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Polycyclic aromatic hydrocarbon concentrations in commercially available infant formulae in Nigeria: Estimation of dietary intakes and risk assessment

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ABSTRACT

The concentrations and profiles of polycyclic aromatic hydrocarbons (PAHs) in commercially available infant formulae and follow-up formulae in Nigeria were determined with a view to providing information on the health risks to children from the consumption of these infant foods. The concentrations of PAHs were measured by means of gas chromatography–mass spectrometry (GC–MS) after extraction by ultrasonication with acetone/dichloromethane and clean-up. The concentrations of the Σ16 PAHs in these infant formulae ranged from 0.102 to 1.98 μg kg^{-1}, 0.054–1.98 μg kg^{-1}, 0.081–2.54 μg kg^{-1} and 0.51–0.70 μg kg^{-1} for infants of ages 0–6 months, 6–12 months, 1–3 years and 0–12 months respectively. The concentrations of benzo(a)pyrene (BaP) in all samples investigated were below the 1 μg kg^{-1} European Commission permissible limit for BaP in foods meant for infants. The estimated daily intake of PAHs based on the European Food Safety Authority (EFSA) suggested indicators of occurrence and effects of PAHs in foods were not detected (nd) to 2.67 ng BaP kg^{-1} bw day^{-1}, nd-5.29 ng PAH2 kg^{-1} bw day^{-1}, nd-11.20 ng PAH4 kg^{-1} bw day^{-1} and nd-34.96 ng PAH8 kg^{-1} bw day^{-1}. The estimated margin of exposure (MOE) values: BaP-MOE, PAH2-MOE, PAH4-MOE and PAH8-MOE values were greater than 10,000 which indicates that there are no health risks from the consumption of these products by infants. The concentrations and dietary exposure to PAHs from these products were similar to values reported in the literature for European Communities.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic organic compounds that are known to have mutagenic, genotoxic and carcinogenic properties and long-range transportation and deposition characteristics. PAHs consist of two or more fused aromatic rings in a linear, angular or cluster formation that are introduced into the environment primarily by incomplete combustion or heat-induced decomposition of organic matter (Tuteja et al., 2011; Alomirah et al., 2009) and from other anthropogenic processes such as inputs from oil spills, ship traffic, urban run-off, waste water discharges as well as atmospheric fallouts of vehicle exhaust and industrial stack emissions (Qiu et al., 2009). PAHs have relatively low water solubility and are highly lipophilic.

Out of more than 100 PAH compounds that exist in nature, the United States Environmental Protection Agency (USEPA) and European Union have identified the following 16 most frequently occurring/or dangerous PAHs as priority pollutants. They include naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chy), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)-pyrene (BaP), indeno(1,2,3-cd)perylene (IndP), dibenzo(a,h)anthracene (DahA) and benzo(ghi)perylene (BghiP). The International Agency for Research on Cancer (IARC) has further classified benzo(a)pyrene as group 1A (carcinogenic to humans), dibenzo (a,h)anthracene as group 2A (probably carcinogenic to humans) and benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene and indeno(1,2,3-cd)perylene as group 2B (possibly

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carcinogenic to human), while other are not classifiable as regards their carcinogenicity to humans (IARC, 2010). PAHs are encountered in the environment as complex mixtures with varying concentrations and profiles. Although not all PAHs have evidence of known oral carcinogenicity, the non-carcinogenic PAHs may, however, synergistically enhance the carcinogenicity of other PAHs (Wenzl et al., 2006; Al-Rashdan et al., 2010). Heavy PAHs have been recognised to induce dioxin-like activity and disruption of oestrogen response (Villeneuve et al., 2002; Al-Rashdan et al., 2010).

Dietary sources are the major route of human exposure to PAH compounds, except for smokers and occupationally exposed populations (White et al., 2008; Iwegbue et al., 2014a,b). The occurrence of PAHs in human foods reflects the conditions of the environment and consequences of some thermal treatments that are used during the preparation and manufacture of foods (Ciecierska and Obiedzinski, 2007; Perello et al., 2009). For example, processing procedures such as smoking, roasting, grilling, baking, frying and drying, especially using the convection method, have been identified as the major sources of potentially high levels of food contamination (Houessou et al., 2008; Perello et al., 2009; Rey-Salgueiro et al., 2008; Suchanová et al., 2008; White et al., 2008; Iwegbue et al., 2014a,b). The level of contamination of infant formulae and follow-on formulae, that is powdered infant milk and follow-on milk, is a function of the environmental contamination in the area from which milk was collected, and drying conditions used (Kishikawa et al., 2003). When baby foods were taken into consideration, according to a producer's declaration, they were produced from fresh and high quality raw materials, subsequently steamed and pasteurized. In this case their contamination by PAHs may also be the result of environmental contamination of raw materials, especially vegetables.

The study of contaminants in infant foods is of significant interest because children are the most vulnerable group to any form of contaminants in the food chain. Exposure to contaminants during growth and development can have significant acute and chronic effects on children. As their bodies are developing, children consume more food on a per body weight basis than their adult counterparts, therefore children are at a higher risk of illness from exposure to toxic chemicals in foods (Iwegbue et al., 2010). In most countries, child-specific data on food consumption and extent of exposure of children to chemical hazards in foods are grossly limited. In many developing countries, data on contamination of food and exposure through food consumption are not collected or may be incomplete or collected in such a manner that may not give room for inter-country comparison (Vracko et al., 2007; Iwegbue et al., 2010). In view of these facts, the concentrations of contaminants in infant and baby foods requires constant monitoring to ensure strict compliance with regulatory control limits in order to protect public health. Relatively few studies have been reported on the levels of PAHs in infant and baby foods in the literature (Aguinaga et al., 2007; FSAI, 2006; Kishikawa et al., 2003; Rey-Salgueiro et al., 2009; Ciecierska and Obiedzinski, 2010; Cho and Shin, 2012) and no data is currently available on the concentrations of PAHs in infant formulae in Nigeria. This study was undertaken to provide information on the concentrations, profiles and hazards of PAHs in some brands of infant and follow-up formulae in Nigeria. Information from such a study is necessary to improve food sanitation and risk management.

2. Materials and methods

2.1. Sample collection

A total of 40 brands of infant formulae, comprising of 16 brands for the ages of 0–6 months, 13 brands for the ages of 6–12 months, 8 brands for ages of 12–36 months, and 3 brands for ages of 0–12 months, were collected from different retail markets in southern Nigeria. Within a given brand at least 4 samples with a different date of manufacture and batch number were collected and mixed together. From this, a subsample was taken for analysis. All samples were stored in the refrigerator prior to the analyses. The countries of origin of the studied commercial brands of infant foods were Nigeria, France, Ireland, The Nertherlands, Switzerland, USA, Spain, Poland, South Africa and India.

2.2. Reagents

All chemicals and reagents used were of analytical grade. Acetone was from Rieldel-de Haën (Seelze, Germany, and purity 99.8%) whilst dichloromethane (LC grade), anhydrous sodium sulfate (purity 99%), alumina and silica gel were obtained from BDH (Poole, UK). n-Hexane was obtained from Sigma–Aldrich (Steinheim, Germany). A PAH standard mixture containing the 16 priority PAHs: namely, naphthalene (Nap) (1000 μg/mL), acenaphthylene (Acy) (2000 μg/mL), acenaphthene (Ace) (1000 μg/mL), fluorene (Flu) (199.9 μg/mL) , phenanthrene (Phe) (99.8 μg/mL), anthracene (Ant) (100.0 μg/mL), fluoranthene (Flt) (200.1 μg/mL), pyrene (Pyr) (99.9 μg/mL), benzo(a)anthracene (BaA) (100.1 μg/mL), chrysene (Chy) (100.0 μg/mL), benzo(b)fluoranthene (BbF) (200.2 μg/mL), benzo(k)fluoranthene (BkF) (99.9 μg/mL), benzo(a)pyrene (BaP) (100.0 μg/mL), dibenzo(a,h) anthracene (DahA) (200.0 μg/mL), indeno(1,2,3-cd)perylene (IndP) (100.1 μg/mL) and benzo (g,h,i)perylene (BghiP) (200 μg/mL), was provided from Sulpelco (Bellefonte, PA, USA). Working mixed standard solutions containing all the PAHs were prepared by dilution of the stock solution with acetone and stored at −20 °C in darkness to avoid volatilization and photodegradation.

2.3. Sample preparation, extraction and clean-up

A mass of 10 g of the subsamples was extracted by ultrasonication with 50 mL of acetone/dichloromethane (DCM) (1:1 v/v) at 30 °C for 30 min. The contents were filtered and the process was repeated three times by sonication of the residue with a fresh mixture of acetone/dichloromethane each time. The solvent extracts were combined and passed through a column packed with anhydrous Na_2SO_4 by using 50 mL of a 1:1 (v/v) mixture of acetone/DCM, evaporated to dryness with a rotary evaporator and dissolved in 2 mL of hexane. The extracts were purified by solid phase extraction with 2 g of aluminium oxide (5% deactivated, upper part) and 2 g of silica gel (5% deactivated, lower part). The PAHs were subsequently eluted with 15 mL of hexane, 5 mL of hexane + DCM (9:1) and 20 mL of hexane + DCM (4:1). The eluted fractions were combined and evaporated to approximately 0.5 mL.

2.4. Chemical analysis

The PAHs in the eluted fraction were measured with a gas chromatograph (HP 6890 Palo Alto, CA) equipped with a HP5 (cross-linked PHME siloxane) column (0.25 μm film thickness, 0.25 μm × 30 m) and a HP 5973 series mass-selective detector. The mass spectrometer was operated in the electron impact ionization mode (ionizing energy of 70 eV) scanning from m/z 50 to 450 at 3.6 scans/s. The ion source and quadrupole temperature were 230 and 150 °C respectively. The operating conditions were as follows: the carrier gas was helium with a linear velocity of 1 mL/min, the injection port temperature was 290 °C, the injection volume was 2 μL in pulsed splitless mode and the GC/MS interface temperature was 250 °C. The column temperature was initially held at 80 °C for 0.5 min and then increased to 230 °C at 80 °C/min and from 230 to 280 °C at 5 °C/min, and held at 280 °C for 18 min; the solvent delay was 6 min. The total analysis time was 46.25 min and the equilibration time was 2 min.

2.5. Quality control/quality assurance and statistical analysis

Quantification was performed by the use of external calibrations which were obtained with PAH solutions at five concentration levels. To evaluate the extraction efficiency for the target compounds, known concentrations of a standard PAH mixture were added to already analysed samples and re-analysed. The recoveries for the PAH compounds were 72.5% (Nap), 79.5% (Acy), 87.9% (Ace), 99.7% (Flu), 90.8% (Phe), 96.3% (Ant), 89.6% (Flt), 98.2% (Pyr), 77.8% (BaA), 89.1% (Chy), 86.9% (Bbf), 93.5% (Bkf), 89.4% (BaP), 92.6% (InP), 95.4% (DahA) and 89.7% (BghiP). The relative standard deviations for replicate analyses ($n = 3$) were less than 9%. The r^2 values for the calibration lines for the PAH compounds were in the range of 0.9992–0.9999. The method detection limits for the PAH compounds ranged between 0.001 and 0.002 μg kg^{-1}. Analysis of variance and Tukey multiple-comparison tests were used to determine whether the concentrations of the PAHs varied significantly within and between the groups. Differences with p values less than 0.05 ($p < 0.05$) were considered to be statistically significant. The statistical calculations were performed with SPSS version 11.5.

3. Results and discussion

The concentrations of the Σ16 PAHs in the different brands of infant and follow-up formulae ranged from 0.102 to 1.98 μg kg^{-1}, 0.054–1.98 μg kg^{-1}, 0.081–2.54 μg kg^{-1} and 0.51–0.70 μg kg^{-1} for