (FL) may affect cellular Ca(2+) levels and subsequently cardiac muscle function, potentially underlying Benzo(a)pyrene (BaP) with fluoranthene (FL) cardiac development toxicity.

6.6 Genotoxicity

Genotoxicity plays a vital role in the carcinogenicity and developmental toxicities. Among iron foundry workers, previous studies correlated elevated levels of mutation at HPRT locus in lymphocytes with levels of DNA adducts (IPCS, 1998). DNA adducts of BaP (mainly the diol epoxide) have been identified in lymphocytes at various workers occupationally exposed to various PAHs. Previous studies demonstrated use of standard tube assay of SOS and chromotest to determine genotoxicity of 32 PAHs in Escherichia coli PQ37 (Mersch-Sundermann et al., 1992; Nylund et al., 1992). Increased genotoxicity on incubation with exogenous metabolic activation mixture was also shown by various PAHs such as benzo(g,h,i)fluoranthene, benzo(j)fluoranthene, benzo(a)pyrene, chrysene, dibenz(a,1)pyrene, fluoranthene and triphenylene (White, 2002). Chromosomal aberrations and dominant lethal in rats through the induction of germ cell effects have been caused by benzo(a)pyrene, benzo(a)anthracene and chrysene (Jung et al., 2013). However, this data could not correlate quantitative genetic risk on exposure to PAHs. Evidence of genotoxicity is limited for other PAH compounds such as anthracene, benzo(ghi)fluoranthene, benzo(c)phenanthrene, 1-methyl phenanthrene, perylene and triphenylene. Martins et al. (2015) reported that low-moderate concentrations of sediment-bound mixed PAHs on peripheral blood cells of the European sea bass may significantly increase the hazard of mutagenesis even when the individual concentrations indicate low risk, especially considering that chromosome-level damage is unlikely to be repaired, leading to the fixation of DNA lesions upon prolonged exposures.

6.7 Intrauterine Growth Restriction (IUGR)

IUGR may be termed as inability of fetus to gain his/her intrinsic growth potential due to various anatomical or functional disorders and disease in maternal-placenta-fetal unit. IUGR can be classified into symmetric and asymmetric. IUGR is referred to as (a) symmetric- if length, head circumference and weight is low, which usually results from process originating early in pregnancies and (b) asymmetric, when sparing of brain takes place as well as head's circumference is within normal limits, which may results due to process occurring during gestation period. Nutritional deficiency and utero-placental function are usually related with asymmetric IUGR. Fetal growth remains normal until growth rate exceeds substrate provision, usually during 3rd trimester. Muscle growth, glycogen levels and body fat also gets limited with a slight decrease in energy substrate levels. Growth of bones and therefore fetal length are affected less whereas cardiac output’s redistribution results in preferential substrate delivery to the brain (Rosenberg et al., 2008; Lapillonne et al., 1997). Therefore, adaptation to unfavourable intrauterine environment represents asymmetric IUGR, which results in long term and pre-natal complications.

Thrifty phenotype hypothesis proposed a relation between poor fetal growth and development of type-2 diabetes metabolic syndrome, which results from poor intrauterine nutrition, producing permanent changes in glucose-insulin metabolism. This change involves reduced insulin resistance and secretion. Epidemiological studies revealed a strong association among IUGR and late metabolic syndrome involving dyslipidemia, coronary heart disease, arterial hypertension, impaired glucose tolerance, visceral obesity and various other diseases like osteoporosis (Rhind et al., 2001). Theory of ‘fetal origins of adult disease’, results from fetal programming. According to this theory, abnormalities in fetal nutrition may lead to developmental adaptation which changes physiology and metabolism of offspring permanently. This predisposes individuals to endocrine, metabolic and cardiovascular disease (Hales et al., 1992; Barker et al., 1998). Fetus utilizes reduced nutrient supply in order to adapt to adverse intrauterine environment to ensure survival. As a result, growth is controlled by redistribution of blood flow and changes occurring during production of placental and fetal hormones (Barker et al., 2002).

PAH exposures due to environmental air pollution are associated with reduced fetal growth and various other morbidities in human pregnancy (Perera et al., 1999; Chattingius, 2004). Low birth weight (LBW) leads to an impaired growth of the infant with its attendant risks of a higher mortality rate, increased morbidity (Ashworth, 1998), impaired mental development (Grantham- McGregor, 1998), and the risk of chronic adult disease (Barker, 1998). In developing countries, including India, the majority of LBW infants because of intrauterine growth restriction (IUGR) are born small at term (>37 wk of gestation) with only 6.7 per cent born prematurely. The prevalence of LBW is a major public health issue in India (30%) and is the highest among South-Asian countries (Chakraborty and Anderson, 2011). Cellular and molecular mechanisms are not clear but it may
involves reprogramming of hypothalamic-pituitary-adrenal axis and insulin-signalling pathways. IUGR childrens and adults have been observed with abnormalities of circulating concentration of insulin, cortisol, growth hormone, catecholamines and insulin-like growth factors (IGFs) (Fowden et al., 2004; Phillips, 2001).

Exposure to PAHs during pregnancy due to place of residence, road traffic, dietary and cooking habits may cause IUGR with delivery of LBW babies and increase a woman’s risk of giving birth to children with congenital anomalies, such as limb defects, nervous system, musculoskeletal or cardiovascular defects, oral clefts etc. The adverse reproductive effects that are non-fatal produce future risks for the individual and for the next generation. Therefore, studies has to be conducted to examine association between IUGR and PAH exposure. Human exposure to BaP is almost ubiquitous and the effect of pollution on 200 normal pregnancies detected BaP in all but one placenta, in amounts up to 6.15 ng/g dry weight (Gladen et al., 2000). During pregnancy, BaP metabolites (mBaP) are capable of forming DNA adducts, causing oxidative damage, or disrupting normal blastocyst development (Miller and Ramos, 2001). BaP and mBaP in the maternal bloodstream may pass to the foetal circulation and expose the developing foetus to PAH-related hazards. Increased risks to foetal development would be expected not only from the transplacental transfer of maternal mBaP, but also from placental and foetal metabolism of BaP. Autrup et al. (1995) observed BaP-DNA adducts in the umbilical cord blood of 21 smoking and 30 non-smoking women, and they reported a positive association between the adduct level in maternal and umbilical cord blood.

7. Biomarkers of Exposure

Various biomarkers have been identified to assess PAHs level in the body. Many methods have been developed to monitor internal levels of PAHs after exposure from workplaces and environment. Most widely used biomarker of PAH exposure in urine is 1-Hydroxypyrene, a metabolite of pyrene. Moreover, pyrene also holds major concentration among mixtures of all PAHs (2-10% of total PAH load). Pyrene content of total PAH is found to be constant in certain environment (Jongeneel, 2001; McClean et al., 2004, 2012; Sobus et al., 2009). Studies have been performed for comparing dietary effects of low and high PAH content meals on 5 male volunteers. Studies concluded increased 100-250 fold dietary benzo(a)pyrene dose which induces 4-12 fold increase in urinary 1-OHP elimination (Buckley and Ljoy, 1992). Likewise, 10-80 fold increase in 1-OHP elimination in urine was also observed in 10 volunteers eating charbroiled beef for 5 days. This increased levels returns back to ground level within 24-72 hours (Kang et al., 1995). Intake of pyrene from diet of normal foods (9 nmol/day) was compared from cigarette smokers (12 nmol/day) (Duarte-salles et al; 2010). Non-smokers are found to have half the level of 1-OHP as compared to tobacco smokers, non exposed to PAH (Hecht, 2002; Srogi, 2007; Van Rooji et al., 1994). As the relative content of pyrene and benzo(a)pyrene may vary considerably, 1-OHP cannot be always used in order to predict extent of exposure to BaP and other carcinogenic PAHs. Another study conducted on concentrations of urinary hydroxylated PAHs metabolites in Asian countries found vital concentration of 6750 pg/ml of OH-PAHs in India (Guo et al., 2013).

Various indicators such as DNA-BaP adduct in present in lymphocytes and tissues with numerous proteins (such as albumin) have been used to indicate dose of reactive metabolites in body. Initiation of cancer is supposed to occur via electrophilic binding of PAH-DNA. Therefore, DNA adduct measurement can be correlated with PAH exposure and dose of ultimate reactive metabolites (Perera et al., 2011). Studies suggested presence of various DNA and reactive metabolites in humans exposed to smoking or polluted areas (Perera et al., 2005).

8. Precautions

Although there is high risk of exposure to PAHs, there are some underline precautions which can reduce the risk of exposure to some extent. Regular pollution check up of vehicles, proper installation of chimneys and exhaust fans at homes, avoiding/quitting cigarettes, chargrilled, barbequed foods, charbroiled or charred portions should be avoided. Proper ventilation of buildings is some safety measure which can reduce PAHs exposure in our day-to-day life.

9. Biological Specimens for PAH Analysis

All organisms involving human’s gets easily exposed to carcinogenic PAHs. Since PAHs are highly lipophilic, they get easily stored in fatty tissues of organisms. Presence of PAHs can easily be determined in various biological specimens for quantitative and qualitative estimation of PAHs in humans. A specimen which confers presence of PAHs is blood, nails, placenta, meconium, teeth, urine, skin, amniotic fluid, amniotic fluid, breast milk and adipose tissue. Furthermore, liver, bile, heart, brain specimens are also used for estimating PAHs levels in an organism. However, latter part is not easily accessible and sample collection is also more laborious as compared to former part.

Blood is used as most important tool for estimating PAHs level in body. After metabolism,
PAHs becomes highly polar and gets easily bound to blood due to polarity and hydrophilicity. PAHs gets easily assimilated and transported throughout the whole body. Skin contains lipophilic nature and contains deposits of fats. Hairs are also used for determining presence of organic pollutants. Cotinine is analysed for the presence of organic pollutant using hairs of chain smokers. After metabolism, PAHs gets converted to polar excretable metabolites and gets excreted via urine. Therefore, urine samples can be used for detecting presence of PAHs. 1-hydroxypyrene, a metabolite of pyrene is mostly found in urine samples. Urine samples can easily be collected and stored. Placenta acts as a medium between maternal and foetus for providing nutrition, blood supply and oxygen. PAHs can be transported to foetus easily via blood and oxygen. Yuan et al. (2013) found 10-fold elevated risk of neural tube defects in high placental PAH levels. Placenta, umbilical cord and amniotic fluid contain fats deposits which enhances deposition of PAHs. Expelled air, nails, breast milk, teeth and saliva are other specimens which evidences presence of PAHs. Nails are dead tissue and hence can be collected easily. Expelled air can be used as an index of PAHs concentration entering human body through inhalation route.

10. Standards and Regulations

PAHs are strongest carcinogenic which reacts with other environmental compounds. Vehicular emissions are globally contributing to PAHs. Marked reduction in PAHs level has been shown by catalytic converters for gasoline engines. Few studies indicate that reduction levels of PAHs were upto 80-90% for BaP (Rogge et al., 1993; Paturel, 1996; Schauer, 2002). Turbo-charging and inter-cooling systems regulate diesel particulate emission in diesel engines. Moreover, trap oxidizers and trap filters has also been used in order to regulate PAHs emission. Aromaticity also affects levels of PAHs in exhaust gases. Fuels without 2-3 benzene rings shows significant low PAHs emission as compared to fuels with 2-membered (7-11%) and 3-membered (1-3%) benzene rings. Reformulating diesel fuel by reducing sulphur content, aromatic rings or adding oxygen species shows significant regulation in PAHs emission. Potential substituents for diesel engines are Dimethyl ether (DME) and oxygen moieties. Due to uncertainty in emission factors, it is quite difficult to control and quantify PAHs emission from agricultural sources. Furthermore, industries are becoming aware of outcomes of PAHs emission. Indeed, improved energy management can lead to improved combustion, which inturn leads to lower emissions. Domestic sources of PAHs emission are highly unregulated. However, they can be regulated by switching on to modern gas and oil burners, hot water systems, solid fuel systems (wood, coal and peet) as well as catalytic devices.

Standards have been established by US agencies which are relevant in order to reduce risk of PAH exposure at workplace and environment. Lowest detectable concentration should be set as a limit for exposure to PAHs at workplace like recommended exposure limit for exposure to coal tar pitch volatile agents is 0.1 mg/m³ for 10 hours workday or 40 hour work week (NIOSH, 2010). In Europe, BaP is the main chemical which is targeted for regulation. BaP is also used as marker compound which is equivalent to total PAH load. The unit risk of lung cancer for lifetime exposure of BaP is $87 \times 10^{-6}$ ng/m³. Guidelines values for BaP exposure have been set up between 0.1 and 1.3 ng/m³ by various countries (WHO, 2003). PAHs have been short listed by many countries as hazardous air pollutants. PAHs with BaP indicator species has been classified as B-2 pollutant, which refers to a probable human carcinogen. Limits of 0.2 mg/m³ has been set up by Occupational Safety and Health Administration (OSHA) (ATSDR, 1995).

11. Future Prospects

Since PAHs are ubiquitous in environment and their presence is highly carcinogenic. PAH is relatively very wider term which needs to be studied thoroughly. Annual average BaP target values have been estimated by most of the countries but there is also a need to study other probable carcinogenic PAHs in urban centres and elucidate their persistence in atmosphere. Values based reports on seasonal and annual ambient levels of PAHs should be prepared. There is insufficient data to correlate concentration related effects of PAHs on human health. Awareness programmes should be conducted by government or doctors for people especially pregnant females regarding exposure to PAHs and probabilities of toxicities. Measurement of PAHs has proven to be a difficult task due to widely varying physical and chemical properties of PAHs. Harmonisation of sampling, analysis and emission estimation methods should be properly done in order to estimate ambient air concentration.

12. Conclusions

Based on above research, environmental PAH pollutants have an adverse impact on human health and it is a matter of serious concern. Concerted efforts should be diverted to address this burning issue and should be solved by negotiations between policy makers, researchers and non-governmental agencies. Hence, it is right time that concerted multidisciplinary and multi-institutional studies should be undertaken to
monitor and establish a reference range of PAH exposure in the Indian population and fully characterize the risk of these compounds to human health. Further to access health hazards and risk of various POPs including PAH on human health including genotoxicity, mutagenicity and carcinogenicity and to determine the effects of oral exposure on pre- and post-natal development in non-rat species. Moreover, it is necessary to examine the effects of PAH exposure on reproductive development, structure and fertility in a multi-generational studies in rodents.

References


Journal of Pathology and Toxicology | Year-2014 | Volume 1 | Pages 16-30

© 2014 Jakraya Publications (P) Ltd

26


Oostingh GJ, Schmittner M, Ehart AK, Tischler U and Duschl A (2012). A high-throughput screening based on stably transformed human cells was used to determine the immunotoxic effects of fluoranthene and other PAHs. Toxicology in Vitro, 22(5): 1301-1310.


Shimada T (2006). Xenobiotic-metabolizing enzymes involved in activation and detoxification of...


