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Comparative study on the occurrence of polycyclic aromatic hydrocarbons in breast milk and infant formula and risk assessment

Serena Santonicola [a], Alessandra De Felice [b], Luigi Cobellis [c], Nicola Passariello [d], Angela Peluso [d], Nicoletta Murru [a], Maria Carmela Ferrante [a], Raffaelina Mercogliano [a, *]

[a] Department of Veterinary Medicine and Animal Production, University of Naples, Italy
[b] Experimental Zooprophylactic Institute of Southern Italy, Portici, Italy
[c] Department of Gynaecology, Obstetrics and Reproductive Medicine, Second University of Naples, Italy
[d] Department of Internal Medicine, Second University of Naples, Italy

HIGHLIGHTS

- Occurrence of polycyclic aromatic hydrocarbons in breast milk and infant formula.
- Carcinogenic and marker PAHs were higher in breast milk than in infant formula.
- Occurrence of light and heavy PAHs from petrogenic and pyrolytic sources.
- PAH contamination needs to be evaluated as a potential hazard for human health.
- Appropriate measures are need for the reduction of pollutants in infant food.

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ABSTRACT

The study compared the polycyclic aromatic hydrocarbons (PAH) profile of human milk collected from Italian mothers and different brands of infant formula available on Italian market. Levels of 14 PAHs most frequently occurred in food, PAH markers listed by Commission Regulation (EC) No. 1881/2006, and carcinogenic PAHs classified by the International Agency for Research on Cancer, were determined by high-pressure liquid chromatography with fluorescence detector.

The average concentrations of total PAHs were 114.93 in breast milk and 53.68 $\mu g\ kg^{-1}$ in infant formula. Furthermore, Benzo(*a*)pyrene (BaP) and the sum of ∑PAH4 markers (BaP, Chrysene, Benzo(a,h) anthracene and Benzo(*b*)fluoranthene) were higher than the permissible limit of 1 $\mu g\ kg^{-1}$ in 43% and 86% for breast milk and in 10% and 76% for infant formula samples, respectively. Breast milk showed higher levels ($P < 0.05$) of carcinogenic, and possible carcinogenic hydrocarbons than infant formula samples.

Both in human and commercial milk, data showed the occurrence of low and high molecular weight PAHs, respectively from petrogenic and pyrolytic environmental sources, characterizing the infant and mother exposure. Particularly, waste incineration could have represented an important exposure source for infants during breastfeeding, through exposition of mothers resident in some areas of Southern Italy. High PAH levels detected in infant formula enriched with LC-PUFA might be related to the contamination of the vegetable oils added as ingredients. Results showed a high percentage of samples of both breast milk and infant formulas with margin of exposure (MOE) value indicating a potential concern for consumer health.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different organic compounds containing two or more aromatic rings (benzene), which are fused together when a pair of carbon

* Corresponding author.
E-mail address: raffaella.mercogliano@unina.it (R. Mercogliano).

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atoms is shared between them (Neff, 1979). PAHs differ in position at which aromatic rings are fused and, in the substituents on the basic ring system (Neff, 1979). Their high chemical stability produces high levels in the environment and enhances bio-accumulation (El Nemr et al., 2007).

PAHs may be formed and released as a result of incomplete combustion of coal, oil, gas, wood, or pyrolysis of organic matter during industrial processes and other human activities (EFSA, 2008).

Pyrolytic and petrogenic inputs are the main sources of these compounds (Veyrand et al., 2013). The occurrence of PAHs in food, as a mixture of low (petrogenic) and high (pyrolytic origin) molecular weight compounds, results both from air pollution and from some manufacture processes such as drying, roasting or smoking (European Commission, 2002; Codex Alimentarius Commission , 2005).

Exposure to PAHs is one of the major concerns for human health, since many PAHs are carcinogenic. PAHs with one- two- and three-rings are acutely toxic, while high molecular weight compounds are genotoxic (Eljarrat et al., 2001; Conte et al., 2016). The International Agency for Research of Cancer (IARC) has designed Benzo(*a*)pyrene (BaP) as compound of Group1-carcinogenic to humans, whereas Dibenzo(a,h)anthracene has been classified as Group 2A-possible human carcinogenic, and other hydrocarbons as B2-probable human carcinogenic (IARC, 2010). In addition, some PAHs classified as no-carcinogenic compounds (Group 3) have shown effects on the immune system and endocrine regulation (Hylland, 2006).

Dietary sources are the major route of human exposure to PAHs. Breast milk and infant formula provide to satisfy the specific nutritional requirements of healthy newborns and infants (children under the age of 12 months) for the first 4–6 months of life (WHO, 2002; EFSA, 2008; Commission Regulation No EC/609/2013). Milk contamination depends of environmental factors, source of exposition, stage of lactation, medical state, rearing system and, for human milk, also life style (Zanieri et al., 2007; Mercogliano et al., 2009). PAHs may be transferred to infant during breastfeeding, being relatively high the milk fat content.

Infant exposure may be the consequence of PAH exposition of mothers for breast milk or raw material contamination for infant formula (Cattaneo, 2013). Unlike breast milk, the infant formula does not afford any protection or mitigation, because can contain the same chemical residues found in breast milk, often at higher levels, but does not contain the same combination of protective and stimulating substances (Cattaneo, 2013).

Infants and children are particularly vulnerable to the effects of exposure to contaminants, because they are at the most sensitive stages of human development (WHO, 2010). Postnatal exposure to chemicals is of great concern. Different reasons may explain the health risk in the infants. In fact, children have high capacity of absorption nutrients and non-nutrients (FIMP, 2011), but a reduced capacity of detoxification, if compared to adults (Ginsberg et al., 2004). Lower activity of some metabolizing enzymes, active in detoxification, might be the reason for their lesser elimination via their natural detoxification mechanism (Armstrong et al., 2004). Infants are getting exposed to the effects of environmental pollutants, which result from their higher uptake, in a mass comparison (as much as six times more than adults) from PAH inhalation and food intake. The vulnerability of humans during the neonatal and prenatal periods, makes the PAH intake assessment more important for infants.

The CONTAM Panel stated that BaP is not a suitable indicator for the occurrence of PAHs in food, and concluded that PAH4 (BaP, Chrysene, Benzo(a,h)anthracene and Benzo(*b*)fluoranthene) and PAH8 (BaP, Chrysene, Benzo(*b*)fluoranthene, Benzo(k)fluoranthene, Benzo(*a*)pyrene, Indeno(1,2,3-*cd*)pyrene, Dibenzo(a,h)anthracene, Benzo(ghi)perylene) are the most suitable indicators of PAHs in food, with PAH8 not providing much added value compared to PAH4 (EFSA, 2008).

Therefore, European Regulations fixed in infant food permissible limits of 1 $\mu g\ kg^{-1}$ both for BaP and the sum of PAH4 ($\sum$PAH4) (Commission Regulation No EC/1881/2006; EFSA, 2008; Commission Regulation No EC/835/2011). No level was included for human milk.

A limited number of studies on PAH concentrations in human milk, from e.g. United States, China, Turkey, Italy and Spain, exists, and all of them are focused on analysing of the most abundant PAHs from the United States Environmental Protection Agency (US EPA) list (Zanieri et al., 2007; Kim et al., 2008; Yu et al., 2011; Çok et al., 2012; Luzardo et al., 2013). Even if food consumption is the major route of human exposition, actually, no exhaustive information on infants' exposure via breastfeeding is available (Pulkrabova et al., 2016). In addition, in some regions where the high concentration of atmospheric PAHs was assessed, mothers are exposed also via inhalation of polluted air (Pulkrabova et al., 2016).

There is no international scientific consensus on what is the best approach for assessing the risk of substances that are both genotoxic and carcinogenic. European Food Safety Authority (EFSA) Scientific Committee adopted the margin of exposure approach (MOE) as a new approach to risk assessment for PAH substances (EFSA, 2005).

The aim of the study was to compare the profile of 14 PAHs, frequently occurred in food, the levels of PAH markers and the occurrence of carcinogenic PAHs in human milk from Italian mothers and infant formula brands available in Italian market. Aim of the study was, also, to perform an exposure assessment and risk characterization study.

2. Material and methods

2.1. Reagents and materials

PAH-mix9 (100 ng μL^{-1} in acetonitrile), a mixture of 14 PAHs, was supplied by Dr. Ehrenstorfer, Reference Materials (Augsburg, Germany), stored at 4 °C, and used for the preparation of working standard solutions (1, 10 and 100 ng mL^{-1} in acetonitrile). Ethanol (ACS, for analysis), cyclohexane (ACS, for analysis), acetonitrile for high performance liquid chromatography (HPLC), water plus for HPLC, potassium hydroxide pellets, and anhydrous sodium sulphate crystals were provided by Carlo Erba (Milan, Italy), and Discovery DSC-18 tubes, 1000 mg/6 mL, were supplied by Supelco (Park Bellefonte, PA USA).

The analyses were carried out using a Jasco HPLC apparatus equipped with a Jasco quaternary pump *2089 plus*, combined with a Jasco fluorescence detector 821-*Fp*. A C18 Envirosep-PP column 5 μm (125 mm × 4.6 mm) by Phenomenex Inc. (Torrance, CA, USA) was used.

2.2. Samples collection

Health pregnant women, volunteers, from general population were recruited in a medical centre in Southern Italy. All of the mothers, in the 25–35 age range, were primiparae, no smokers and no exposed to occupational source of contamination. Participating women gave an informed consent, and the ethics committee of the medical centre approved the study protocol.

A food diary was delivered to evaluate eating habits. Women who participated in the breast-feeding study were trained in procedures for collecting breast milk, which minimized the risk of contamination. The samples were collected during the fourth and the tenth d after delivery. Thirty breast milk samples (30–60 mL)