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PAHs in Size Fractionate Mainstream Cigarette Smoke, Predictive Deposition and Associated Inhalation Risk

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ABSTRACT

Size fractionated mainstream cigarette smoke (MCS) samples were collected with variable configuration cascade impactor (VCCI). Samples were extracted ultrasonically and analysis of sixteen priority polycyclic aromatic hydrocarbons (PAHs) was performed using high performance liquid chromatography (HPLC) coupled with UV-visible detector. Identification of PAHs were also carried out using gas chromatography coupled with mass spectrometry (GC-MS) technique. Data of size fractionate PAHs in MCS were used to calculate size dependent deposition in different compartment of human respiratory tract using multiple path particle dosimetry (MPPD) model. All brand cigarette smoke showed similar trends of particle mass and PAHs size distribution peaked at two sizes 0.3–0.1 μm and 0.75–1.13 μm aerodynamic diameter. All tested brands of MCS recorded around 48.75% of two and three-ring PAHs, 23 to 25% four-ring PAHs and 26–27% five and higher rings PAHs of total analyzed PAHs. Benzo[a]pyrene equivalent ($B[a]P_{eq}$) PAHs emission in MCS was found to be 110 ± 12 (μ ± 1σ) ng per cigarette for tested brands. Total deposition fraction in respiratory tract was found to be 0.69 ± 0.26 for tested size ranges. Average 5th and 95th percentile values of incremental lifetime cancer risk (ILCR) for smoker due to PAHs exposure were found to be 1.3×10^{-5} to 3.9×10^{-5} respectively for tested cigarette brands.

Keywords: Carcinogens; Liquid chromatography/Mass spectrometry; Respiratory health; ILCR; MPPD.

INTRODUCTION

The tobacco epidemic is one of the biggest public health threats the world has ever faced, killing nearly six million people a year. More than five million of those deaths are the result of active smoking, while more than 600,000 are the result of non-smokers being exposed to second-hand smoke (WHO, 2014). The United States Environmental Protection Agency (U.S. EPA) have consistently ranked indoor air pollution among the top 5 environmental risks to public health. One of the most important contributors to indoor air pollution is cigarette smoke which contains thousands of chemical compounds, many of which are known carcinogens (Brownson *et al.*, 2002). Cigarette smoke is a complex and dynamic chemical mixture containing neutral constituents, free radicals, and certain ions, as a result of pyrolysis and combustion reactions of tobacco products (Green and Rodgman, 1996; Pan *et al.*, 2013). Lung, skin and bladder cancers have been associated with polycyclic aromatic hydrocarbons (PAHs) exposures (Boffetta *et al.*, 1997). The relationship between cancer and the environment is largely conditioned by investigations involving PAH exposures (Armstrong *et al.*, 2004). Several individual PAHs such as Benzo[a]pyrene (B[a]P), chrysene, indeno[1,2,3-c,d]pyrene, and Benzo[b]Fluoranthene have produced carcinogenic, mutagenic, and genotoxic effects in animal experiments (Somers *et al.*, 2002; Farmer *et al.*, 2003; Luch, 2005).

Cigarette smoking is an established cause of a variety of cancer types and the involvement of environmental tobacco smoke (ETS) in human lung cancer is no longer a matter of dispute (Besaratinia *et al.*, 2002). Smoking is a major risk factor for endothelial cell injury and subsequent coronary artery disease (Culea *et al.*, 2005). Experimental evidence indicates that airborne PAHs exposures such as diesel exhaust particles have pro-inflammatory effects on airways of respiratory system. Epidemiology studies have also shown associations between allergic responses or asthma following exposure to ambient air pollutant mixtures containing PAHs, black smoke, and environmental tobacco smoke (Delfino, 2002). Some PAHs present in cigarette smoke can be metabolized to diol-epoxides, which are potent mammary carcinogens, such as Benzo[c] Phenanthrene. PAHs may also play a role in human hepato-carcinogenesis (Chen *et al.*, 2002; Hecht, 2002). ETS consists of mainstream smoke (MS),

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the portion that leaves the mouth end of a cigarette; sidestream smoke (SS), the portion releases in the smoldering between puffs. Several reports have examined the SS to MS ratio of smoke components including selected PAHs. Depending on cigarettes types and experimental conditions, the SS to MS ratio ranges from 2–20. Such results may be caused by the incomplete combustion due to the limited oxygen available to the cigarette's fire coal during the smoldering (Baker *et al.*, 1999; Ding *et al.*, 2005; Lu and Zhu, 2007).

The mainstream smoke produced by a cigarette is a commonly encountered aerosol of considerable physiological significance. The particle size distribution of cigarette smoke is an important factor in predicting the deposition fraction of the inhaled particles in various regions of the respiratory tract. Smoking can be simulated as a two-step process. The first step involves puffing of the cigarette and drawing smoke into the mouth where it is held for a finite period of time, as the palate generally closes off the mouth from the rest of the airways during puffing. The second step is inhalation of the smoke into the lungs. Therefore the puffing process can be decoupled from the inhalation process. Various mechanisms have been put forward to explain the deposition pattern of cigarette smoke in respiratory tract, including coagulation, hygroscopic growth, condensation and evaporation, changes in composition, or changes in inhalation behavior. The disparity which exists between experimental and predicted data from deposition models has still not been fully addressed in the literature. This may be due to incomplete incorporation of parameters in predictive models for cigarette smoke deposition in respiratory track. The three main mechanisms that affect the behavior of cigarette smoke particles in the respiratory tract are gravitational sedimentation, inertial impaction and Brownian motion (diffusion). Sedimentation and impaction are 'aerodynamic' effects that are important above about 1 μm and increase with increasing size. Aerodynamic effects are negligible for very small particles and thermodynamic effects are negligible for large particles (ICRP, 1994; McGrath *et al.*, 2009; Sahu *et al.*, 2013).

The International Commission on Radiological Protection (ICRP) has proposed a semi-empirical model for regional deposition and clearance from the human respiratory tract (ICRP, 1994).This type of models consider human respiratory tract as a series of anatomical compartments through which aerosol pass during inhalation and exhalation. Each respiratory compartment is treated as a filter in which deposition is calculated by semi-empirical equations derived from fitting experimental data as a function of particle size and flow rate (Stahlhofen *et al.*, 1986). The advantage of such semi-empirical models is that they are easy to use with less computational work. Yet, they can neither be used for deposition calculations at the airway generation level, nor for flow rates and particles sizes be-yond the limits of the experimental data sets on which the semi-empirical model is based upon (Hussain *et al.*, 2011). The Multiple Path Particle Dosimetry (MPPD) model is a higher tier exposure assessment model utilizing a computational model of human and rat's specific anatomical differences in the respiratory tract (the nasal cavity and lung airways). The MPPD allows the direct extrapolation of laboratory animal data to human exposure and is capable to estimate dose-related kinetics of inhaled material (Garcia, 2009; Schroeter, 2009). The MPPD model allows the specific determination of the dose deposited at various sites of the respiratory tract, and to calculate the dose which can be systemically up taken across the tissue surface in the lung (Steiling *et al.*, 2014). The MPPD model calculates the deposition and clearance of monodisperse and polydisperse aerosols in the respiratory tracts for particles ranging in size from ultrafine (0.01 μm) to coarse (20 μm). The models are based on single-path and multiple-path methods for tracking air flow and calculating aerosol deposition in the lung. Within each airway of respiratory tract, deposition is calculated using theoretically derived efficiencies for deposition by diffusion, sedimentation, and impaction within the airway or airway bifurcation. Filtration of aerosols by the nose and mouth is determined using empirical efficiency functions. The MPPD model also includes calculations of particle clearance in the lung following deposition (Anjilvel and Asgharian, 1995; RIVM, 2002).

Objectives of present study are (1) to quantify particle mass/PAHs size distributions for mainstream cigarette smoke; (2) PAHs ring number distribution and diagnostic ratios in mainstream cigarette smoke (MCS) (3) to evaluate the deposition fraction and PAHs distributions in various compartments of the human respiratory tract for MCS; and (4) ILCR values for PAHs exposure through first hand smoking.

METHODS

Cigarette Samples

Three popular commercial cigarette brands in India were selected for present study. The tar content of the cigarettes used in this study was in the range of 11 to 17 mg per cigarette. The tar deliveries are pack tar values which are generated using ISO puffing conditions. In order to avoid marketing use of our results the trade-marks of the cigarettes have been omitted. All of them belong to well-known and appreciated brands. Cigarettes used for these experiments were conditioned in a humidified chamber at 65% relative humidity (RH) at room temperature (22°C) for at least 24 h prior to smoking. The cigarettes were smoked under the ambient laboratory conditions (45% RH, 24°C). Mainstream smoke generated under a standard smoking protocol (60-s puff interval, 2-s puff duration, and 35-mL puff volume) was collected on VCCI. All the cigarettes were fitted with filter tips approximately 20 mm in length. The cigarettes were smoked to a butt length of 23 mm or to the length of the filter overwrap plus 3 mm. For measurable quantity of MCS mass and subsequent analysis of PAHs ten cigarettes were smoked in VCCI in single experiment. Eight number of replicate of each brands cigarettes were smoked to obtain the average smoke particulate level for each of the 16 PAHs listed below in size fractionate cigarette smoke.

Experimental Setup

The experimental setup for collection of size segregated mainstream smoke is described in Fig. 1. The mainstream cigarette smoke was passed through a dilution chamber

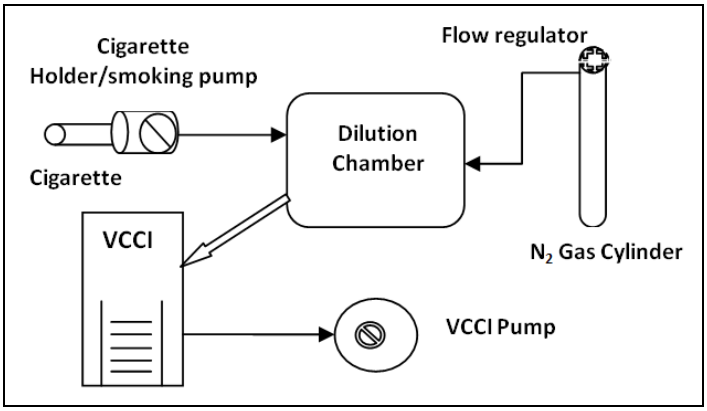

Fig. 1. Experimental set for collecting mainstream cigarette smoke (MCS) using variable configuration cascade impactor.

prior to being sampled by the variable configuration cascade impactor (VCCI). Particulate free nitrogen was used as dilution media and it did not contribute any particulate signature in VCCI. The setup was checked for leaks and the mass flow meter was calibrated using master meter (flow meter) with high accuracy. All flows were controlled with mass flow controllers (Model 2179A; MKS, Andover, MA) with accuracies > 1% of their operating flow rate. The sampling flow rate for smoke was kept 1.1 L min^{-1} so that we can collect realistic sample. As cigarette smoke is hygroscopic in nature so dilution is applied as aerosol drying technique. The dilution chamber was flushed with particulate free air (using HEPA filters) between the measurements to ensure that no particulates were present in the dilution chamber before the subsequent sample was taken. A dilution factor of 14.3 was maintained in chamber during sampling, which was very similar to puff volume (35 mL) and average lung tidal volume (500 mL).

Variable Configuration Cascade Impactor

Particle mass size distribution of mainstream cigarette smoke was evaluated on variable configuration cascade impactor (VCCI) (Singh *et al.*, 2010). Glass fiber paper discs (EPM2000, Whatman) were used as an impaction surface for particles as well as a backup filter in the cascade impactor. Glass fibre filters baked in high temperature in furnace more than 450 °C to remove any PAHs. Glass fiber paper was numbered, and again dried in an oven at 100 °C for 2 h, kept in a desiccator for 24 h and weighed prior to sampling. Air was drawn through the impactor at a flow rate of 10 L min^{-1}. The size ranges (μm) collected from the different stages of cascade impactor were as > 21.3, 21.3–15.1, 15.1–11.2, 11.2–7.38, 7.38–5.47, 5.47–2.23, 2.23–1.13, 1.13–0.75, 0.75–0.50, 0.50–0.30, 0.30–0.10 and 0.10. The pressure at stage seven was continuously measure through the course of sampling so the flow rate and stage cut off can be monitored.

Extraction and Sample Preparation

After collecting the size fractionated mainstream cigarette smoke, loaded glass fiber filter paper was chemically extracted for their polycyclic aromatic hydrocarbon content. All chemicals used in extraction and cleanup step were HPLC grade. Amber glass wares were used during extraction process to prevent photo degradation of polycyclic aromatic hydrocarbon. The loaded glass fiber filter paper was chopped in small pieces and kept in a 100 mL conical flask with 40 mL n-hexane. The same was exposed to ultra sonication for a period of 1 h. The extracts then filtered through Whatman 542 filter paper. The filtrates were evaporated under a gentle stream of nitrogen to reduce the volume to 1 mL. Then for further cleanup the samples were passed through a silica column of 10 cm × 2 cm size with 50 mL of n-hexane and dichloromethane at ratio of 1:1 (v/v). The eluents were further evaporated to near dryness under a gentle stream of nitrogen.

Analysis of PAHs Using High Performance Liquid Chromatography (HPLC)

The identification and quantification of PAHs in size fractionated cigarette smoke were performed on a high performance liquid chromatography (HPLC) system (Shimadzu LC-10 AD) with UV-visible detector. The detector wavelength set at 254 nm, which shows maximum absorbance response to the polycyclic aromatic hydrocarbons (Escriv *et al.*, 1991). The output of the detector was processed using a chromatography workstation (Jasco–Borwin). The analysis is carried out reverse phase and isocratic mode (Acetonitrile: H_2O, 85:15), C-18 column (5 μm totally porous Octa decyl silane packing, Merck Germany), 250 mm × 4.6 mm i. d. with a C-18 guard column. Synthetic standard of PAHs was purchased from Supelco, Belle-fonte, USA. The method was optimized using synthetic standards. Peaks were also confirmed using gas chromatography coupled with mass spectrometry (Shimadzu GCMS-QP2010 Ultra) technique.

Respiratory Tract Deposition Model

Deposition of MCS particle in human respiratory airways is a complex process involving multiple mechanisms, such as impaction, sedimentation, diffusion, hygroscopic growth, coagulation, as well as possible cloud motion and charge effect (Zhang *et al.*, 2012a).The Multiple-Path Particle Dosimetry (MPPD V2.11) model developed jointly by the Hamner Institutes for Health Sciences and the Dutch National Institute for Public Health and the Environment has been used for prediction of aerosol deposition fractions

in different compartments of the human respiratory tract. During smoking, the cigarette puff is mixed with a volume of ambient air equal to the average lung tidal volume (500 mL) that delivers the smoke to the lungs (Ingebrethsen *et al.*, 2011). This volume mixes with and dilutes the puff smoke as it travels into the respiratory tract. There may be no, complete, or partial mixing of the puff with the dilution air. If x denotes the fraction of the puff volume that mixes with the dilution air, the deposition fraction of the inhaled puff (DF) is found by tracking the MCS particles in the puff and dilution volumes and calculating their losses.

$$DF = DF_D + (1 - x)\frac{V_t}{V_p}(DF_t - DF_D) \quad (1)$$

where, V_P and V_t are the puff and tidal volumes, respectively, DF_t is the deposition fraction when the dilution air and smoke from the cigarette puff are completely mixed, and DF_D is deposition fraction when the dilution air is filled with smoke particles but the puff volume is void of any particles (i.e., no mixing). The existing version of MPPD allows particle loss calculations for DF_t and DF_D by invoking oral only and oral plus tracheal breathing route options respectively. The modified version of MPPD automatically calculated these deposition fractions to find the deposition fraction of fully mixed cigarette smoke in the lung. MCS contains a significant amount of semi volatile components that are distributed between the particulate and vapor phases (Kane *et al.*, 2010; Zhang *et al.*, 2012b). As the smoke puff mixes with air during smoking, the vapor phase becomes unsaturated, causing evaporation from the particle to establish the particle-vapor equilibrium. MCS also contains many hygroscopic components which may cause inhaled smoke particles to grow in size when they enter the humid environment of the respiratory tract. This growth may directly impact the site of deposition in the respiratory tract and the overall deposition in the airways. The model for hygroscopic growth was used in MPPD to include the effect of hygroscopic growth on deposition (Asgharian, 2014).

Inhalation Exposure

The carcinogenic risk of a PAHs mixture is often expressed by its BaP equivalent concentration (B[a]Peq). The B[a]Peq of MCS PAHs (BEC) was calculated according to Eq. (2):

$$BEC = \sum_{i=1}^{n} Ci \times TEFi \quad (2)$$

where Ci = concentration of PAH congener i (ng cigarette^{-1}); TEFi = the toxicity equivalency factor (TEF) of PAH congener i. The carcinogenic potencies of 16 PAHs were estimated as the sum of each individual B[a]Peq. Daily intake (I) of PAHs (mg day^{-1}) through inhalation of $B[a]P_{eq.}$ was calculating using following Eq. (3).

$$I = BEC \times N \quad (3)$$

N is average of cigarette smoked. BEC was calculated for each size bin of measured MCS after multiplication of deposition fraction for size been as calculated using MPPD model.

Health Risk Assessment

Toxicity equivalent (TEQ) method was used to assess the inhalation risk for MCS exposure. The total BaP equivalent concentration (BaPeq) was calculated by the sum of BaP_{eq} for each PAH using toxicity equivalent factors (Ohura *et al.*, 2004). Monte Carlo Analysis (MCA) is the most widely used probabilistic method of risk assessment. The MCA technique treats any uncertain parameter as random variable that obeys a given probability distribution. This technique is widely used for probabilistic uncertainty. In MCA computer simulations are used to combine multiple probability distributions associated with the risk equation. Thus we get a probabilistic distribution of risk. Inhalation risk due to exposure PAH from MCS is calculated using following Eq. (4).

$$Risk = \frac{1 \times CSF \times EF \times ED}{BW \times AT} \quad (4)$$

I is daily intake of PAHs (mg day^{-1}) through inhalation of $B[a]P_{eq}$, CSF is cancer slop factor for B[a]P in (mg kg^{-1} day^{-1})$^{-1}$, EF exposure frequency (day year^{-1}), ED exposure duration (year), BW is average body weight (kg), and AT is averaging time (day) (Chen and Liao, 2006; Xia *et al.*, 2012). Assumptions here made are 1) 20 cigarettes are being smoked by an adult daily; 2) The number of years of smoking as 30 years and average lifetime as 70 years. 3) Cancer slop factor (CSF) for B[a]P is taken as log normally distributed with a geometrical mean 3.14 (mg kg^{-1} day^{-1})$^{-1}$ and a geometrical standard deviation 1.80; 4) Adult body weight LN(59.78, 1.07) kg (Collins *et al.*, 1991; Behera *et al.*, 2014).

Quality Control and Quality Assurance

Several dilutions corresponding to 0.2–20 ng absolute of synthetic standard mixture of Naphthalene (NAPH), Acenaphthylene (ACY), Acenaphthene (ACP), Fluorene (FLR), Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLT), Perylene (PERY), Benzo (a) Pyrene (BaP), Indeno(1,2,3-cd) Pyrene (IcP), Benzo (ghi) Perylene (BgP), Pyrene (PYR), Chrysene (CHR), Benzo(a) Anthracene (BaA), Benzo (b) Fluoranthene (BbF), Benzo (k) Fluoranthene (BkF) (Supelco, Belle-fonte, USA) dissolved in HPLC grade acetonitrile was used for determining the retention data and for studying the linearity of the detector. The recovery efficiencies were determined by spiking filter paper samples with PAH standard mixture. The mean recovery of PAHs varied from 82.6 ± 1.96% to 93.4 ± 1.17%, recovery values for each PAHs are represented in Table 1. The efficacy of the extraction process has been evaluated with NIST SRM-1649 urban dust sample, and the results obtained were comparable with the certified values (Table 1). For the data analysis, Student's t-test was applied to determine the statistical significance ($P < 0.05$,

Table 1. Recovery (mean ± 1SD)% of PAHs, analysis results of NIST SRM-1649 urban dust sample.

PAHs	Recovery (%), Mean ± SD	Certified value (μg kg^{-1})	Measured value (μg kg^{-1})
NAPTH	85.5 ± 1.23	-	-
ACY	87.9 ± 1.74	-	-
ACE	88.6 ± 0.74	-	-
FLUO	83.7 ± 1.32	-	-
PHEN	87.4 ± 0.72	4.14 ± 0.37	4.69 ± 0.42
ANTH	90.2 ± 0.56	0.43 ± 0.08	0.45 ± 0.07
FLT	83.7 ± 0.88	6.45 ± 0.18	5.89 ± 0.20
PYR	82.6 ± 1.96	5.29 ± 0.25	4.79 ± 0.32
BAA	89.3 ± 0.85	2.21 ± 0.07	1.94 ± 0.69
CHRY	91.3 ± 1.20	-	-
BBF	84.5 ± 1.05	6.45 ± 0.64	5.93 ± 0.46
PERY	85.2 ± 0.78	0.65 ± 0.07	0.72 ± 0.09
BKF	84.5 ± 1.98	1.91 ± 0.03	2.17 ± 0.39
BAP	92.5 ± 0.74	2.51 ± 0.09	2.84 ± 0.62
BGHIP	88.6 ± 1.75	4.01 ± 0.91	3.88 ± 0.64
INDO	93.4 ± 1.17	3.08 ± 0.63	3.88 ± 0.64

two tailed) of the differences between the means determined for both sites (Tiwari *et al.*, 2013).

RESULTS AND DISCUSSION

Particle Mass Size Distribution

The particle mass size distributions of three popular brands cigarette smoke is depicted in Fig. 2(a), which shows most of cigarette smoke particle are emitted in sub-micron particle size. All three brands cigarette smoke show similar trends of particle mass size distribution peaked at two (multimode) sizes 0.3 and 0.75 μm aerodynamic diameters. Total particle mass for brand 1, brand 2 and brand 3 cigarettes is 26.3, 57.8 and 34.58 mg per cigarette respectively collected in VCCI. Total particle mass concentration of MCS found to be two to three fold higher compare to tar content; which is possible due to hygroscopic nature of tar. The average mass percentage of particles having aerodynamic diameter < 11.2 μm (inhalable size) of the total cigarette smoke was found to be around 98% for all three brand, which indicates that a major part of cigarette smoke is capable of getting into respiratory track. The mass percentage of fine particle (< 2.23 μm aerodynamic diameter), which is capable of deep pulmonary infiltration and alveolar deposition, was found to be 91 to 95% to the total. The mass percentage of coarse particles in cigarette smoke was less than 5% of total particle mass, and this fraction was not considered for health risk assessment. The contribution of ultrafine particle i.e., particle having aerodynamic diameter < 0.1 μm (also referred to as submicron mode, ultrafine mode, vaporization mode, condensation mode, etc.) to the total particle mass emitted as a smoke was 1.45% for all three brands.

Size Fractionated PAHs in Mainstream Cigarette Smoke

Total particle phase PAHs content in mainstream cigarette smoke were found to be 1.70, 2.01, and 1.41 mg per cigarette respectively for brand 1, 2 and 3. Order of average PAHs emission (ng cigarette^{-1}) was found to be PERY < ACP < CHR < BkF < IcP < BaA < BaP < ACY < ANT < BbF < NAPH < FLR < FLT < PYR < PHE < BgP. Average size fractionated PAHs concentration (ng per cigarette) in mainstream cigarette smokes is represented in Table 2. Scattered plot of MCS concentrations in different size bin and corresponding total PAHs (ng per cigarette) is shown in Fig. 2(d). Total PAHs in size bin are well match with particle mass distributions of cigarette smoke (R^2 = 0.95). The order of average total PAHs emission in different size bin was found to be as 0.1–0.3 μm > 0.75–1.13 μm > 0.3–0.5 μm > 0.5–0.75 μm > 1.13–2.23 μm > 2.23–5.47 μm > (< 0.1 μm) > 5.47–7.38 μm > 7.38–11.2 μm > 11.2–15.1 μm > 15.1–21.3 μm > (> 21.3 μm) aerodynamic diameter (Fig. 2(b)). Most of PAHs were found to be associated with fine size fraction i.e., < 2.23 μm aerodynamic diameter particles for all brands. Benzo (ghi) Perylene was found to be most abundant PAHs in particle phase for all tested brands. For tested cigarette brands average Benzo (ghi) Perylene emissions were found to be 279 ± 35 ng per cigarette. Perylene was found to have the lowest concentration in cigarettes smoke among tested priority sixteen PAHs and its average value ranges from 25 to 47 ng per cigarette for brands under investigations. Mainstream Cigarette smokes PAHs content were found to be accumulated in bimodal distributions with size bin of 0.5–0.1 μm and 0.75–1.13 μm aerodynamic diameter for tested brands (Fig. 2(b)). Fluorene, Phenanthrene, Anthracene, Fluoranthene and Pyrene were also found to associate with coarse fraction as in fine smoke particles for all tested brands. High ring number PAHs were found to be mostly in fine fraction.

PAHs ring Number Distribution and Diagnostic Ratios

Fig. 3 shows the ring number wise distribution of PAHs for all tested brands mainstream cigarette smoke. PAHs can be classified by the number of aromatic rings into PAHs of two and three rings (NAPH, ACY, ACP, FLR, PHE, ANT, FLT), four rings (PYR, BaA, CHR, BbF, BkF), five and higher rings (BaP, PERY, IcP, BgP). All tested brand of MCS recorded up to around 48.75% of two and three rings PAHs, which have low molecular weights. Middle molecular

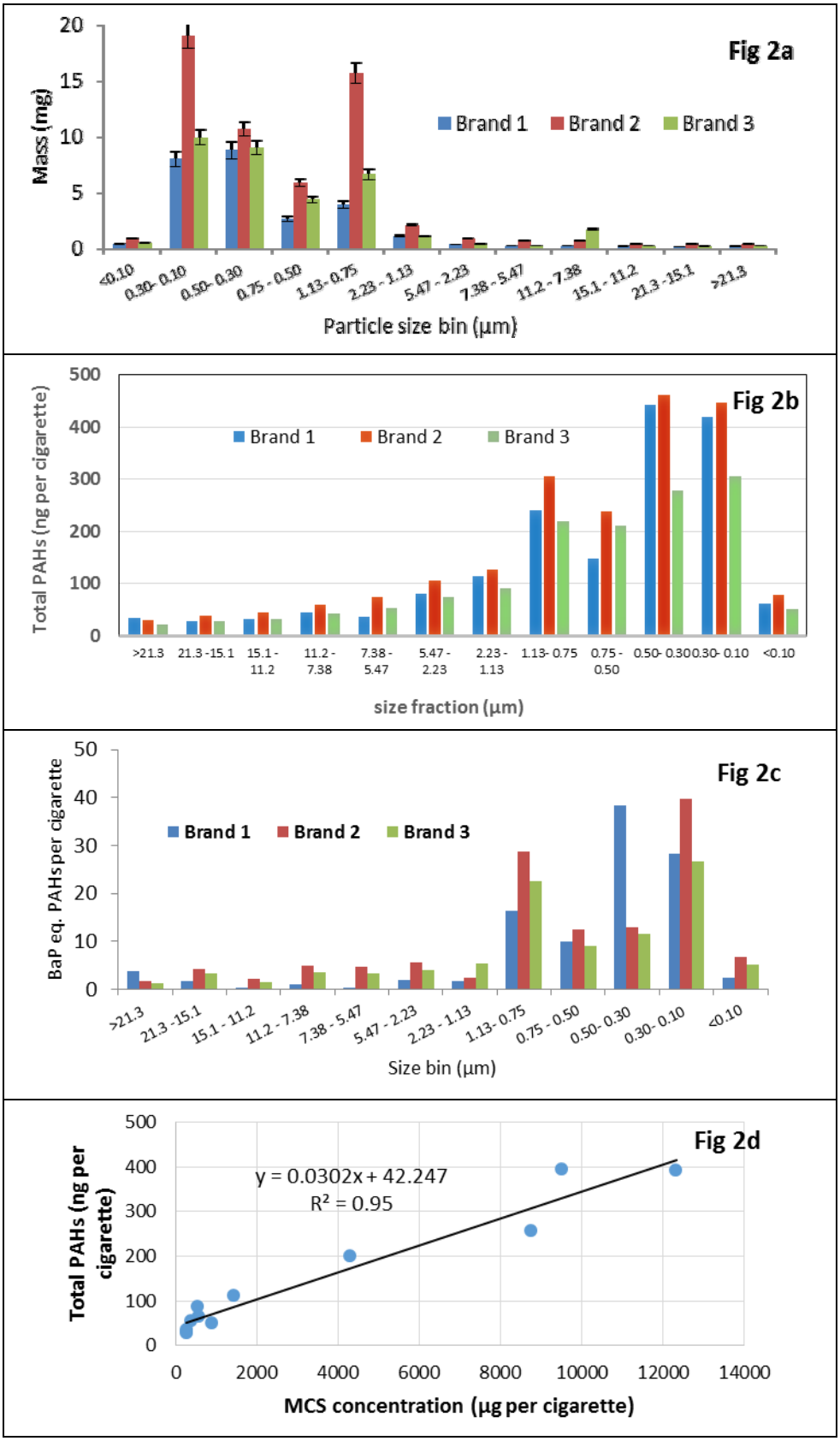

Fig. 2. (a) Particle mass size distribution of mainstream cigarette smoke obtained using VCCI for tested brands. (b) Size fractionated total PAHs emission (ng per cigarette) for different brands cigarette (c) Size segregated B[a]P equivalent emission (ng per cigarette) for different brands cigarette. (d) Scattered plot of MCS concentration and corresponding total PAHs (ng per cigarette) independent of size bin.

Table 2. Size fractionated PAHs emission (ng cig^{-1}) ($\mu \pm 1\sigma$, n = 240) for tested brands mainstream cigarette smoke.

Dp (μm)	> 21.3	21.3–15.1	15.1–11.2	11.2–7.38	7.38–5.47	5.47–2.23	2.23–1.13	1.13–0.75	0.75–0.50	0.50–0.30	0.30–0.10	< 0.10
NAPTH	1.03 ± 0.13	1.02 ± 0.13	1.69 ± 0.23	2.58 ± 0.36	3.11 ± 0.40	5.37 ± 0.71	8.29 ± 1.05	18.6 ± 13.1	17.04 ± 9	22.9 ± 8.06	13.3 ± 7.25	2.55 ± 0.19
ACY	0.50 ± 0.06	0.40 ± 0.15	2.07 ± 0.23	2.68 ± 0.87	3.04 ± 0.47	4.96 ± 0.52	12.3 ± 3.60	13.8 ± 4.74	10.2 ± 1.98	17.5 ± 2.4	5.97 ± 1.01	3.79 ± 0.51
ACE	0.37 ± 0.14	0.55 ± 0.31	0.57 ± 0.14	0.86 ± 0.18	2.03 ± 0.26	2.22 ± 1.40	3.42 ± 0.40	7.63 ± 2.53	9.87 ± 4.56	11.9 ± 2.67	6.16 ± 0.82	1.38 ± 0.64
FLUO	5.57 ± 0.91	8.47 ± 1.38	8.72 ± 1.42	14.9 ± 2.43	13.17 ± 2.99	22.4 ± 6.21	26.2 ± 6.53	28.6 ± 5.21	21.3 ± 3.57	19.8 ± 4.65	11.8 ± 0.62	9.28 ± 3.54
PHEN	3.09 ± 0.50	3.60 ± 0.59	5.11 ± 0.83	10.8 ± 1.76	7.11 ± 5.00	14.5 ± 2.36	16.4 ± 3.51	15.6 ± 4.55	20.6 ± 2.11	51.8 ± 6.86	45.5 ± 6.02	9.39 ± 2.34
ANTH	2.17 ± 0.35	1.26 ± 0.30	2.19 ± 1.33	1.93 ± 0.31	2.45 ± 0.40	2.26 ± 1.31	1.41 ± 0.63	8.89 ± 1.19	10.7 ± 4.20	31.6 ± 5.43	14.6 ± 2.71	6.39 ± 0.86
FLT	2.59 ± 2.05	2.72 ± 0.73	2.42 ± 0.39	3.29 ± 0.54	9.35 ± 1.52	9.53 ± 2.49	8.19 ± 1.22	25.6 ± 9.41	11.5 ± 5.78	25.3 ± 2.32	27.7 ± 3.67	2.65 ± 0.70
PYR	3.78 ± 0.80	2.23 ± 0.39	3.35 ± 0.55	3.14 ± 0.94	3.49 ± 1.20	6.83 ± 1.11	1.36 ± 0.20	13.7 ± 4.53	12 ± 4.42	37.9 ± 6.23	50.8 ± 8.27	2.97 ± 0.40
BAA	0.74 ± 0.09	0.95 ± 0.58	0.57 ± 0.31	0.64 ± 0.33	1.51 ± 0.87	2.37 ± 1.27	1.25 ± 0.73	2.08 ± 0.28	24.5 ± 7.81	10.34 ± 0.7	25.6 ± 3.39	0.70 ± 0.66
CHRY	0.98 ± 0.41	0.92 ± 0.42	1.12 ± 0.55	0.92 ± 0.41	1.49 ± 0.81	2.21 ± 1.32	1.39 ± 0.15	3.27 ± 0.44	4.6 ± 1.32	8.59 ± 3.77	23.4 ± 6.73	7.37 ± 3.55
BBF	0.37 ± 0.18	0.80 ± 0.09	0.82 ± 0.10	1.62 ± 0.19	1.45 ± 0.44	1.51 ± 0.45	1.15 ± 0.12	20.9 ± 2.79	12.7 ± 1.68	5.25 ± 0.69	37.7 ± 4.99	1.75 ± 0.24
PERY	0.44 ± 0.27	0.60 ± 0.36	0.73 ± 0.38	0.88 ± 0.51	1.16 ± 0.83	0.82 ± 0.55	0.74 ± 0.86	6.32 ± 2.86	4.83 ± 0.69	14.1 ± 4.20	4.02 ± 1.23	1.21 ± 0.95
BKF	1.07 ± 0.17	1.00 ± 0.65	2.13 ± 1.47	0.78 ± 0.36	0.72 ± 0.51	2.86 ± 1.56	2.58 ± 0.31	7.13 ± 4.70	5.91 ± 3.49	31.1 ± 16.1	3.93 ± 2.71	1.41 ± 0.63
BAP	1.89 ± 1.06	2.47 ± 1.12	0.78 ± 0.43	2.64 ± 1.50	2.17 ± 1.42	2.93 ± 1.11	2.11 ± 1.50	11.3 ± 3.60	7.2 ± 0.96	17.8 ± 7.16	21.5 ± 2.85	3.88 ± 1.56
BGHIP	2.86 ± 0.36	1.46 ± 0.20	2.15 ± 0.29	0.47 ± 0.06	0.82 ± 0.11	5.63 ± 0.76	21.9 ± 2.67	64.6 ± 2.32	21.3 ± 16.5	73.6 ± 26.4	78.4 ± 17.1	6.55 ± 4.34
INDO	1.42 ± 0.19	2.86 ± 1.64	1.48 ± 0.84	1.34 ± 0.72	2.16 ± 1.43	0.85 ± 0.47	2.49 ± 1.75	8.07 ± 3.23	4.65 ± 0.62	14.9 ± 6.26	20.94 ± 3.1	2.31 ± 1.24

weight (4-rings) PAHs, on the other hand, accounted for only 23 to 25% of the total PAHs. High molecular weight PAHs (5 and higher rings) accounted for 26-27% of total analyzed PAHs. Earlier study by Skoropinski *et al.* (1985) measured approximately 28% of the BAP in the mainstream smoke, 46–48% in the sidestream smoke from the burning end of the cigarette, and 7% in the butt and ash (Skoropinski *et al.*, 1985). The proportion of PAHs in particulate phase proportion increased with increasing molecular weight. Low molecular weight PAHs are more volatile compared to high molecular weight PAHs and are therefore partitioned between particulate matter and the gas phase, which reveals that gas phase low molecular weight PAHs are in high concentration (Lu and Zhu, 2007). PAH ratios have been used to determine PAH sources. The diagnostic ratio for particle phase PAHs of MCS were calculated and found to be 0.56, 0.18 and 0.26 for BaA/(BaA + CHRY), IcP/(IcP + BgP), BaP/BgP respectively. The diagnostic ratios BaA/(BaA + CHRY), IcP/(IcP + BgP), BaP/BgP for all tested brands values indicate cigarette smoke PAHs has similar isomeric composition as combustion, petrogenic and traffic sources (Pandey *et al.*, 1999; Yunker *et al.*, 2002).

Predicted Size Fractionated Deposition

Deposition fractions of size fractionated MCS were calculated for different respiratory compartments viz. head airways (H), Trachea and bronchi (TB) and pulmonary (P); using Multiple-Path Particle Dosimetry (MPPD V2.11) model. The deposition fraction were calculated for each size bin of sampling after calculating geometrical mean diameter of the size ranges of variable configuration parameters. Deposition fractions for each size bin in different compartment of respiratory tract were shown in Fig 4. The deposition fraction (DF) were found less than 0.15 for smoke particle below 3.5 μm aerodynamic diameter in head airways of respiratory tract. Particle having more than 3.5 μm aerodynamic diameter were found to more deposition in head airways. For coarse particle with size around 25 μm diameter deposition fraction was determined more than 0.90. In trachea and bronchi regions values of deposition fraction was found more for coarse mod particle i.e., dp > 3.5 μm. For fine particle (dp < 3.5 μm) deposition fraction value was less than 0.10, while for ultrafine particle DF values were near 0.4. DF of smoke particle having aerodynamic diameter more than 6.3 μm in pulmonary region was estimated very less (DF < 0.1), while for fine smoke particle DF values varies between 0.1 and 0.45. Total deposition in respiratory tract was found to be varying from 69 ± 26% for tested size ranges. In size bins from 0.3 to 0.75 μm deposition fraction values were found least compare to other size bin.

Distribution of PAHs in Different Compartments of Respiratory Tract

Deposition of individual PAHs (ng per cigarette) in different compartment of respiratory tract viz. head airways, trachea and bronchi, and pulmonary regions were calculated. It is done by integrating the result of size fractionated PAHs concentration (Table 2) and deposition fractions calculated

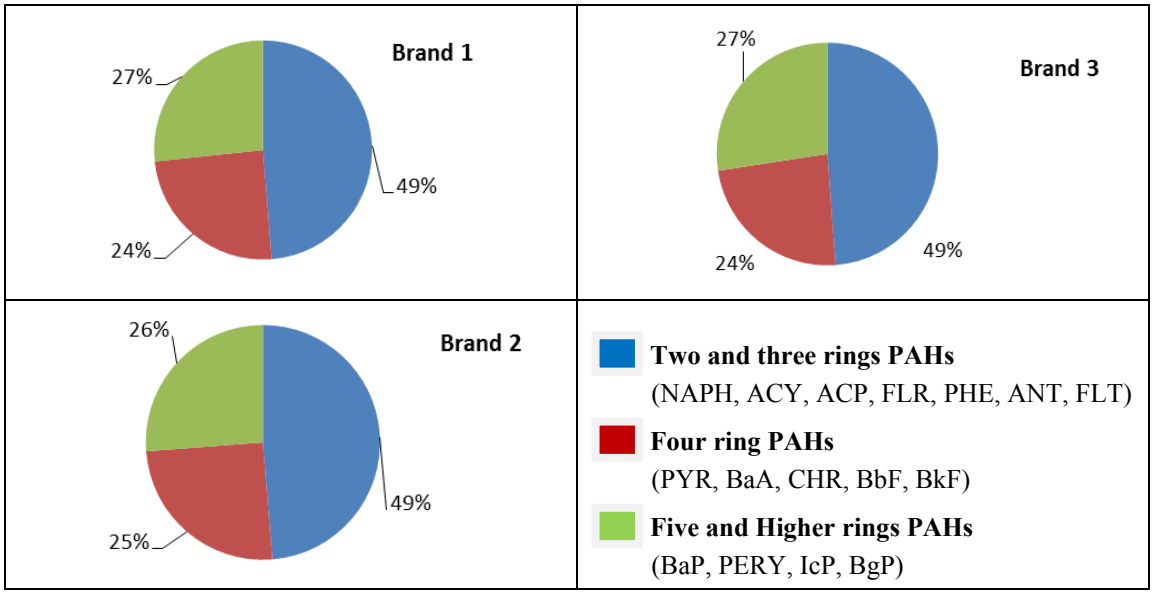

Fig. 3. Ring number wise distribution of PAHs in mainstream cigarette smoke.

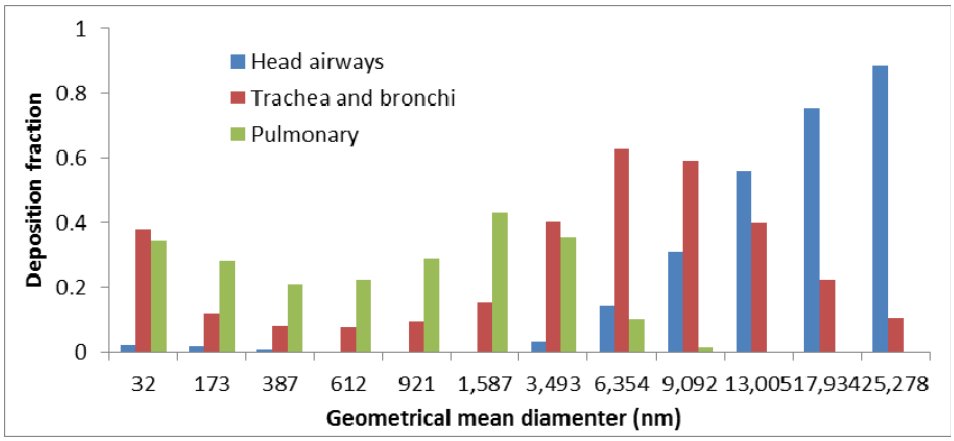

Fig. 4. Size fractionated deposition fraction for mainstream cigarette smoke in different respiratory tract using MPPD model.

using MPPD model. For all 16 monitored PAHs in MCS; respiratory compartmental distribution results are represented in Fig. 5. Percentage distribution of total deposition in head airways, trachea and bronchi, and pulmonary regions are respectively 13.5, 29.4 and 51.6%. High DF values in pulmonary region indicates more than half of smoke particle penetrating up to pulmonary where gaseous exchange between the inhaled air and the blood occurs (Till and Grogan 2008). Benzo (ghi) perylene is most accumulating PAHs with a mean value of 14.13%, closely followed by Fluorene (14.26%) and Phenanthrene (12.65%) of the total deposited PAHs in respiratory tract.

Inhalation Exposure and Risk Assessment

Incremental lifetime cancer risk (ILCR) assessments calculation were carried out for exposure of MCS of tested cigarette brands. Monte Carlo based approach is used for calculation of ILCR probability and cumulative probability distribution. Random number were generated for lognormal distribution of parameter viz. body weight and cancer slope factor of B[a]P to calculate carcinogenic risk. B[a]P_{eq} PAH concentration for each size range is calculated using toxicity equivalent factor for each PAHs with respect to B[a]P, depicted in Fig. 2(c). For tested brands MCS B[a]P_{eq} emission per cigarettes were founds to be 106, 126 and 97 ng for brand 1, 2and 3 respectively. Daily intake (I) of B[a]P_{eq} concentration for each size bin calculated and using deposition fraction and number of cigarette smoked daily. Average daily intake of B[a]P_{eq} PAHs through MCS were founds to 0.88, 1.26 and 0.97 mg per day for brand 1, 2 and 3 respectively. Random number from lognormal distribution, were generated for cancer slop factor with a geometrical mean 3.14 (mg kg^{-1} $day^{-1})^{-1}$ and a geometrical standard deviation 1.80. Risk for each brand MCS were calculated to 10 run (each includes 5000 random numbers) and average probability and cumulative frequency distribution (CFD) were generated and shown in Fig. 6. Probability distribution were found be positively skewed for incremental lifetime

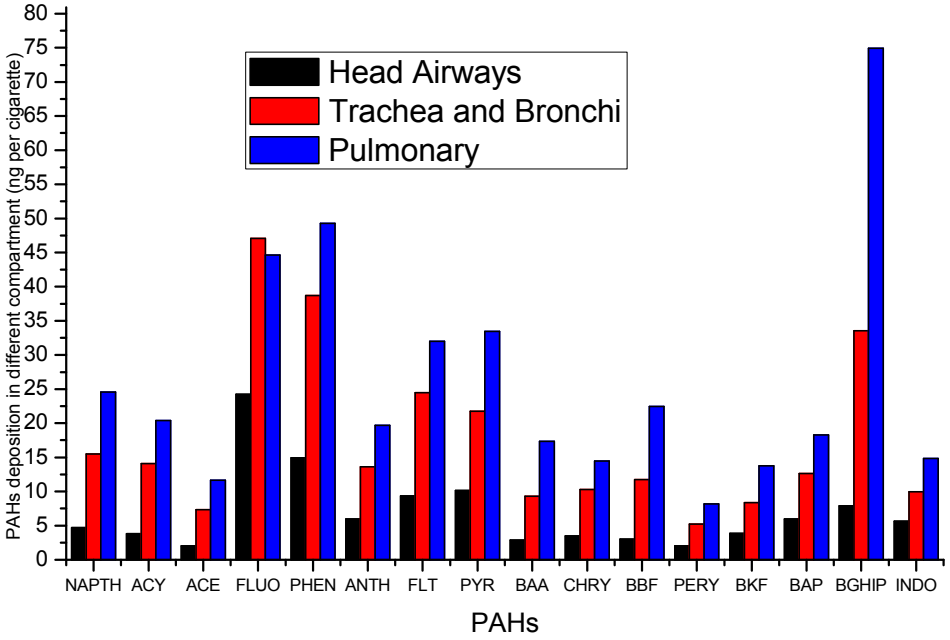

Fig. 5. Distribution of PAHs (ng per cigarette) in different compartments of respiratory tract (head airways, trachea and bronchi, and pulmonary regions).

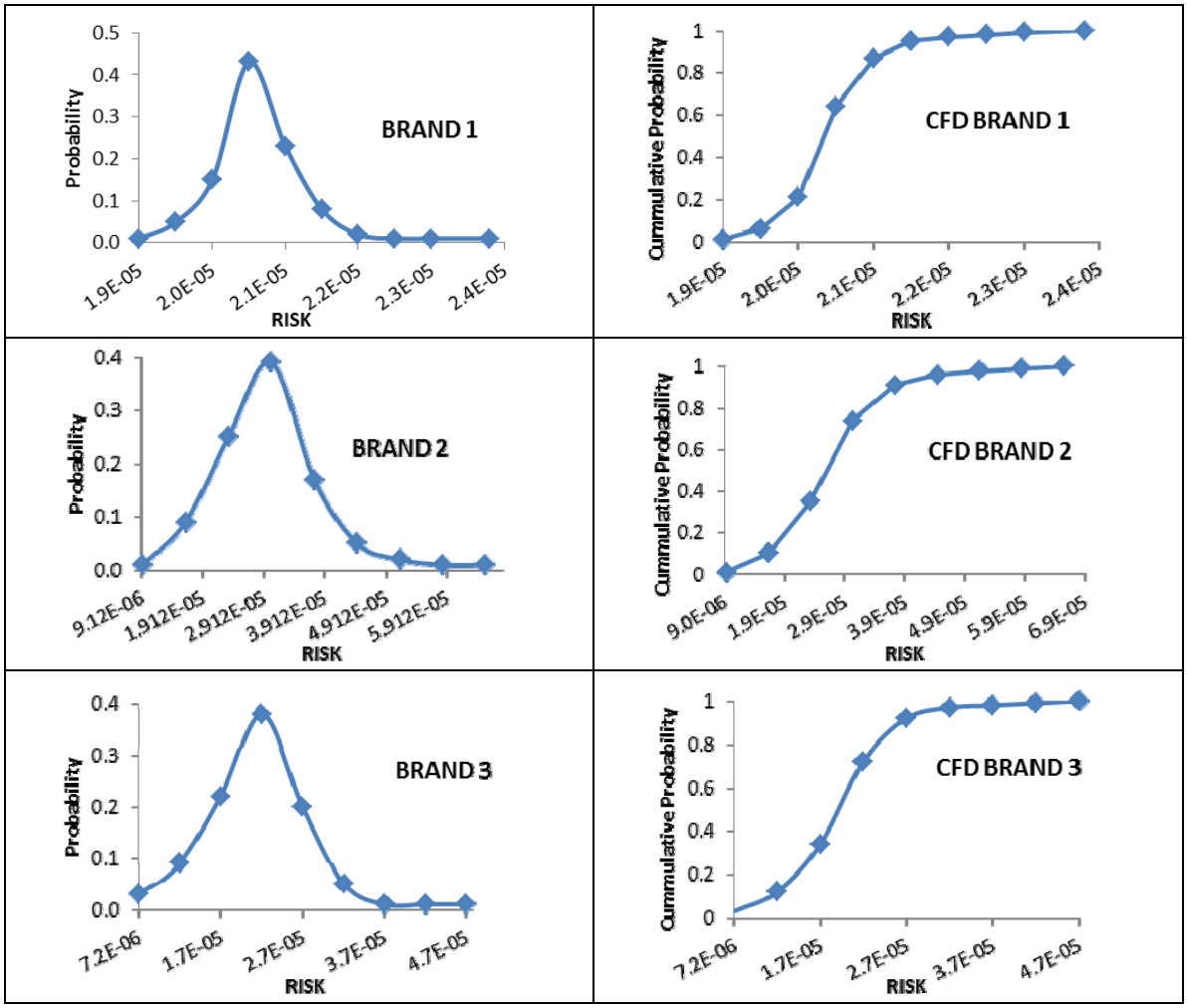

Fig. 6. Probability and cumulative frequency distributions (CFD) of ILCR for exposure of particle phase MCS PAHs.

Table 3. 5th, 50th and 95th percentile of ILCR probability distributions for different brands MCS PAHs exposure.

Cigarette Type	5th Percentile	50th Percentile	95th Percentile
BRAND 1	1.94×10^{-5}	2.06×10^{-5}	2.32×10^{-5}
BRAND 2	1.11×10^{-5}	2.94×10^{-5}	5.28×10^{-5}
BRAND 3	8.60×10^{-6}	2.27×10^{-5}	4.08×10^{-5}

cancer risk of PAHs through MCS exposure. It was found that the cumulative ILCR for PAH MCS exposure through first hand smoking investigated in this study was order of 10^{-5} for all tested brands. Average 5th and 95th percentile values of incremental lifetime cancer risk (ILCR) for MCS PAHs in there probability distribution were found to be 1.3×10^{-5} to 3.9×10^{-5} respectively in tested cigarette brands. 50th percentile values of risk in there probability distribution were founds to be 2.06×10^{-5}, 2.94×10^{-5} and 2.27×10^{-5} respectively for brand 1, brand 2 and brand 3. 5th, 50th and 95th percentile values of risk for all three tested brands were represented in Table 3.

CONCLUSIONS

There was not much variation observed in particle mass and PAHs size distribution with brands of cigarettes. More than 90% of particle mass for MCS associated with fine particle (< 2.5 μm aerodynamic diameter) and which capable to penetrated deep alveoli of respiratory tract and subsequent deposition. Benzo (ghi) Perylene emissions were found to be among tested priority sixteen PAHs. The concentration of BaP in MCS (ng per cigarette) was found higher than of Kentucky 3R4F cigarette reference value. B[a]P_{eq} PAHs emission in MCS was found to be 110 ± 12 ng cigarette^{-1} for tested brands. The PAHs diagnostic ratios values indicate cigarette smoke PAHs has similar isomeric composition as combustion, petro-genic and traffic sources. Generated data are also helpful in source apportionment study for pattern contribution of size fractionated PAHs due to cigarette smoke. Coarse particle were seem to deposited mostly in head airways for fine particle the pattern of deposition also found in pulmonary and trachea and bronchi region of respiratory tract. ILCR values for MCS PAHs is significant when we calculate the societal risk for millions of smokers.

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