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Polycyclic aromatic hydrocarbons in breast milk of nursing mothers: Correlates with household fuel and cooking methods used in Uganda, East Africa

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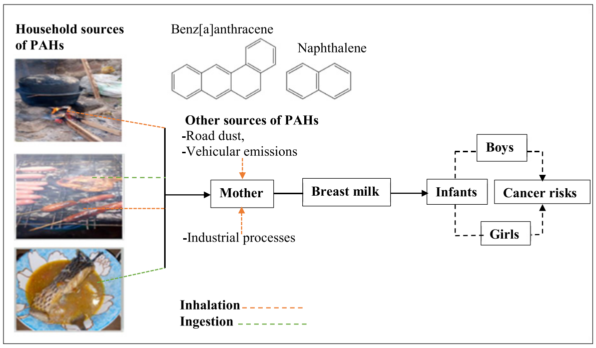
HIGHLIGHTS

- Influence of cooking methods on PAH levels of breast milk has been investigated.
- Firewood and charcoal were the widely used fuels for cooking by the donor mothers.
- PAHs in breast milk samples of urban mothers were higher than in rural mothers.
- Incremental cancer risk estimates suggest tolerable health risks to infants.

GRAPHICAL ABSTRACT



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ABSTRACT

Maternal breast milk, which is a complete food for the infant's growth, development, and health, contains fats and lipids making it susceptible to accumulation of lipophilic compounds like polycyclic aromatic hydrocarbons (PAHs). This study aimed at analyzing correlates of measured levels of PAHs in breast milk of nursing mothers to frequently used household fuels and cooking methods in Uganda, and estimate the potential health risks of PAHs to infants through breastfeeding. Sixty breast milk samples were collected from healthy and non-smoking mothers who had lived in Kampala capital city (urban area) and Nakaseke district (rural area) for at least five years. Sample extracts were analyzed for PAHs using a gas chromatograph coupled with a triple quadrupole mass spectrometer. Σ_{13}PAHs in samples from Kampala ranged from 3.44 to 696 ng/g lw while those from Nakaseke ranged from 0.84 to 87.9 ng/g lw. PAHs with 2–3 rings were more abundant in the samples than PAHs with 4–6 rings. At least 33 % of the variance in the levels of Σ_{13}PAHs in the breast milk samples was attributable to the fuel type and cooking methods used. Nursing mothers who used charcoal for cooking accumulated higher levels of Σ_{13}PAHs in their breast milk

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samples compared to those who used firewood. Levels of Σ_{13}PAHs in breast milk of mothers increased depending on the cooking methods used in the order; boiling< grilling< deep-frying. In all samples, hazard quotients for PAHs were <1 and estimated incremental cancer risks were all between 10^{-6} and 10^{-4}, indicating that the health risks to infants due to the ingestion of PAHs in breast milk was tolerable. Further studies with large datasets on PAHs and their derivatives and, larger samples sizes are needed to confirm these findings.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) refer to a class of chemicals that consist of two or more fused aromatic hydrocarbon rings (Ingenbleek et al., 2019; Srogi, 2007). PAHs are contained in crude oil, refined petroleum products, and coal but are also emitted from combustion of organic matter, waste, and fossil fuels (Achten and Hofmann, 2009; Lima et al., 2005; Lindén and Pålsson, 2013). Spillage of the oil, refined petroleum and combustion processes (i.e., forest fires, cooking activities, household heating, industries, power plants, vehicles) are major contributors to the environmental burden and human exposure to PAHs (Abayi et al., 2021; Lima et al., 2005; Srogi, 2007; Zakaria et al., 2002). PAHs are persistent, bioaccumulative, mutagenic, carcinogenic, teratogenic, and estrogenic with several of them classified as human carcinogens (Sun et al., 2021). Sixteen of the pollutants have been included in the United States Environmental Protection Agency (U.S. EPA) list of priority pollutants (Abayi et al., 2021; Andersson and Achten, 2015; Domingo and Nadal, 2015; Keith, 2015).

Consumption of PAHs contaminated foods is considered to be a major route for human exposure to PAHs (Domingo and Nadal, 2015; Phillips, 1999). In processed foods, PAHs could be due to the different fuels, cooking methods, and ingredients used, therefore exposing humans to PAHs (Abdullahi et al., 2013; Bandowe et al., 2021; See et al., 2006). This exposure may be detrimental to human health especially in vulnerable populations such as nursing mothers and infants. Humans could also get exposed to PAHs via inhalation of contaminated air and through dermal contact with the contaminated materials especially in occupational environments (Sun et al., 2021). Fumes from the heat processing of food ingredients and combustion of fuels (e.g., firewood, kerosene, charcoal, coal) used in the cookstoves, household lighting and heating are the important sources of PAHs and human exposure (Abdullahi et al., 2013; Bandowe et al., 2021; Du et al., 2020; Piedrahita et al., 2017).

Combustion of solid fuels for household activities (e.g., cooking) generally releases more PAHs and other combustion products into such homes than in households using electricity or cleaner fuels (Chen et al., 2016; Munyeza et al., 2020). Previous studies have shown that indoor air contains substantial levels of PAHs and alarmingly indoor environments in rural areas show higher concentrations of PAHs than in urban areas (Munyeza et al., 2020). About 80.4 % of low-income households in Kampala city use wood fuel and about 8.2 % use electricity for cooking/lightening (Nzabona et al., 2021). Exposure to pollutants in indoor environments (like kitchens) with toxic combustion products (e.g., PAHs) is a major source of human exposure because people spend more time indoors than outdoors. This is especially true for women and girls in sub-Saharan Africa and other developing countries in Latin America and Asia (Adetona et al., 2013; Weinstein et al., 2017). For example, in sub-Saharan Africa, women are mostly responsible for cooking in households or on a commercial basis (restaurant operators, roasting, and smoking of fish, meat, etc.) mostly using solid fuelwood or charcoal (Okello et al., 2018; Xu et al., 2019).

Many areas in East Africa, such as Kampala, are undergoing rapid economic development, industrialization, and socio-demographic transition (Barinov and Sharova, 2021). Such developments lead to environmental degradation and significantly increase ambient air pollutant levels (Abera et al., 2021; Singh et al., 2021). PAHs concentrations have been reported in outdoor air of Uganda, Rwanda and Kenya (Kalisa et al., 2018; Munyeza et al., 2020) with urban sites having higher levels of PAHs than rural sites, potentially due to vehicular emissions (Kalisa et al., 2018; Munyeza et al., 2020). Street dust sampled from urban areas in the sub-Saharan Africa also contain higher levels of PAHs (Bandowe and Nkansah, 2016). We hypothesize that the urban populations in sub-Saharan Africa might be exposed to higher levels of PAHs from outdoor environment than rural populations.

Moreover, in many urban areas of sub-Saharan Africa, women are overwhelming engaged in occupations such as selling of items in open markets and street vending that might expose them to high levels of combustion pollutants emitted from vehicles (Amegah and Jaakkola, 2014). The socio-economic factors underlie the choice of fuels for cooking, lighting and heating. The urban poor are over represented in occupations such as street vending. Consequently, the socio-economic gradients might also be a driver for the PAHs exposure in sub-Saharan Africa. However, to the best of our knowledge, this hypothesis has never been tested.

In East Africa, recent studies have reported maternal occupational exposure to some classes of organic pollutants such as polybrominated diphenyl ethers (e.g., Matovu et al., 2019). Infant exposure to polychlorinated biphenyls and dioxins through breastmilk has also been reported (Matovu et al. (2021). To the best of our knowledge, to date, there have been no studies on PAHs in human breast milk in East Africa. Moreover, there are still large data gaps in the literature reviews pertaining to the levels of PAHs, and POPs in general, not only in the breast milk of mothers (Khanverdiluo et al., 2021) but in human matrices in East Africa, and Africa at large (Ssebugere et al., 2019). This underscores the compelling need for biomonitoring of PAHs and other POPs in the less studied regions on the African continent. Since breast milk represents a noninvasive lipid-rich matrix for human biomonitoring, focused studies on the exposure of nursing mothers to PAHs are needed to inform policymakers about the potential effects of indoor and outdoor pollution.

The objectives of this study are: (i) To quantify the levels and composition profiles of 13 PAHs in breast milk of mothers residing in the capital city of Uganda and a rural area. (ii) To estimate possible health risk to infants who are breastfed with this milk. (iii) To determine the association and/or influence of household fuel type and cooking method used on the body burden of PAHs in nursing mothers in Uganda.

2. Materials and methods

2.1. Study areas

The study was carried out in two settings in Uganda, Kampala city, and Nakaseke district (Fig. 1) with differing levels of urbanization and socio-economic status. Kampala city is located between Latitude 0° 20′51.3456″ N, and Longitude 32° 34′ 57.072″ E at an elevation of 1200 m above sea level (Kajjoba et al., 2021). The city was chosen because of its diversity in the socio-economic status among the population, with the majority of households (the urban poor) still relying on wood fuels for cooking/heating which results into indoor and outdoor pollution (Mugabi and Kisakye, 2021; Ssemugabo et al., 2021). The city's pollution levels are further exacerbated by the significant rise in vehicular/motorization emissions, fumes from industries, and solid wastes (Amulen et al., 2022; Bassi et al., 2021). Kampala being a very cosmopolitan city with a large proportion of residents originally from the neighboring countries such as South Sudan, Burundi, Ethiopia, Eritrea, Democratic Republic of Congo, Rwanda, and Somalia (Matovu et al., 2021) presents a suitable case for studying the paired maternal-infant body burdens of PAHs and their health effects in the East African region.

The rural Nakaseke district is located at Latitude 0°43′29″ N, and Longitude 32° 54′04″ E, with altitudinal range of 1086–1280 m above sea level. This area, which falls within the Central Wooded Savannah agro-ecological

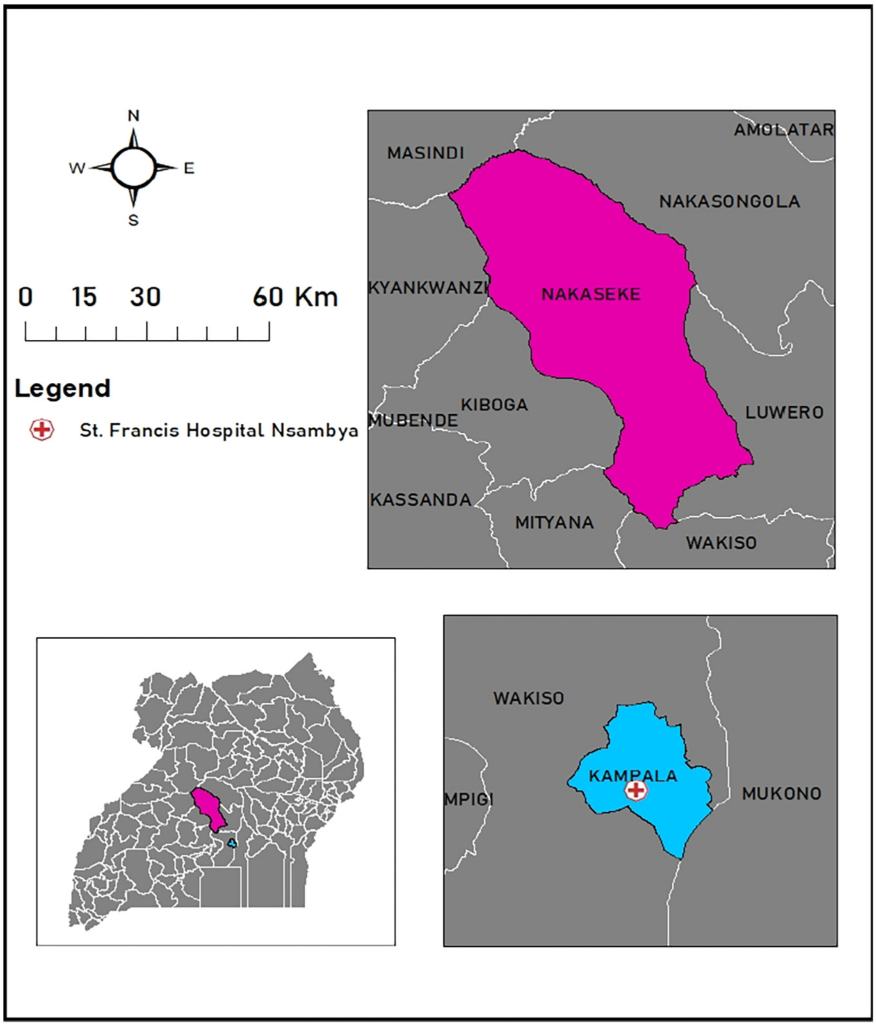

Fig. 1. Map of Uganda showing the locations of Kampala capital city and Nakaseke district.

zone has a population size of approximately 191,000 people (Dougherty et al., 2018; Nankya et al., 2021).

2.2. Ethical consideration and study population

The study protocol was reviewed and approved by Ethics and Research Committees of Uganda National Council of Science and Technology (approval number HS 2263), and St. Francis Hospital Nsambya, Uganda. Recruitment into the study was solely based on the willingness of mothers to participate, their time of residence in the study areas and health status. Only healthy and non-smoking mothers who had lived in Kampala city and Nakaseke district for more than five years were requested to provide their breast milk. Prior to recruitment, researchers explained the purpose of the study to the mothers in English and/or their respective native languages. Only mothers who signed an informed consent form were included in the present study. Information about the maternal age, pre-pregnancy weight, number of household persons, monthly income earnings, education level, relationship status, type of fuel, cooking methods, and lifestyle at the time of sample collection were obtained from the mothers by filling a questionnaire modified from WHO guidelines (Matovu et al., 2021; WHO, 2007). The characteristics of the participants in our study are summarized in Table 1. All the information provided by the donor mothers were kept confidential.

2.3. Sample collection and storage

A total of 60 samples of breast milk (30 from each study area) were collected between February and October 2019. Samples of breast milk (7 mL) were collected from each mother by manual expression during postnatal care with the help of a physician. Breast milk samples were collected in chemical-free glass bottles with aluminium seals and then transferred in cooling boxes with ice packs for transportation to the Pesticides Laboratory, Department of Chemistry, Makerere University, Uganda. Upon delivery, samples were frozen at −20 °C to avoid microbial degradation and to keep their physical and chemical properties intact, before extraction. All the breast milk samples were collected between 2 and 9 days after postpartum (Matovu et al., 2021).

2.4. PAHs reference standards, solvents, reagents and glassware

The thirteen reference standards [naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP) and dibenzo[*a*, *h*]anthracene (DaA)] were purchased from Chiron (Trondheim, Norway). All the standards were above 99 % purity. The standards were separately kept in a freezer at -18 °C before use. Dichloromethane

Table 1
Characteristics of participating mothers.

Maternal characteristics	Nakaseke ($N = 30$)		Kampala (N = 30)		All ($N = 60$)	
	Mean (median)	Min-max	Mean (median)	Min-max	Mean (median)	Min-max
Age (years)	29.9 (29.5)	16.0–39.0	29.0 (29.0)	22.0–40.0	29.5 (29.0)	16.0–40.0
BMI (kg/m^2)	28.2 (27.6)	19.1–38.2	29.9 (28.7)	23.2–49.5	29.0 (28.1)	19.1–49.5
BMI ranges (persons)						
18.5–< 25	8 (26.7 %)		4 (13.3 %)		12 (20 %)	
25–< 30	13 (43.3 %)		16 (53.3 %)		29 (48.3 %)	
30–< 35	6 (20.0 %)		20 (20.0 %)		12 (20.0 %)	
35–< 40	3 (10.0 %)		6 (20.0 %)		12 (20.0 %)	
>40	0		1 (3.3 %)		1 (1.7 %)	
Relationship status (persons)						
Married	28 (93.3 %)		27 (90.0 %)		55 (91.7 %)	
Single	2 (6.7 %)		3 (10.0 %)		5 (8.3 %)	
Education level						
No education	6 (20.0 %)		1 (3.3 %)		7 (11.7 %)	
Primary	18 (60.0 %)		2 (6.7 %)		20 (33.3 %)	
Secondary	5 (16.7 %)		9 (30.0 %)		14 (23.3 %)	
Tertiary	1 (3.3 %)		9 (30.0 %)		10 (16.7 %)	
University	0		9 (30.0 %)		9 (15.0 %)	
Number of persons in household						
<5	6 (20.0 %)		19 (63.3 %)		25 (41.7 %)	
≥5	24 (80.0 %)		11 (36.7 %)		35 (58.3 %)	
Monthly earnings in USD (persons)						
<13.6	7 (23.3 %)		0		7 (11.7 %)	
13.6–< 40.6	17 (56.7 %)		7 (23.3 %)		24 (40.0 %)	
40.6–<81.6	4 (13.3 %)		4 (13.3 %)		8 (13.3 %)	
81.6–<135.6	1 (3.3 %)		7 (23.3 %)		8 (13.3 %)	
135.6–< 270.6	1 (3.3 %)		10 (33.3 %)		11 (18.3 %)	
≥270.6	0		2 (6.7 %)		2 (3.3 %)	
Cultural influence (persons)						
No	23 (76.7 %)		28 (93.3 %)		51 (85.0 %)	
Yes	7 (23.3 %)		2 (6.7 %)		9 (15 %)	
Fuel type used (persons)						
Charcoal	6 (20.0 %)		22 (73.3 %)		28 (46.7 %)	
Firewood	23 (76.7 %)		4 (13.3 %)		27 (45.0 %)	
Electricity	0		3 (10.0 %)		3 (5.0 %)	
LPG	1 (3.3 %)		1 (3.3 %)		2 (3.3 %)	
Cooking methods used ≥3 times a week (persons)						
Boiling	13 (43.3 %)		7 (23.3 %)		20 (33.3 %)	
Deep-frying	7 (23.3 %)		12 (40.0 %)		19 (31.7 %)	
Grilling	10 (33.3 %)		9 (30.0 %)		19 (31.7 %)	
Microwaving	0		2 (6.7 %)		2 (3.3 %)	

BMI- pre-pregnancy body mass index; LPG- liquefied petroleum gas.

(DCM), *n*-hexane, ethyl acetate, cyclohexane and isooctane used were all of pesticide residue grade purchased from Sigma-Aldrich. Analytical grade anhydrous magnesium sulfate, anhydrous sodium sulfate, sodium chloride and silica gel used were all supplied by Sigma-Aldrich. High purity ethanol used was bought from Altia, Rajamäki, Finland. All the glassware used were thoroughly cleaned by first soaking them for 2 h in tap water mixed with a detergent, and then rinsed with hot water followed by acetone. The glassware were then dried in an oven for 4 h at 105 °C before use.

2.5. Analysis of the samples

2.5.1. Extraction of PAHs from the breast milk samples

Firstly, the breast milk samples were allowed to thaw and homogenized. The samples were then extracted following a method described by Pulkrabova et al. (2016), with slight modifications. Briefly, breast milk (5 mL) was mixed with distilled water (3 mL) and the mixture was shaken with ethyl acetate (20 mL) in a polypropylene centrifuge tube for 2 min. Anhydrous magnesium sulfate (1 g) and sodium chloride (0.5 g) were added to the mixture and shaken for another 2 min. The mixture was centrifuged (5 min; 10,000 rpm) and the upper organic layer was removed. The extract was concentrated to 2 mL using a rotary evaporator and to near dryness using a gentle stream of nitrogen gas. The extracts in the tube were reconstituted in *n*-hexane (1 mL) and kept for clean-up.

2.5.2. Clean-up of breast milk extracts

The evaporated extracts were then purified using a glass column (1 cm internal diameter, 30 cm length) as described by Pulkrabova et al. (2016). The column was plugged with a pre-cleaned glass wool followed by the silica gel (5 g) and anhydrous sodium sulfate (1 g). The column was conditioned with 15 mL of a mixture of *n*-hexane and DCM (3:1, *v*/*v*) followed by *n*-hexane (12 mL). Analytes were eluted with 24 mL of a mixture of *n*-hexane and DCM (3:1, v/v). The eluate was then concentrated to 2 mL using a rotary evaporator and further to near dryness using a gentle stream of nitrogen gas. The concentrated eluate was reconstituted in isooctane (1 mL), transferred to glass vials, vortexed and then kept for instrumental analysis.

2.5.3. Determination of lipid content of breast milk samples

The lipid content of each breast milk sample was gravimetrically determined. The details can be found in our previous publication (Matovu et al., 2019). Briefly, a breastmilk sample (2 mL) was extracted with a mixture of *n*-hexane/ethanol (1:1, *v*/*v*). The organic layer was then dried by passing it through a 3 mL polypropylene cartridge filled with anhydrous sodium sulfate. The resulting eluate was then evaporated to dryness using a gentle stream of nitrogen gas and the lipid weight determined gravimetrically.

2.5.4. Instrumental analysis

The target PAHs were analyzed using a Shimadzu gas chromatograph coupled with the mass spectrometer triple quadrupole (GC–MS/MS) and an auto-injector (Shimadzu, AOC-20i) using electron impact ion source under 70 eV and operated in a multiple reactions monitoring (MRM) mode. Separation was achieved with a ZB-5MSi capillary column (30 m × 0.25 mm i.d × 0.25 μm film thickness). Details of the chromatographic conditions are presented in the Online supplementary material (Table S1a). PAHs identification in the sample extracts was based on a comparison of the retention times and fragmentation patterns of the peaks in the chromatograms of measured sample extracts to those in the calibration standards. After confirmation, quantification of PAHs was done using an external calibration method ($R^2 = 0.999$ or better).

2.6. Quality assurance and quality control

The analytical method was validated in terms of robustness, precision, accuracy, limits of detection (LOD) and limits of quantification (LOQ). Calibration plots were prepared using blank solvent samples spiked with PAHs standards at seven different concentrations ranging from 0.1 to 25 ng/mL. Least square method was used to calculate the linearity of the assay, which was expressed as coefficient of determination (R^2). R^2 of 0.99 or better was always used. Precision of the GC–MS/MS method for each PAH analyte investigated was determined by analyzing the seven concentrations in triplicates. For each analyte, LOD was calculated as a signal to noise ratio of 3, while LOQ was calculated by 10 times signal to noise ratio. LOD values ranged from 0.001 (benzo[*k*]fluoranthene) to 0.15 ng/mL (benzo[*b*]fluoranthene), while the LOQ ranged from 0.003 (benzo[*k*]fluoranthene) to 0.45 ng/mL (benzo[*b*]fluoranthene) (Table S1b). Blank powdered milk samples were spiked with PAH standards at levels of 0.2 and 0.8 ng/mL per PAH analyte and analyzed in triplicate using the same analytical method as the breast milk samples. Recoveries of 62–91 % and 72–97 % were obtained at 0.2 ng/mL, and 0.8 ng/mL spike levels, respectively. No adjustment was made on PAHs data since majority of the recoveries were above 70 % (Matovu et al., 2019). In addition, procedural solvent blanks were analyzed for every batch of 5 samples to check for any cross-contamination. The PAHs data generated were blank-corrected in a way that an average of all procedural blank values for PAHs were subtracted from the sample values (Matovu et al., 2021).

2.7. Statistical data analysis

All statistical analyses were performed using SPSS statistic software, version 20 (IBM SPSS Inc., Chicago, IL, USA). Medians were reported only for those PAHs with a detection frequency of ≥50 %; analytes with a detection frequency of <50 % were reported with the ranges only. Preliminary analysis revealed that lipid-normalized PAHs concentrations did not follow a normal distribution, and the data were natural log-transformed before further analysis. The normality of the log-transformed data was confirmed using the Shapiro-Wilk test, while the Levene's test was used to test for homogeneity of variance. A two-way analysis of variance (ANOVA) followed by Tukey's post hoc test were used to evaluate relationship between fuel type and cooking practices used on maternal body burdens of PAHs. Spearman's rank-order correlation coefficients were used to evaluate the bivariate associations between lipid content (%) and ΣPAHs (ng/mL milk) in the breast milk samples of donor mothers.

A multinomial logistic regression (MLR) model was used to evaluate the relationship between the multiple independent variables and household practices (fuel type and cooking methods). A linear model was first run on the responses as a function of predictors to ensure that there were no problems with the multicollinearity. The odds ratios (OR) and 95 % confidence intervals (CI) were calculated for each factor or covariate included in the MLR model. In all cases, statistical significance was considered at a 5 % significance level.

2.8. PAHs health risks to nursing infants

WHO strongly recommends exclusive breastfeeding for the first 6 months of the infant's life, followed by continued breastfeeding with appropriate complementary foods for up to 2 years (WHO, 2017). However, breastfeeding on PAHs-contaminated milk may be detrimental to the infants' health. In the present study, the health risks due to PAHs in breastmilk posed to the nursing infants were estimated using hazard quotients (HQ) for the first three months, and incremental cancer risk (ICR) calculations for non-cancer and cancer-related outcomes in the first two years of infant life. For each PAH, HQ was estimated using Eq. (1) (Torres-Moreno et al., 2022).

$$HQ = \frac{EDI}{RfD} \tag{1}$$

where, HQ is the hazard quotient, EDI is the estimated daily intake dose (ng/kg/day) per PAH, and RfD is the oral reference dose as defined by the US EPA's integrated risk information system (IRIS) as the maximum acceptable oral daily dose for which no adverse non-cancer effects are expected (https://iris.epa.gov/AtoZ/?list_type=alpha). For each of the seven PAHs for which RfDs are defined by EPA (Nap, Ace, Flu, Ant, Flt, Pyr, and BaP), EDIs were calculated according to Eq. (2).

$$EDI = \frac{C_i\ x\ F\ x\ IR}{BW} \tag{2}$$

where, C_i is the average milk concentration of the PAH (ng/mL), F is the percent lipid content in the milk samples (%), and IR is the milk ingestion rate by the infant (700 g milk/day), and BW is infant body weight at birth (5 kg) (Asamoah et al., 2019). Normally, a HQ <1 shows little to no health risks associated with PAHs ingestion, while HQ ≥1 shows potential health risks to the infants due to PAHs.

The carcinogenic risk assessment in infants consuming the PAH-contaminated milk was conducted using the BaP toxic equivalent concentrations (ΣTEQ_{BaP}) approach (Nisbet and Lagoy, 1992). Because BaP is considered the most carcinogenic PAH, it is assigned a toxicity equivalency factor of 1. TEQ_{BaP} of each of the PAHs were calculated by multiplying the average concentration of the individual PAH (C_i) with its corresponding toxicity equivalent factor (TEF_i) value as shown in Eq. (3) (Asamoah et al., 2019; Torres-Moreno et al., 2022).

$$\mathrm{TEQ_{BaP}} = \sum(\mathrm{C_i} \times \mathrm{TEF_i}) \tag{3}$$

The incremental cancer risk (ICR) was calculated as recommended by US. EPA for the exposure periods of between 2 weeks and 7 years (Li et al., 2020; USEPA, 1989).

$$\mathrm{ICR} = \mathrm{EDI}_{TEQBaP} \times \mathrm{CSF} = \frac{\mathrm{TEQBaP \times F \times IR \times EF \times ED}}{\mathrm{BW \times AT}} \times \mathrm{CSF} \tag{4}$$

where $TEQ_{BaP,}$ F, IR, BW are as defined in Eqs. (2) and (3) above, EF is the exposure frequency, ED is the exposure duration, AT is the average life span and CSF is the oral carcinogenic slope factor for BaP (7.3 per mg/kg/day). To account for changing infant weight and the infants' reduced reliance on breastmilk after six months, in this study, we used a breastfeeding mode of 2 years divided into three stages of infant life: 0–6, 7–12, and 13–24 months, using an IR of 750, 600, and 500 mL/day, respectively (Li et al., 2021; Li et al., 2020). EF of 365 days/year was considered for each group, while ED of 0.5-year, 1 year, and 2 years were used for the exposure periods of 0–6, 7–12, and 13–24 months, respectively. In this study, we used BW of 7.9 kg (boys) and 7.3 kg (girls) at age 6 months; 9.6 kg (boys) and 8.9 kg (girls) at 12 months; 12.2 kg (boys) and 11.5 kg (girls) at 24 months (retrieved from WHO, 2006). AT of 182.5, 365, and 730 days were used for the exposure periods of 0–6, 7–12, and 13–24 months, respectively (Asamoah et al., 2021). Under most regulatory programs, cancer risk is considered negligible when CR < 10^{-6}, but when CR > 10^{-4}, the risk is unacceptable, and when CR is between 10^{-6} to 10^{-4}, the risk is tolerable (Khanverdiluo et al., 2021; Wang et al., 2011).

3. Results and discussion

3.1. A multinomial logistic regression (MLR) model

To examine the impacts of independent variables (i.e., age, monthly earnings, education level, relationship status, the number of household persons, and cultural influence) on the fuel type, and cooking methods used by the nursing mothers in our study, an MLR analysis was performed (Tables S4 & 5). Overall, the fitted model information indicated a good model fit for the final model ($p < 0.001$). Our model, based on fuel type as the independent variable accounted for 55.7 % to 77.3 % of the observed variance (Cox and Snell = 0.664; Nagelkerke = 0.773; McFadden = 0.557), and correctly classified 83.3 % of the known observations, which was relatively higher compared to the cooking methods, which accounted for 32.3 % to 59.5 % of the variance (Cox and Snell = 0.542; Nagelkerke = 0.595; McFadden = 0.323).

Our model revealed that choice of the fuel type was significantly impacted by the mother's age ($p < 0.05$), monthly earnings ($p < 0.05$), education level ($p < 0.001$), and number of persons in household ($p < 0.05$). The choice of cooking methods was significantly impacted by mother's age ($p < 0.05$), monthly earnings ($p < 0.05$), and number of persons in household ($p < 0.05$), but there was no significant impact by the education level ($p > 0.05$). For each additional $10 in monthly earnings, the odds of mothers opting to deep-frying rather than boiling foods increased by 16.0 % (OR = 1.016; 95 % CI = 0.994–1.038). However, for each additional year of life, the odds of mothers opting to deep-frying rather than boiling decreased by 15.2 % (OR = 0.848; 95 % CI = 0.704–1.023), and the odds of opting to grilling rather than boiling foods decreased by 11.1 % (OR = 0.889; 95 % CI = 0.768–1.028).

In terms of education level, the odds of mothers who received university and tertiary education opting for electricity and liquefied petroleum gas (LPG) rather than charcoal and firewood were higher than those of mothers who received lower education, but not statistically significant ($p > 0.05$). In addition, the users of electricity and LPG were all from urban setting (Kampala). Our findings are consistent with the energy ladder model, which presupposes that households switch from the traditional energy systems (animal wastes, agricultural wastes, and firewood) at the bottom to modern ones higher up the ladder (Adamu et al., 2020). The shift depends on factors such as household incomes, fuel prices, and equipment costs, availability, and accessibility of fuels. The model further suggests that as the families acquire improved socio-economic status, they abandon the first stage technologies that are characteristically inefficient, less costly, and more polluting. Instead, the pattern gradually shifts toward the second phase transition fuels (charcoal, kerosene, and coal), and ultimately, to the higher order fuels (LPG and electricity) in the third phase (reviewed in Kay et al., 2021; Nzabona et al., 2021).

3.2. Lipid content and occurrence of PAHs in breast milk samples

The lipid content in breast milk samples ranged from 1.05 to 11.9 % with mean of 6.65 ± 3.20 % for donor mothers from Kampala city, while those from Nakaseke district ranged from 1.09 to 11.4 % with mean of 7.59 ± 3.08 %. The relatively high variation in lipid content observed in our study has also been reported in similar studies elsewhere (Çok et al., 2012; Matovu et al., 2021; Pulkrabova et al., 2016). The variation in the lipid content might be influenced by several factors such as mother's nutrition, lactation period, sampling time, and physical conditions of the donor mother (reviewed in Kuang et al., 2020; Pulkrabova et al., 2016). Furthermore, we obtained a weak positive correlation between lipid content (%) and Σ_{13}PAHs (ng/mL milk), which was not statistically significant for the investigated breast milk samples from Kampala city (Spearman's rho, $\rho = 0.274$, $p > 0.05$), and Nakaseke district ($\rho = 0.051$, $p > 0.05$). Our findings were similar to those reported by Tsang et al. (2011), and Zanieri et al. (2007). In this paper, concentrations of detected PAHs were lipid-normalized to enable easy comparison of our data with other studies elsewhere since majority of published studies on PAHs have reported concentrations of PAHs in breast milk on lipid weight basis.

As shown in Table 2, the levels of Σ_{13}PAHs in all investigated breast milk samples ranged from 25.85 to 726.59 ng/g lipid weight (lw), with a median level of 247.87 ng/g lw. In general, levels of low molecular weight PAHs (ΣLPAHs: defined as PAHs with 2–3 aromatic rings) which ranged from 19.08 to 675.3 ng/g lw (median = 212.19 ng/g lw) were significantly higher than the levels (median = 39.24 ng/g lw, range: 6.78 to 75.59 ng/g lw) of high molecular weight PAHs (ΣHPAHs: defined as PAHs with 4–5 aromatic rings). Other authors elsewhere have also reported the predominance of the LPAHs over HPAHs in breast milk (Asamoah et al., 2019; Oliveira et al., 2020; Torres-Moreno et al., 2022), as well as in other human matrices such as maternal blood, placenta tissues and umbilical cord blood (Drwal et al., 2017; Yu et al., 2011; Zajda et al., 2017).

Predominance of LPAHs over HPAHs in human breast milk can be attributed to physico-chemical properties of LPAHs, especially their low molecular weight coupled with their lipophilicity which are crucial for their selective transfer in the mammary cells (Cavret et al., 2005; Khanverdiluo et al., 2021; Santonicola et al., 2017). In comparison with other studies elsewhere, the levels of total PAHs in our study, which ranged from 25.9 to 727 ng/g lw were higher than that reported for breast milk of mothers from Turkey i.e., 22.32 to 363.01 ng/g lw (Çok et al., 2012), and Czech Republic i.e., 0.71 to 378 ng/g lw (Pulkrabova et al., 2016).

Table 2
Levels of PAHs (ng/g lw) in breast milk samples collected from Nakaseke district and Kampala capital city, Uganda.

PAHs	Nakaseke (N = 30)				Kampala (N = 30)				All samples (N = 60)			
	DF (%)	Median	Min	Max	DF (%)	Median	Min	Max	DF (%)	Median	Min	Max
Nap	83	3.76	bdl	51.53	93	69.38	bdl	505.33	88	13.22	bdl	505.33
Acy	57	0.85	bdl	12.59	97	14.52	bdl	23.44	77	8.07	bdl	23.44
Ace	73	0.82	bdl	11.05	90	14.29	bdl	252.52	82	9.40	bdl	252.52
Flu	70	0.83	bdl	8.26	93	23.48	bdl	127.69	82	5.34	bdl	127.69
Ant	53	0.66	bdl	66.44	83	40.36	bdl	236	68	3.83	bdl	236
Flt	67	10.63	bdl	21.83	97	13.6	bdl	40.37	82	12.14	bdl	40.37
Pyr	63	0.35	bdl	12.58	87	2.4	bdl	23.85	75	1.21	bdl	23.85
BaA	70	1.93	bdl	3.33	90	1.76	bdl	5.91	80	1.84	bdl	5.91
Chr	47		bdl	7.96	63	0.54	bdl	8.15	55	0.24	bdl	8.15
BbF	0				60	0.28	bdl	5.44	30		bdl	5.44
BkF	30		bdl	1.1	57	0.42	bdl	9.15	44		bdl	9.15
BaP	0				57	0.55	bdl	2.59	29		bdl	2.59
DaA	47		bdl	2.83	63	0.22	bdl	3.12		0.12	bdl	3.12
Σ_{13}PAHs		24.48	0.84	87.85		217.95	3.44	695.77		247.87	25.85	726.59
ΣLPAHs		14.16	0.14	75.05		194.98	1.46	658.29		212.19	19.08	675.3
ΣHPAHs		15.25	0.57	27.44		24.95	1.98	69.56		39.24	6.78	75.59

DF (%)-Detection frequency (percent); bdl-below detection limit; LPAHs-Low molecular weight PAHs; HPAHs-High molecular weight PAHs;
Σ_{13}PAHs = ΣLPAHs + ΣHPAHs.

However, our values were lower than those reported for human breast milk samples from Portugal i.e., 55.2 to 1119 ng/g milk fat (Oliveira et al., 2020), New Mexico i.e., non-detectable (n.d) to 950.7 ng/g milk fat (Acharya et al., 2019), Hong Kong i.e., n.d to 1477 ng/g milk fat (Tsang et al., 2011), and Ghana i.e., n.d to 15,936.57 ng/g lw (Asamoah et al., 2019). The differences in the levels of PAHs in human breast milk samples investigated in our study and previous studies elsewhere might probably be due to the variations in the specific metabolic activity of each donor mother, sampling seasons and time, as well as the different exposure sources affecting each donor mother, such as indoor and outdoor pollution, type of meal consumed before milk collection, and as regards the smokers, the number and type of cigarettes smoked (Pulkrabova et al., 2016; Santonicola et al., 2017; Wang et al., 2018; Zanieri et al., 2007).

Pertaining to breast milk PAHs contamination in our study locations, visualization of Fig. 2 showed that the levels of PAHs were higher among breast milk samples collected from Kampala city (urban area) compared to Nakaseke district (rural area). As shown in Table 2, the levels of Σ_{13}PAHs in breast milk samples collected from Kampala city ranged from 3.44 to 695.77 ng/g lw (median = 217.95 ng/g lw), while those from Nakaseke district ranged from 0.84 to 87.85 ng/g lw (median = 24.48 ng/g lw). The levels of ΣLPAHs and ΣHPAHs in breast milk samples from Kampala city ranged from 1.46 to 658.29 ng/g lw (median = 194.98 ng/g lw), and 1.98 to 69.56 ng/g lw (median = 24.95 ng/g lw), respectively. For breast milk samples collected from Nakaseke district, levels of ΣLPAHs and ΣHPAHs ranged from 0.14 to 75.05 ng/g lw (median = 14.16 ng/g lw), and 0.57 to 27.44 ng/g lw (median = 15.25 ng/g lw), respectively.

The observed variances in the levels of PAHs in breast milk samples from Kampala and Nakaseke can be attributed to the different exposure sources affecting each donor mother like indoor and outdoor pollution, and type of meal consumed before milk collection (Santonicola et al., 2017; Wang et al., 2018). With respect to the former, residents from urban areas are more exposed to a different profile of PAHs emissions like traffic, vehicular and industrial emissions which usually result in a different profile of PAHs mixtures (Ali et al., 2021; Davoudi et al., 2021). Furthermore, food processing procedures depend on a number of parameters like time, type of fuel, distance from heat source, drainage of fat, and cooking methods, which produce many hazardous compounds including the PAHs (Abdel-Shafy and Mansour, 2016; Bandowe et al., 2021). In recent times, the impacts of the different cooking methods and fuel type used on the generation of PAHs during food processing procedures has attracted considerable attention from the researchers worldwide (Bandowe et al., 2021; Kim et al., 2021; Mirzazadeh et al., 2021; Oliveira et al., 2021; Siddique et al., 2021).

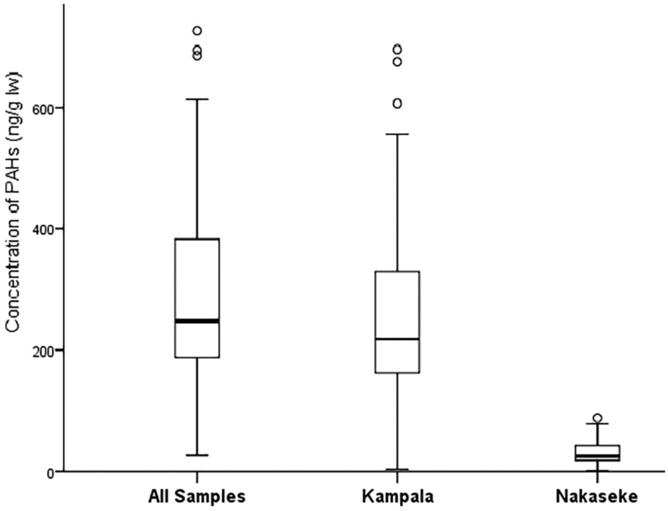

Fig. 2. Five number summary plots (minimum, lower quartile, median, upper quartile, and maximum) of total PAHs (ng/g lw) in breast milk samples collected from Kampala city and Nakaseke district. The dots represent outliers in the concentration of PAHs.

3.3. Influence of cooking methods, and fuel types on PAHs levels in breast milk

From our questionnaire data (Table 1), the frequently used cooking methods by the non-smoking mothers were; boiling (33.3 %), grilling (31.7 %), and deep-frying (31.7 %), while the frequently used fuels were; charcoal (46.7 %), and firewood (45 %). In our study design, we divided 54 non-smoking mothers into 2 groups of frequently used wood fuels i.e., charcoal (27), and firewood (27), equally split by frequently used cooking methods i.e., boiling, grilling and deep-frying, with 9 participants in each group.

A two-way ANOVA (Table S6) was performed to find the impacts of fuel type and cooking methods on the levels of Σ_{13}PAHs in breast milk samples. A statistically significant effect was found for fuel type ($p < 0.05$), and cooking methods ($p < 0.05$), but there was no statistically significant interaction ($p > 0.05$). As presented in Fig. S1, the levels of Σ_{13}PAHs in breast milk samples of different sub-groups increased depending on the cooking methods used by donor mothers in the order; boiling< grilling< deep-frying. In addition, mothers that used charcoal for cooking always accumulated higher levels of PAHs compared to those that used firewood, hence both fuel type and cooking methods seemed to have a main effect on the levels of PAHs in the breast milk of donor mothers.

In our ANOVA model, 33.6 % of the variance in the levels of Σ_{13}PAHs in the investigated breast milk samples is attributed to fuel type and cooking methods used. Studies on different cooking methods have demonstrated that the deep-frying process produced significantly higher levels of PAHs (Siddique et al., 2021; Yao et al., 2015). This is because deep-frying procedure consumes bigger volumes of edible oil, and involves higher oil temperatures (Yao et al., 2015). Moreover, increased amounts of fatty acids in meat products, which arise from the use of frying oils, results into greater absorption of PAHs during heat treatment (Mirzazadeh et al., 2021). Furthermore, grilling, a high temperature cooking method has been reported to produce high levels of PAHs in meat, chicken, and fish (Kafouris et al., 2020; Kim et al., 2021; Sahin et al., 2020).

A study by Gholizadah et al. (2021) found that the median levels of BaP (7.85 μg/kg) and sum of BaP, Chr, BaA and BbF levels (15.74 μg/kg) in grilled Iranian foods exceeded the maximum level imposed by the Commission of the European Communities. In addition, Gholizadah et al. (2021) reported a significant relationship between the Iranian foods prepared with charcoal and amounts of PAHs generated. Another study by Siddique et al. (2021) found that during boiling procedure, majority of PAHs were produced below detection limit in all boiled samples. Solyakov and Skog (2002) also reported that boiling of chicken did not produce large number of carcinogenic compounds because it required low temperatures (<100 °C). In the present study, higher detectable levels of Σ_{13}PAHs in breast milk of mothers from the boiling group can be attributed to the consumption of boiled foods with background contamination and/or inhalation of fumes from the combustion or pyrolysis of charcoal and firewood during the cooking procedures and from the other exposure sources in their surroundings.

Furthermore, we observed higher levels of Σ_{13}PAHs in breast milk samples from donor mothers that used charcoal for cooking compared to those that used firewood as cooking fuel. Although our literature review did not find similar studies for direct comparison, the impacts of using charcoal and firewood as cooking fuels on the levels of PAHs generated in foods are well documented. For example, Silva et al. (2011) reported higher levels of Σ_{16}PAHs in samples of fish cooked using firewood compared to those cooked using charcoal. Akpambang et al. (2009) also reported higher levels of PAHs in the meat and fish grilled using firewood compared to those grilled using charcoal. In addition, studies have also quantified higher levels of PAHs, including the carcinogenic HPAHs in the smoke produced from firewood compared to charcoal (Kim Oanh et al., 1999; Zou et al., 2003).

In contrast, the accumulation of higher levels of Σ_{13}PAHs in breast milk samples of mothers that belonged to the charcoal group compared to firewood group in our study seemed to be greatly influenced by the donor's location. From our questionnaire data, 78.6 % of the charcoal users were from Kampala city, while 85.2 % of the firewood users were from Nakaseke district. Unlike in rural areas, PAHs profiles in breast milk samples from the mothers in Kampala could have possibly been exacerbated by their exposure to intense road dust, vehicular emissions, and industrial processing (Ali et al., 2021; Arinaitwe et al., 2012; Davoudi et al., 2021; Kerebba et al., 2017; Wang et al., 2021a, 2021b; Zhou et al., 2014). Further studies are however needed to confirm this hypothesis.

3.4. Infant's health risk assessment

The potential health risks to infants resulting from exposure to PAHs in breastmilk were estimated based on daily oral intake values and associated hazard quotients relative to RfDs, and incremental cancer risk calculations. There were variations in the daily exposure levels, potentially due to differences to the extent and nature of the sources of PAH exposure among the donor mothers. Overall, Naphthalene had the highest exposure levels in both sampling areas with mean levels of 74 and 837 ng/kg/day for Nakaseke and Kampala, respectively, while BaP had the lowest daily exposure levels with mean 5.31 and 5.11 ng/kg/day, in Nakaseke and Kampala, respectively (Table S2).

Assuming 100 % gastrointestinal absorption of the PAHs in breastmilk, hazard quotients (HQs) were estimated using the oral RfD values for non-carcinogenic effects due to seven PAHs (Table S2). On average, the HQs for the PAHs ranged from 1.65×10^{-4} for Ant to 1.33×10^{-2} for BaP in Nakaseke. For Kampala, the HQs ranged from 1.97×10^{-3} for Ant to 4.19×10^{-2} for Nap. Since all the HQs were far below the safe threshold value of 1, the non-carcinogenic health risk to infants feeding on the PAH-contaminated breastmilk is low.

The incremental carcinogenic risk (ICR) values to the infants due to exposure to the 13 PAHs, based on gender and age groups exposure are presented in Table S3. Across all age groups in our study locations, ICR were all between 10^{-6} to 10^{-4}, indicating that the cancer risk to infants due to the ingestion of PAHs in breast milk was tolerable (Kiani et al., 2021; Qiao et al., 2021). The highest ICR of 1.80×10^{-4} and 8.14×10^{-5} was calculated for infants from Kampala city and Nakaseke district, respectively, implying that PAHs-associated cancer risks could be higher in urban areas than rural areas (Ali et al., 2021; Davoudi et al., 2021), but further studies are needed to confirm this in the case of East Africa, and Africa at large. In addition, for the three age categories considered in this study, both ICR values were in the order; 0–6 > 7–12 > 13–24 months, and in all cases, ICR values were higher for girls than boys. These results could be explained by high intake rate of milk (750 mL/day) and relatively low body weights (7.3–7.9 kg) for infants aged 0–6 months, compared to those aged 7–12, and 13–24 months (Qiao et al., 2021; Wang et al., 2021a, 2021b). The observed differences between girls and boys can be attributed to the relatively lower body weights of the girls (7.3–11.5 kg) compared to boys (7.9–12.2 kg) across all the age categories (Wang et al., 2021a, 2021b; WHO, 2006).

3.5. Study strengths, limitations, and recommendations

To the best of our knowledge, this is the first study to analyze the correlates of measured PAHs in breast milk of nursing mothers to frequently used wood fuels (charcoal, and firewood) and cooking methods (boiling, grilling, and deep-frying) in East Africa, and Africa at large. This study also showed the relevance of using human matrices like breast milk for biomonitoring of indoor, and outdoor pollution. Nonetheless, there were some study limitations encountered; firstly, due to financial constraints, we did not use isotope-labeled standards or certified reference materials to validate our analytical method. However, we conducted several quality assurance and quality control strategies (such as triplicate analyses, recovery tests, spiked blanks, LODs, and LOQs) to ensure the robustness, accuracy, and reliability of our analyses. Our QA/QC results showed that our method was sufficiently robust and accurate. Secondly, the sample sizes ($N = 30$ for Kampala, and $N = 30$ for Nakaseke) were also relatively small. In addition, PAHs and their derivatives from emissions of different fuels, kitchen environments, and cooking methods were not quantified in this study. To generate more generalizable findings, future studies may wish to quantify these pollutants in a bigger population, different environments, and establish their associations with the different cooking methods, and potential health risks in the exposed individuals.

4. Conclusions

We investigated the occurrence and concentrations of Σ_{13}PAHs in the breast milk of the nursing mothers from Kampala city, and Nakaseke district. The levels of Σ_{13}PAHs in investigated breast milk samples ranged from 3.44 to 696 ng/g lw. Levels were higher in samples from urban setting compared to rural setting. PAH mixtures in the breast milk samples were dominated by LPAHs. Based on our ANOVA model results, there was a significant main effect of fuel type and cooking methods on the levels of PAHs in breast milk samples. Donor mothers who used charcoal for cooking accumulated higher levels of Σ_{13}PAHs in their breast milk compared to those that used firewood. The levels of Σ_{13}PAHs in breast milk samples of donor mothers increased depending on cooking methods in the following order; boiling< grilling< deep-frying. The hazard quotients for non-carcinogenic health risks and incremental cancer risk values were all below threshold values suggesting that the breast milk in Uganda is still safe for infant consumption with respect to PAHs. Mothers should be encouraged to breastfeed their babies.

CRediT authorship contribution statement

Fred Ssepuya: Conceptualization, Methodology, Formal analysis, Investigation, Writing-original draft. **Silver Odongo**: Conceptualization, Methodology, Investigation, Writing-review and editing. **Benjamin A. Musa Bandowe**: Conceptualization, Methodology, Investigation, Writing-review and editing. **Juma John Moses Abayi**: Writing-review and editing. **Chijioke Olisah**: Writing-review and editing. **Henry Matovu**: Conceptualization, Methodology, Investigation, Writing-review and editing. **Edward Mubiru**: Conceptualization, Writing-review and editing, Supervision. **Mika Sillanpää**: Writing-review and editing. **Ibrahim Karume**: Writing-review and editing. **Charles Drago Kato**: Writing-review and editing. **Victor Odhiambo Shikuku**: Writing-review and editing. **Patrick Ssebugere**: Conceptualization, Writing-review and editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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