Polycyclic aromatic hydrocarbons in infant formulae, follow-on formulae, and baby foods in Iran: An assessment of risk

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A R T I C L E   I N F O

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A B S T R A C T

Twenty-seven samples of infant formulae and follow-on formulae and fifteen samples of baby food from Iranian markets were analyzed for concentrations of four polycyclic aromatic hydrocarbons (PAH4) determined by use of gas chromatography coupled to mass spectrophotometry. An assessment of risks posed to infants and toddlers was conducted by calculating the margin of exposure and incremental lifetime cancer risk (ILCR) by use of the Monte Carlo Simulation Method. Benzo (a) anthracene, was not detected in any of the samples, while approximately 64.3% samples contained detectable amounts of benzo (a) pyrene, while chrysene was observed in three samples and benzo (b) fluoranthene was detected in one sample. One of the samples contained 1.43 μg PAH4/kg, which was greater than the maximum tolerable limit (MTL; 1 μg/kg) stated in Commission Regulation (EU) 2015/1125. Accordingly, the 95% ILCRs in the infants/toddlers due to ingestion of milk powder and baby foods were determined to be 1.3 × 10\(^{-6}\) and 7.3 × 10\(^{-7}\), respectively. Also, the 95th centiles of the MOEs due to ingesting milk powder or baby foods by infants/toddlers were estimated to be 3.6 × 10\(^{4}\) and 7.2 × 10\(^{4}\), respectively. In Iran, infants and toddlers are not at serious health risk (MOE ≥ 1 × 10\(^{4}\) and ILCR < 1 × 10\(^{-4}\)).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are comprised of two or more fused aromatic rings (Wang et al., 2018). It has been shown that PAHs are produced by pyrolysis of organic substances and incomplete combustion (Belo et al., 2017; Wang et al., 2018). Except for smokers, injection of food is the predominant route of exposure of humans to these carcinogens (Iwegbue et al., 2014; Rozentale et al., 2017). PAHs occur in foods, not only due to environmental conditions (i.e. deposition on vegetable leaves), but can be created by heat treatment such as grilling, smoking, and smoke-drying (de Lima et al., 2017; Rozentale et al., 2017).

Based on toxic potency, benzo (a) anthracene (BaA), chrysene (Chr), benzo (b) fluoranthene (BbF) and benzo (a) pyrene (BaP) are the most important PAHs, and their sum has been defined as PAH4 (Rozentale et al., 2017). Chronic exposure to PAHs can result in skin inflammation, cataracts, asthma-like symptoms and kidney damage. Also, teratogenic, genotoxic, and immunotoxic effects of these compounds have been
documented (Rengarajan et al., 2015). The International Agency for Research on Cancer (IARC) has categorized BaP as a known carcinogen to humans (group 1A). In addition, BaA, Chr, and BbF are listed as possibly carcinogenic to humans (group 2B) (Iwegbue et al., 2014).

Safe and sufficient diets are essential for growth of babies. Because of their vulnerability, infants and toddlers are sensitive to noxious effect of toxicants. In comparison with adults, infants exhibit greater absorption capacity, lesser ability to detoxify and lesser ability to metabolize residues. Once carcinogenic compounds, such as PAHs are absorbed, they are able to distribute into various tissues, particularly those of greater lipid content (Rey-Salgueiro et al., 2009). As children grow their bodies need greater quantities of food. Therefore, they are at greater risk of dietary exposure to food contaminant. Hence, postnatal exposure to food contaminant is of concern (Santonicola et al., 2017b).

Products consumed by infants and toddlers, such as formula milk, canned fruits and vegetables, and baby food are used as alternatives to breast milk (Nepalia et al., 2017). In accordance with conclusions by the medical community, when breast feeding is not sufficient or possible, infant formulas are the best choice for feeding. Baby food is defined as any food which can be used by infants and toddlers during the period of ages 6 months to 2 years (Nepalia et al., 2017). European regulations have set 1 μg/kg as permissible concentration for the sum of the four PAHs constituting PAH4 in infant formulae, follow-on formulae, processed cereal-based foods and baby foods (Purcaro et al., 2013).

Manufacturing conditions and environmental contamination are two important factors affecting PAHs content of milk powders (Amiridvani et al., 2019; Ciecierska and Obiedziński, 2010).

Food standards agency (FSA) evaluated PAHs levels in 97 samples of infant formulae milk, collected from across the UK. In 39 samples, BaP level was found to be lower than 1.0 μg/kg (FSA (Food Standards Agency), 2006). Kishikawa and co-workers determined PAHs concentration in baby food samples. They found that PAHs levels were about 2.0 ± 0.30 μg/kg (Kishikawa et al., 2005; Yebra-Pimentel et al., 2015). In 2009, by using Sep-Pack silica plus cartridges as SPE mini columns, PAHs were quantified in infant foods (Rey-Salgueiro et al., 2009). In silica-based cartridges, triglycerides are retained, while PAHs can be eluted by n-hexane as the solvent mobile phase (Purcaro et al., 2013). Currently, several instruments, including high performance liquid chromatography (HPLC), liquid chromatography tandem mass spectroscopy (LC-MS/MS), and gas chromatography mass spectroscopy (GC-MS) have been employed for identification and quantification of PAHs (Nyiri et al., 2017; Rey-Salgueiro et al., 2009; Wang et al., 2018).

Since health is associated with various variables and factors, evaluation of risk is a complicated process. Estimation of risk by single-point values can result in uncertainties (Fathabad et al., 2018). Monte Carlo Simulation (MCS) can be applied to evaluate uncertainties during assessment of risks associated exposure of humans to contaminants. MCS is proposed for quantification of uncertainty and variability (Keramati et al., 2018; Razzaghi et al., 2018; Yousefi et al., 2018). Moreover, Sensitivity analysis is performed to identify the most important variable which affect accuracy of risk assessment (Xia et al., 2013).

In the study, results of which are presented here, concentrations of PAH4 were quantified in common brands of infant formula, follow-on formula, and baby-food in Iran. In addition, risks to health due to injection of food by infants and toddlers were determined by calculating the margin of exposure (MOE), while incremental lifetime cancer risk (ILCR) was estimated by use of the MCS method.

2. Material and methods

2.1. Reagents and materials

All chemicals and reagents were of analytical grade. benzo (a) anthracene (BaA), chrysene (Chr), benzo (b) fluoranthene (BbF), benzo (a) pyrene (BaP) and triphenyl phosphate (TPP) were procured from Sigma-Aldrich (Steinheim, Germany). Anhydrous sodium sulfate (purity 99%) obtained from BDH (Poole, UK). Sep-Pak silica plus long cartridges (690 mg sorbent per cartridge, 55–105 μm particle size) supplied by Waters (Wexford, Ireland).

Stock solutions of PAHs at 1000 mg L−1 were prepared in n-hexane wrapped in aluminum foil. In addition, TDP stock solution was provided in acetone at 1000 mg L−1. To prevent volatilization and photodegradation, stock solutions were stored in the dark at 4 °C. Also, more diluted solutions at 10 mg L−1 were prepared monthly. A working standard mixed solution was prepared daily by diluting with n-hexane.

2.2. Instruments

Identification and quantification were accomplished by use of GC analyses with a Agilent 6890 N (Agilent, Waldbronn, Germany) gas chromatograph, coupled to a Agilent 5975 quadrupole mass selective spectrometer equipped with an inert ion source and provided with a split–splitless injection port. The carrier gas helium was maintained at a constant pressure of 13.00 psi and purge flow of 30.0 ml/min. A DB-5MS (Agilent) capillary column (5% diphenyl- 95% dimethyl poly-siloxane) (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. Operating conditions were as follows: helium carrier gas 1.5 ml/min (constant flow); injector temperature 300 °C, pulsed splitless, injection volume of 1 μL; the ion source, transfer line, and quadrupole temperature 300 °C, 280 °C, and 180 °C, respectively; the GC oven temperature program: 55 °C (1 min), 55–290 °C (25 °C/min), (3 min). The quadrupole analyzer measured the abundance of ions of m/z from 45 to
450 and detector voltage was 1294 V. Electron ionization (70 eV) with selected ion monitoring mode was used, and the most abundant fragment from the molecular ion cluster was measured for each analyzed compound (Szlewniowski, 2005). PAHs were identified based on comparisons of observed GC retention time with those of standard solutions of PAHs and use of characteristic ions.

2.3. Sample collection and preparation

Twenty-seven samples of baby milk powder and fifteen samples of baby food were collected from retail markets of Iran. Samples of baby milk included: infant milk formulae (0–6 months) coded as A1, B1, and C1 (n = 3); Follow-on formulae (6–12 months) coded as A2, B2, and C2 (n = 3); Follow-on formulae (1–2 years) coded as A3, B3, and C3 (n = 3). Samples of baby food included wheat + milk, honey + wheat with milk, wheat + palm with milk, banana + wheat with milk and fruit + wheat with milk (n = 3). Within a given brand, 3 samples with different dates of manufacture were purchased and composited. From this, a subsample was taken for analysis. All samples were refrigerated prior to analyses (Iwegbue et al., 2014).

The pre-analytical treatment was relied on a method for the determination of PAHs in infant foods, smoked foods, and instant coffee previously reported by other researchers (Falcón et al., 1996; García-Falcón et al., 2005; Rey-Salgueiro et al., 2008; Rey-Salgueiro et al., 2009). 1.0 g of samples spiked with TPP as an internal standard (IS) (Szlewniowski, 2005) were subjected to ultrasound-assisted solvent extraction with 3 × 10 mL n-hexane/acetone (95:5 v/v) for 10 min each in an amber glass vials (Oh et al., 2016; Ciecierska and Obiedziński, 2010). Solvent extracts were filtered (filter paper) and passed through a column packed with anhydrous Na2SO4 (Ciecierska and Obiedziński, 2010). In next step, solutions were cleaned-up, using Sep-Pack silica plus cartridges" (Wexford, Ireland) that had been preconditioned with 10 mL n-hexane. Then, 10 mL hexane/acetone (95:5 v/v) added to avoid loss. With the help of a gentle stream of nitrogen, the elute was evaporated until about 1 mL. The obtained elute filtered with PTFE filter (0.45μm) and transferred to amber vial glass and evaporated till full dryness. Finally, re-dissolved in 0.2 mL n-hexane for GC-MS analysis.

2.4. Quantification and method validation

Quality assurance and Quality control were ensured by quantification of recoveries from matrix spikes. Compounds quantification was performed by using an internal standard method. Calibrations were obtained with PAH solutions at six concentrations.

The calibration curve for each compound was constructed by plotting the ratio of the peak area of standards to peak area of internal standard against the concentration (Moudgil et al., 2019; Wang et al., 2018). Coefficients of determination (R2) values obtained were between 0.991 and 0.997. The limit of detection (LOD), defined as signal to-noise 3:1, and the limit of quantification (LOQ), defined as signal to-noise 10:1 (Lee et al., 2018), as well as recovery experiments were carried out. Accuracies were determined by spiking samples of infant milk formula and infant food with three different concentrations of PAH4 standards (Ciecierska and Obiedziński, 2010). Method precision was analyzed by intraday and inter-day assays at three concentrations each day for three consecutive days (Table 1).

2.5.5. Characterization cancer risk

Assessment of ILCR, recommended by United States Environmental Protection Agency and MOE suggested by World Health Organization and EFSA Scientific Committee (EFSA, 2005) were employed (Tables 2 and 3) (Lee et al., 2018). Daily dietary intakes of PAHs (EDI) were calculated based on published methods (Li et al., 2016a; Tian et al., 2018) (Equations (1–3)).

\[
E_D = \sum_{i=1}^{n} \text{BEC} \times \text{IRi}
\]  
(1)

\[
\text{BEC} = \sum_{i=1}^{n} C \times \text{TEFi}
\]  
(2)

Concentrations of PAH4 were converted to concentrations of benzo(a) pyrene equivalents (BaPeq; μg kg\(^{-1}\)), (BEC) in infant formulae, follow-on formulae, and baby foods by use of toxicity equivalency factors (TEFs). Values of TEF, for BaA, Chr, BbF and BaP are 0.1, 0.001, 0.1 and 1, respectively (Xia et al., 2010). IR and C are ingestion rate of food and concentration of PAH congener in food, respectively (Xia et al., 2010).

2.5.1. Calculation of ILCR

Cancer risk correlated with consumption of infant formulae, follow-on formulae and baby foods were calculated, based on previously published methods (Li et al., 2016a; Tian et al., 2018) (Equation (3)).

\[
\text{ILCR} = E_D \times ED \times EX \times CF/(\text{BW} \times AT)
\]  
(3)

Where ILCR is incremental lifetime cancer risk (dimensionless); E_D is the daily dietary PAH exposure level (ng day\(^{-1}\)), EX is exposure frequency (365 day year\(^{-1}\)), ED is duration of exposure (years), CSF is the oral cancer slope factor of BaP with geometric mean of 7.3 \([\text{mg/kg/day}]\) (Srivastava et al., 2017), CF is conversion factor (10\(^{-6}\) g ng\(^{-1}\)), BW is the body weight and AT is mean time for development of carcinogens (25 550 days) (Fakhri et al., 2018; Li et al., 2016b; Shahrbabki et al., 2018; Taghizadeh et al., 2017, 2018).

2.5.2. Calculation of MOE

The MOE, which is the inverse of the risk, is another approach to evaluate the risk compounds such as PAH4, which exhibited both carcinogenic and genotoxic effects (Santonicola et al., 2017b). By dividing the Benchmark Dose Lower Limit (BMDL\(_{10}\)) with the estimated daily intake (EDI), MOE can be calculated (Suparmi et al., 2018). BMDL\(_{10}\) is the lower bound of a 95% confidence interval on the benchmark dose corresponding to a 10% tumor incidence in test animal (Iwegbue et al., 2014). EDI was calculated by use of previously published methods (Rahmani et al., 2018; Santonicola et al., 2017b) (Equation (4)).

\[
\text{EDI} = \frac{CI \times \text{IRi}}{\text{BW}}
\]  
(4)

2.6. Uncertainty analysis for carcinogenic risk

MCS (n = 10 000) was used to evaluate the uncertainties and its impact on the risk estimation. This probabilistic modeling, employing the entire range of input variable to develop a probability distribution of exposure or risk rather than a single point data (Zhou et al., 2011). The model input parameters applied in the simulation was showed in Table 3. The MCS and sensitivity analysis were all implemented by Oracle Crystal Ball (version 11.1.4512.0). All values were extracted from Oracle Crystal Ball and then figures plotted by employing Origin 2018 software (version: 9.50.00).

2.7. Sensitivity analysis

Sensitivity analysis was conducted to identify the most significant input data and risk model factors that affected the output values (Zhou et al., 2011). Input variables were BaPeq, ED, EF, IR, CSF, CF, BW, AT and BMDL\(_{10}\).
3. Results

3.1. Method validation

European Commission Regulation (EC) No. 333/2007 defines the conditions for approaches for analysis for BaP as follows: LOD < 0.3 μg/kg, LOQ < 0.9 μg/kg, recovery from 50 to 120%. LOD of BaA, Chr, BbF and BaP calculated 0.02, 0.05, 0.04 and 0.04 μg/kg, respectively. LOQ values for these compounds were 0.11, 0.17, 0.14 and 0.16 μg/kg, respectively. Recoveries for PAH4 were in the acceptable range, which demonstrated accuracy of the analyses (Soceanu et al., 2016) (Table 1).

3.2. PAH4 profile in infant formulae and follow-on formulae

Results, which are presented here, revealed that none of the infant formulae or follow-on formulae were contaminated with BaA (Table 4). Chr was detected in 22.2% of samples with concentrations from 0.53 ± 0.06 to 0.95 ± 0.11 μg/kg. BbF was determined in 11.1% of infant formulae and follow-on formulae. Concentrations of BaP in 55.5% of samples exceeded the limit of quantification.

3.3. PAH4 profile of baby foods

Similar to infant formulae and follow-on formulae, in baby foods, concentrations of BaA were less than the limit of quantification. Concentrations of Chr were detected in 20% of baby foods with a mean of 0.18 ± 0.01 μg/kg. BaP detected in 80% of baby food samples, at concentrations ranging from 0.190 ± 0.02 to 0.48 ± 0.04 μg/kg. Concentrations of BbF did not exceed the limit of quantification in any sample of baby foods.

3.4. Risk assessment of PAH4 based on ILCR

Results demonstrated that ILCR values for infant formulae and follow-on formulae ranged from $1.7 \times 10^{-7}$ to $1.2 \times 10^{-6}$ (obtained from single point values) (Table 5). C3 and A2 exhibited the greatest values for ILCR, which were $1.3 \times 10^{-6}$ and $1.1 \times 10^{-6}$, respectively. In baby foods, ILCR values ranged from $3.1 \times 10^{-7}$ to $7.1 \times 10^{-7}$. Based on MCS, 95th centiles for the ILCR in infants/toddlers exceeding were estimated to be $5.6 \times 10^{-6}$ and $3.1 \times 10^{-7}$, respectively (Figs. 1 and 2). 50th centiles (mean) ILCR values for infants and toddlers were $1.1 \times 10^{-6}$ and $1.4 \times 10^{-7}$, respectively (Figs. 1 and 2).

3.5. Risk assessment of PAH4 based on MOE

MOE, which are the inverse of risk quotients (RQ) were calculated for infants and toddlers (Table 5). Rely on single point values, least values for MOE values were observed for A2, for which MOEs were $3.3 \times 10^{3}$, $7.0 \times 10^{3}$, and $1.2 \times 10^{4}$, for BaP, PAH2 (sum of BaP and Chr) and PAH4, respectively. Maximum values observed for MOE in A3 and B3 were $2.7 \times 10^{4}$, $3.1 \times 10^{4}$ and $3.6 \times 10^{4}$ for BaP, PAH2, and PAH4, respectively. In baby food samples, the greatest MOEs were observed in samples of wheat + milk were $4.8 \times 10^{4}$, $5.7 \times 10^{4}$, and $6.4 \times 10^{4}$ for BaP, PAH2, and PAH4, respectively. Also, the least MOEs for BaP, PAH2, and PAH4, which were $1.6 \times 10^{4}$, $2.9 \times 10^{4}$, and $4.2 \times 10^{4}$, respectively were observed in samples of wheat + palm. Employing MCS, 95th centiles MOE for consumption of milk powders and baby foods for infants/toddlers were estimated to be $2.1 \times 10^{5}$ and $6.1 \times 10^{5}$, respectively (Figs. 3 and 4). The 50th centiles MOE for intake of milk powders and baby foods for infants and toddlers was predicted to be $3.4 \times 10^{5}$ and $3.5 \times 10^{5}$, respectively (Figs. 3 and 4).

3.6. Parameters sensitivity analysis

The quantitative sensitivity analysis was conducted to determine the greatest effects of input parameters on the health risk assessment of milk powder and baby food (Yang et al., 2015). The most significantly influential parameters which determined by sensitivity analysis during the MCS were shown (Fig. 5–8). Results revealed that $\Sigma$ BaP$\varepsilon_{0.69}$, ED (0.42) and IR (0.30) were the most sensitive variables. In both samples of milk powder and baby food, BW contributed $-0.19$ and $-0.07$, respectively. When risk analysis conducted by MOE, BW had the greatest effect on risk estimates.
Concentrations of \( \Sigma \) BaP equivalency (\( \mu g/kg \)), ILCR, EDI (ng/kg bw/day) and MOE for infant formula, follow-on formula and baby food.

**Table 4**

Concentrations (Mean ± SD, \( \mu g/kg \)) of individual PAHs included in PAH4 in samples of infant formula, follow-on formula, and baby food (\( n = 3 \)).

<table>
<thead>
<tr>
<th>Sample</th>
<th>BaA</th>
<th>Chr</th>
<th>BbF</th>
<th>BaP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>&lt;0.11</td>
<td>0.95 ± 0.11</td>
<td>&lt;0.14</td>
<td>&lt;0.16</td>
</tr>
<tr>
<td>B1</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>C1</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>A2</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>B2</td>
<td>&lt;0.11</td>
<td>0.53 ± 0.06</td>
<td>&lt;0.14</td>
<td>&lt;0.16</td>
</tr>
<tr>
<td>C2</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>A3</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>B3</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>C3</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>Wheat + hony</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>Wheat + fruit</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>Wheat + palm</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>Wheat + milk</td>
<td>&lt;0.11</td>
<td>&lt;0.17</td>
<td>&lt;0.14</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>Wheat + banana</td>
<td>&lt;0.11</td>
<td>&lt;0.18 ± 0.01</td>
<td>&lt;0.14</td>
<td>0.19 ± 0.02</td>
</tr>
</tbody>
</table>

A1, B1 and C1 are infant formula (0–6 months). A2, B2 and C2 are follow-on formula (6–12 months). A3, B3 and C3 are follow-on formula (1–2 years).

### 4. Discussion

Concentrations of PAH4 listed by the EU Scientific Committee on Food were determined in the most consumed brands of infant formulae, follow-on formulae, and baby food in Iran.

According to the EU 2015/1125 dated 10 July 2015, the maximum tolerable limit for BaP and sum of PAH4 in the group of infant formulae, follow-on formulae, and baby foods has been set at a concentration of 1 \( \mu g/kg \) (Commission, 2015). In this study, except for one product, A2, which contained 1.43 ± 0.30 \( \mu g/kg \) (sum of PAH4), the other samples complied with EU criteria. The results of this study were consistent with results of previous studies. In 2017, concentrations of 14 PAHs most important PAH listed by Commission Regulation (EC) No. 1881/2006 were detected by high-pressure liquid chromatography with fluorescence detector in 30 samples of the Italian infant formulae. In this study, concentrations of BaP ranged from 0.00 to 1.66 \( \mu g/kg \) (with mean of 0.46 \( \mu g/kg \)) (Santonicola et al., 2017b). In a study, using GC-MS, performed in Nigeria, concentrations of PAHs in various infant formulae and follow-up formulae. Herein, different infant formulae included 6–12 months, 1–3 years and 0–12 months were analyzed. Concentrations of BaP in all samples was less than 1 \( \mu g/kg \), with a range of < 0.001–0.165 \( \mu g/kg \). Also, the concentration of PAH4 in these products varied from < 0.001 to 0.651 \( \mu g/kg \) (Iwegbue et al., 2014). In a study conducted in Poland, infant formulae, follow-on formulae and baby foods were monitored for PAHs presence. In most samples, none of the 15 PAHs were observed, while BaP was only determined in two kinds of soups at lesser concentrations than the legal limit (Ciecierska and Obiedziński, 2010). In another study, occurrences of 11 PAHs and their hydroxylated metabolites were quantified in 17 infant cereals and 19 infant formulae. Only benzo (k) fluoranthene (BkF) was found at concentrations of 0.30 and 0.10 \( \mu g/kg \) in one sample of cereal and milk, respectively (Rey-Salgueiro et al., 2009).

Recent study, in a study of presence of PAHs in milk and meat/fish based baby food from Italy showed that concentrations of BaA, Chr, BbF and BaP in milk based baby foods were in the range 0.00–4.05, 0.00–6.68, 0.00–2.94 and 0.06–2.09 \( \mu g/kg \), respectively. In addition, these contaminants in meat/fish based baby foods were in the range of 0.00–2.39, 0.00–2.11,

### Table 5

Concentrations of \( \Sigma BaP \) equivalency (\( \mu g/kg \)), ILCR, EDI (ng/kg bw/day) and MOE for infant formula, follow-on formula and baby food.

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample</th>
<th>( \Sigma BaP ) eq.</th>
<th>ILCR(^a)</th>
<th>EDI</th>
<th>MOE(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant formula (0-6a)</td>
<td>A1</td>
<td>0.19</td>
<td>2.0 × 10^-7</td>
<td>3.25</td>
<td>22.60</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>0.57</td>
<td>6.1 × 10^-7</td>
<td>11.12</td>
<td>14.56</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>&lt;0.11</td>
<td>6.5 × 10^-7</td>
<td>11.95</td>
<td>15.41</td>
</tr>
<tr>
<td>Follow-on formula (6-12a)</td>
<td>A2</td>
<td>1.27</td>
<td>1.1 × 10^-6</td>
<td>21.36</td>
<td>24.29</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>0.18</td>
<td>1.7 × 10^-7</td>
<td>2.75</td>
<td>11.93</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>0.74</td>
<td>6.6 × 10^-7</td>
<td>12.30</td>
<td>15.22</td>
</tr>
<tr>
<td>Follow-on formula (1-2b)</td>
<td>A3</td>
<td>0.18</td>
<td>3.2 × 10^-7</td>
<td>2.62</td>
<td>5.41</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>0.18</td>
<td>3.2 × 10^-7</td>
<td>2.62</td>
<td>5.41</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>0.75</td>
<td>1.3 × 10^-6</td>
<td>11.94</td>
<td>14.72</td>
</tr>
<tr>
<td>Baby food (6a – 2h)</td>
<td>Wheat + honey</td>
<td>0.37</td>
<td>5.3 × 10^-7</td>
<td>3.14</td>
<td>4.69</td>
</tr>
<tr>
<td></td>
<td>Wheat + fruit</td>
<td>0.21</td>
<td>3.1 × 10^-7</td>
<td>1.73</td>
<td>3.27</td>
</tr>
<tr>
<td></td>
<td>Wheat + palm</td>
<td>0.50</td>
<td>7.1 × 10^-7</td>
<td>4.333</td>
<td>5.89</td>
</tr>
<tr>
<td></td>
<td>Wheat + milk</td>
<td>0.18</td>
<td>2.6 × 10^-7</td>
<td>1.45</td>
<td>3.29</td>
</tr>
</tbody>
</table>

\(^a\) represents month.
\(^b\) shows year.
\(^c\) means obtained from single point values.
0.00–1.16 and 0.06–1.66 μg/kg, respectively (Santonicola et al., 2017a).

Except for smokers and occupational exposure, ingestion via food is one of the important routes of PAHs uptake along with dermal exposure. Concentrations of PAHs in dietary sources are a function not only of environmental state, but also thermal conditions. In the case of infant formulae and follow-on formulae, occurrence of PAHs is a function of conditions of drying as well as contamination of the source products (Iwegbue et al., 2014). PAHs can contaminate grass and feed ingredients of ruminants. When compared with rural areas, grass in industrial regions can contain these compounds at concentrations as much as eight-fold greater (García Londoño et al., 2017). Contamination of vegetables and fruits as the result of air deposition, irrigation with polluted water and soil uptake are other routes which affect concentration of PAH in final products (Cho and Shin, 2012). Alternatively, heat processes such as spray drying and evaporation can result in

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**Fig. 1.** Estimation of ILCR for infants due to consumption of PAH4 occurred in milk powder (E means × 10).

**Fig. 2.** Estimation of ILCR for infants due to consumption of PAH4 occurred in Baby food (E means × 10).
in formation of carcinogenic or genotoxic PAHs (Cho and Shin, 2012).

Determination of eight compounds (PAH8) including BaP, Chr, BkF, BaA, BbF, dibenz (a,h) anthracene (DahA), indeno (1,2,3-cd) pyrene (IcdP), benzo (ghi) perylene (BgHiP) is appropriate for monitoring of PAHs in food. However, when compared with PAH4, PAH8 does not offer much added value (Zelinkova and Wenzl, 2015).

Due to vulnerability of infants and toddlers to carcinogenicity of PAH4, the assessment of potential health impacts of PAH4 in infant formula and infant foods seems crucial (Benford et al., 2010). USEPA defines that a level of risk where there is a lifetime cancer risk of one in ten thousand or greater (10^{-5}), is considered serious (Nie et al., 2014). In our study, the 95% ILCRs in the infants/toddlers due to ingestion of milk powder and baby foods were determined to be 5.6 × 10^{-6} and 3.1 × 10^{-7}, respectively. Hence, the consumption of milk powders and baby foods does not possess serious risk for infants and toddlers.

Use of the MOE is recommended by EFSA Scientific Committee to estimate risk level of carcinogenic and genotoxic PAH (EFSA, 2005). The MOE of 1 × 10^{4} or higher signifies low public health concern (Yousefi et al., 2018). The 95th centiles of the MOEs, due to ingesting

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**Fig. 3.** Estimation of MOE toddlers due to consumption of PAH4 occurred in milk powder.

**Fig. 4.** Estimation of MOE for toddlers due to consumption of PAH4 occurred in Baby food.
milk powder or baby foods by infants/toddlers were estimated to be $2.1 \times 10^5$ and $6.1 \times 10^5$, respectively. Hence, the consumption of milk powders and baby foods does not possess serious risk for infants and toddlers.

It should be mentioned that a significant difference was observed between MOE values obtained from single point values and data obtained by MCS. This is because of input variable employed for each models. For example, when MOE obtained by single point data, IR assumed 100 g/day. However, this value in MCS expected 39 ± 3 g/day. This difference will affect final output, remarkably.

Uncertainties are inherent in evaluation of risk, which could refer to toxicity assessment and exposure. Although MCS was applied to assess the uncertainties and its impact on risk estimation, there were still other uncertainties during the processes of risk characterization, especially for the variables determined by sensitivity analysis. Because of the limited dose–response data on carcinogenic compounds, the risk assessment of PAHs mixture for human remains changeable. Herein, the TEFs were used to convert PAHs concentrations to BaPeq level. These values have acquired from animal studies. It was found that TEF values obtained from experimental data always led to in different TEF values, especially for high weight PAHs. Specific-chemical parameters such as CSF and BMDL10 were obtained from animal studies. Uncertainties may occurred during the extrapolation of animal data to human. In addition, probability distributions of BW was obtained from previous published studies, which can results in uncertainty (Wu et al., 2011).

Similar to our findings, in 2016, Li and co-workers exhibited that concentration of PAHs, ED, CSF and IR are important variables that sensitively correlated with ILCR (Li et al., 2016a). The results were consistent with the report of Xia et al. (2010) which estimated the carcinogenic risk of PAHs in twenty-five kinds of seven categories of food in China. They showed that CSF and ED are the most important factors in health risk assessment. Several studies reported that ED was the sensitive parameter in the risk assessment (Wu et al., 2011; Yang et al., 2014, 2015). Another study showed that concentration of PAHs
and ED are the most influential variable in quantitative evaluation of carcinogenic potential of PAHs (Yang et al., 2015). Hence, based on the sensitivity results, we can enhance the accuracy of the risk assessment by improving the accuracy of $\Sigma$ BaP eq., ED, CSF and IR.

5. Conclusions

In the current study, the levels of PAH4 were determined in commonly used brands of infant formula, follow-on formula, and baby foods in Iran, using GC-MS. In addition, risk assessment for infants and toddlers performed, with the help of incremental lifetime cancer risk (ILCR) and margin of exposure (MOE) formulas in Monte Carlo Simulation (MCS) method. The results of MCS showed that there is no serious risk for infants and toddlers by ingestion of infant formula, follow-on formula and baby foods. Furthermore, based on the sensitivity results in this study, we found that $\Sigma$ BaP eq., ED, CSF and IR are the most influential factors in risk assessment. Due to presence of PAH4 in analyzed brands, particular attention is required. Presence of PAHs in infant formulae and follow-on formulae products depend both on environmental pollution of the area in which milk was produced and conditions during processing. Although production of PAHs requires temperatures of 500–700°C, PAHs can be produced at 100–150°C. Packaging material is another factor from which contents can become contaminated by PAHs. It is recommended to select non-polluted raw material and establish good manufacturing practice to minimized contamination of final products. Because of low sample size in our study, further research on PAHs content in such products is still needed to continuously control the exposure of infants and babies to these compounds.
Conflicts of interest
The authors declare that there are no conflicts of interest.

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