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Polycyclic aromatic hydrocarbons and pesticides in milk powder

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This Research Communication reports analysis of 37 compounds comprising polycyclic aromatic hydrocarbons (PAHs), organochlorine and organophosphate pesticides (OCPS and OPPS) in milk powder (one brand each of commercial infant formulae, follow-on formulae and baby formulae purchased from a local supermarket in Romania). The selected analytes were investigated using gas chromatography–mass spectrometry (GC-MS), gas chromatography with electron capture detector (GC-ECD) and gas chromatography with thermionic sensitive detection (GC-TSD). The estimated limits of detection for most target analytes were in the µg/kg level (range 0·001–0·320 µg/kg). The purpose of the study was to determine the selected analytes, to assess the exposure of babies and infants and to produce data for comparison with tolerable limits according to the European Union Regulations. In most of the samples the organochlorine pesticides values were under the limit of detection. Exceptions were heptachlor epoxide and endosulfan sulphate, the last of which was found in all analysed samples at low concentrations. We also found detectable levels of ethoprophos, parathion-methyl, chlorpyrifos, prothiofos, guthion, disulfoton and fenchlorphos in most of the analysed samples. Benzo[a]pyrene, which is used as an indicator for the presence of PAHs, was not detected in selected samples. The low level of exposure to contaminants indicates that there are no health risks for the infants and babies that consume this brand of milk powder formulae.

Keywords: OCPs, OPPs, PAHs, powder milk.

Milk is an interesting biomarker for assessing population exposure to pollution, since it is obtained non-invasively and contains a high proportion of lipids in which persistant organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) are liable to accumulate because of their lipophilic nature.

Because of their vapour pressures and partitioning behaviour under ambient conditions, organochlorine pesticides (OCPs) are mobile in the environment and thus bioaccumulate in the environment and consequently in the food chain. OCPs can cause environmental damage and may pose a risk to human health due to their high resistance to degradation and long half-lives These compounds can accumulate in human tissue and can cause chronic toxicity after long-term exposure. The half-life of most organochlorine pesticides can range from a few years to more than 10 (Zakaria et al. 2003).

Organophosphate pesticides (OPPs) are used widely for agriculture, vector control and domestic purposes. Despite the apparent benefits, these pesticides are the most important cause of severe toxicity and death from acute poisoning worldwide (Roberts & Aaron, 2007).

Polycyclic aromatic hydrocarbons constitute a large class of organic compounds that are composed of two or more fused aromatic rings. The concentration of benzo(a)pyrene (BaP) has been used as an indicator of total contamination by PAHs, the maximum level for BaP established by European Union (EU) being of 1 µg/kg in infant formula food (The EFSA Journal, 2008).

The presence of pollutant residues in food has aroused the concern of scientists and health officials and the aim of this study was to determine the contamination level of selected baby powder milk formulae (in the most popular brand of milk powder from the Romanian market) by 15 PAHs, 8 OPPs, 8 OCPs and their isomers and metabolites. Quantitative OCPs, OPPs and PAHs determinations were performed by GC-ECD, GC-TSD and GC-MS, respectively.

As far as we know, there are no published data about the OCPs, OPPs and PAHs in milk powder formulae from Romania.

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Materials and methods

Reagents

Standards of pesticides: Aldrin, Endrin, α-HCB, β-HCB, γ-HCB, λ-HCB, Heptachlor, Heptachlor epoxide, α-endosulfan, β-endosulfan, endosulfan sulphate, p,p'- DDT, p,p'- DDE, p,p'- DDD, and dichlorvos, ethoprophos, parathion-methyl, chlorpyrifos, prothiofos, guthion, disulfoton, fenchlorphos were supplied by International Atomic Energy Agency, Monaco laboratory.

Standards of PAHs: Acenaphthene (Ace), acenaphthylene (Acy), fluorene (F), naphthalene (Np), anthracene (An), fluoranthene (Fl), phenanthrene (Ph), benz[*a*]anthracene (B[*a*]An), benzo[*k*]fluoranthene (B[*k*]Fl), chrysene (Chry), pyrene (Py), benzo[*ghi*]perylene (B[*ghi*]Pe), benzo[*a*]pyrene (B[*a*]Py), dibenz[*a,h*]anthracene dB[*a,h*]An, indeno[*1,2,3–cd*]pyrene (I[*1,2,3–cd*]Py) were supplied by LGC Promochem, Germania.

For clean-up step we used two usual sorbent materials of variable polarities for PAHs: silica gel (0·2–0·5 mm) and aluminium oxide 90 (0·063–0·200 mm) supplied by Merck, Darmstadt, Germany and for pesticides: florisil from Fluka (packed in Switzerland) that was activated 12 h at 130 °C before use. Silica gel and aluminium oxide were activated at 420 °C for 4 h before use. Anhydrous sodium sulphate (granulated for residue analysis) was activated at 200 °C for 2 h before use. As eluents we assayed two organic solvents: n-hexane, supplied by Merck, Darmstadt, Germany and dichlormethane supplied by J.T. Baker. All glassware was washed with detergent, rinsed with deionised water and acetone before use.

Samples

Five samples of baby milk powder formulae were purchased at a supermarket in Romania, in the year 2014. Samples include: infant formulae (one sample marked as sample 1), follow-on formulae (two samples marked as sample 2 and 3), baby formulae (two samples marked as sample 4 and 5). Infant formulae means milk intended for infants from birth until the first 6 months of life. Follow-on formulae contain probiotics and are only for babies over 6 months, as part of a mixed diet. A baby formula is for babies over 1 year and covers the daily requirement of nutrients recommended for young children.

It should be mentioned that in the Romanian market there are three main brands of milk powder formulae and one brand was selected for the research, which is the most popular as well as the most available in the market.

Extraction

Samples of 1 g powdered milk were extracted in an ultrasound bath with 3 × 10 ml of hexane for 10 min, then the extracts were centrifuged for 10 min at 9000 rcf and the supernatants were collected and filtered. Then ~30 ml of the filtered extract was applied to 5 g of activated florisil column for pesticides and 5 g of activated aluminium oxide and 5 g of activated silica-gel for polycyclic aromatic hydrocarbons, both topped with 1 cm of anhydrous sodium sulphate, which was pre-washed with n-hexane. The columns were eluted with *n*-hexane-dichloromethane (3:1). Each fraction was concentrated to 1 ml using the Kuderna–Danish concentrator. The concentrated aliquot was blown down with nitrogen, the internal standards: 2,4,5 trichlorobiphenyl for pesticides and 9,10 dihidroanthracene for PAHs were added, and the final volume was injected.

Instrumental analysis

A Hewlett-Packard 5890 gas chromatograph (GC) equipped with an electron capture detector (ECD) and a HP–5 fused–silica capillary column (29·6 m × 0·32 mm × 0·25 μm) was used for OCPs analysis. Helium was used as the carrier gas with flow rate 1·86 ml/min and nitrogen makes-up gas at 40 psi. The injector and detector temperature was 250 °C. The initial temperature was 60 °C, after the temperature was increased to 300 °C at a ramp rate of 20 °C/min and then held for 10 min.

For PAHs analysis, a Hewlett- Packard 5890 gas chromatograph (GC) equipped with a Hewlett-Packard 5972 mass spectrometer (MS) was used. The gas chromatograph was installed with an HP-5 fused-silica capillary column (29·6 m × 0·25 mm × 0·25 μm) (Hewlett- Packard, Germany). The temperature programme was initially set at 60 °C, held at 60 °C for 1 min after the temperature was increased to 300 °C at a ramp rate of 20 °C/min and then held for 10 min. Helium was used as a carrier gas at a flow rate of 1·86 ml/min. The analyses were operated using selected ion monitoring and electronic ionisation.

A Varian gas chromatograph (model 520) equipped with an thermionic specified detector (TSD) and a fused–silica capillary column 29.6 ml × 0.32 mm i.d. × 0.25 μm film thickness were used for organophosphorus pesticides analysis. Operating conditions were as follows: initial temperature 50 °C (2 min), increased at a rate of 25 °C/min to 300 °C and finally held for 8 min; injector temperature: 250 °C; carrier gas: He; column flow-rate: 1·86 ml/min; detector temperature: 300 °C; operation mode: split (electronic pressure control); split/splitless inlet vent –17·14 ml/min; purge time on: 2·5 min; purge time off: 7 min; injection volume: 1 μl.

Calibration curves were prepared for commercial milk samples which were spiked with standard PAHs, OPPs and OCPs. In all the samples, good linearities ($r > 0{\cdot}993$) were obtained between the peak height ratios and concentrations. Recoveries were calculated as differences in PAHs content in spiked and un-spiked samples relative to the spiked level. The recovery studies, with results between 95 and 98% indicated a high accuracy of the method. LOD values were determined using calibration standards and the values of LODs for PAHs were in the range 0·07 and 0·29 μg/kg and the values of LODs for pesticides were in the range 0·001–0·320 μg/kg.

Table 1. OCPs values for studied samples

	Mean concentrations of OCPs ± standard deviation (ppm)				
OCPs	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Aldrin	<LOD	<LOD	<LOD	<LOD	<LOD
Endrin	<LOD	<LOD	<LOD	<LOD	<LOD
α-HCB	<LOD	<LOD	<LOD	<LOD	<LOD
β-HCB	<LOD	<LOD	<LOD	<LOD	<LOD
γ-HCB	<LOD	<LOD	<LOD	<LOD	<LOD
λ-HCB	<LOD	<LOD	<LOD	<LOD	<LOD
Heptachlor	<LOD	<LOD	<LOD	<LOD	<LOD
Heptachlor epoxide	<LOD	0·002 ± 0·001	<LOD	<LOD	<LOD
p,p'-DDD	<LOD	<LOD	<LOD	<LOD	<LOD
p,p'-DDE	<LOD	<LOD	<LOD	<LOD	<LOD
p,p'-DDT	<LOD	<LOD	<LOD	<LOD	<LOD
α-endosulfan	<LOD	<LOD	<LOD	<LOD	<LOD
β-endosulfan	<LOD	<LOD	<LOD	<LOD	<LOD
Endosulfan sulphate	0·001 ± 0·00	0·003 ± 0·01	0·001 ± 0·00	0·001 ± 0·00	0·001 ± 0·02

LOD-limit of detection, Values are means (*n* = 3) ± SD.
HCB, hexachlorobenzene; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; DDD, dichlorodiphenyldichloroethane.

Table 2. OPPs values for studied samples

	Mean concentrations of OPPs ± standard deviation (ppm)				
OPPs	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Dichlorvos	<LOD	<LOD	<LOD	<LOD	<LOD
Ethoprophos	0·004 ± 0·001	0·014 ± 0·001	0·001 ± 0·00	0·001 ± 0·001	0·001 ± 0·001
Chlorpyrifos	0·005 ± 0·01	0·0002 ± 0·001	0·0001 ± 0·02	<LOD	0·0001 ± 0·01
Prothiofos	<LOD	0·0001 ± 0·001	0·0009 ± 0·003	0·0005 ± 0·002	<LOD
Guthion	0·0008 ± 0·00	<LOD	<LOD	0·0008 ± 0·001	<LOD
Disulfoton	0·0002 ± 0·02	0·002 ± 0·00	<LOD	<LOD	<LOD
Fenchlorophos	<LOD	0·0002 ± 0·01	0·00004 ± 0·01	0·0004 ± 0·01	<LOD
Parathion- Methyl	0·0002 ± 0·00	0·0001 ± 0·01	<LOD	0·00008 ± 0·00	0·00004 ± 0·00

LOD-limit of detection, Values are means (*n* = 3) ± SD.

Results and discussion

The values of OCPs, OPPs and PAHs concentrations are presented in Tables 1–3.

In most of the samples the OCPs values were under the limit of detection whilst in all samples endosulfan sulphate was found at a concentration between 0·001–0·003 ppm.

Nigam and Siddiqui have reported 100% contamination with DDT in milk collected from two dairies in India with the range of concentration being 0·01 ± 0·03 mg/kg (Nigam & Siddiqui, 2001). Different branded and unbranded milk from various cities of Maharashtra contained DDT residues varying from 0·016 to 0·338 mg/kg but none exceeded the FAO/WHO tolerance level (Pandit et al. 2002). DDT contamination was present in only six samples (6·52%) out of 92 liquid milk samples obtained from Ludhiana district of Punjab (Battu et al. 2004). Kumar et al. found pp- DDE as the major constituent with a mean value of 0·055 mg/kg, followed by pp- DDT (0·04 mg/kg) and op- DDT (0·01 mg/kg), as the composition of total DDT residues found in milks from India (Kumar et al. 2005). In our study the concentrations of DDT were under the limit of detection in all studied samples.

Reports of the occurrence of endosulfan residues in milk are very rare because the use of endosulfan is very much restricted to the field of agriculture and it is not as persistent as the other OPCs because it metabolises at a much faster rate to water-soluble metabolites and has a lower partition coefficient. Residues of endosulfan are estimated in terms of α-endosulfan, β-endosulfan and its toxic metabolite endosulfan sulphate. Among the two stereoisomers, the β-isomer is reported to be more persistent than it's α counterpart as the latter partly isomerises to the β isomer and also converts into sulphate at a faster rate in different substrates (Nag, 2010). In one monitoring study with 147 milk samples taken from different districts of Haryana, α and β-endosulfan were detected in 7 and 44% of samples, respectively, with concentrations varying from BDL (below detectable level) to 0·0079 and from BDL to 0·028 µg/ml, respectively (Sharma et al. 2007). Our endosulfan sulphate

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Table 3. PAHs values for studied samples

PAHs	Mean concentrations of PAHs ± standard deviation (μg/kg) Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Np	<LOD	<LOD	<LOD	<LOD	<LOD
Acy	<LOD	<LOD	0·28 ± 0·00	0·14 ± 0·02	<LOD
Ace	0·14 ± 0·02	0·15 ± 0·02	0·13 ± 0·03	0·09 ± 0·02	<LOD
F	<LOD	<LOD	<LOD	<LOD	<LOD
Ph	<LOD	<LOD	<LOD	<LOD	<LOD
An	<LOD	0·09 ± 0·01	0·22 ± 0·01	0·21 ± 0·00	<LOD
Fl	<LOD	<LOD	0·17 ± 0·01	0·07 ± 0·00	<LOD
Py	0·24 ± 0·01	0·18 ± 0·01	<LOD	0·08 ± 0·04	<LOD
B[*a*]An	<LOD	<LOD	<LOD	<LOD	<LOD
Chry	0·16 ± 0·00	0·17 ± 0·01	0·28 ± 0·04	0·25 ± 0·06	0·35 ± 0·01
B[*k*]Fl	0·04 ± 0·04	0·37 ± 0·02	0·012 ± 0·03	0·35 ± 0·03	0·11 ± 0·02
B[*a*]Py	<LOD	<LOD	<LOD	<LOD	<LOD
B[*ghi*]P	<LOD	<LOD	<LOD	<LOD	<LOD
dB[*a,h*]An	<LOD	<LOD	<LOD	<LOD	<LOD
I[*1,2,3–cd*]Py	0·21 ± 0·03	0·36 ± 0·05	<LOD	0·21 ± 0·001	0·015 ± 0·01

LOD-limit of detection, Values are means ($n = 3$) ± SD.
Np, naphthalene; Acy, acenaphthylene; Ace, acenaphthene; F, fluorine; Ph, phenanthrene; An, anthracene; Fl, fluoranthene; Py, pyrene; B[*a*]An, benz[*a*]anthracene; Chry, chrysene; B[*k*]Fl, benzo[*k*]fluoranthene, B[*a*]P, benzo[*a*]pyrene; B[*ghi*]P, benzo[*ghi*]perylene; dB[*a,h*]An, dibenz[*a,h*]anthracene; I[*1,2,3-cd*]Py, indeno[*1,2,3-cd*]pyrene.

concentrations are in concordance with these reported in literature.

In spite of being less stable and less persistent than the OCPs, there are some reports alluding to the presence of OPP residues in milk.

The OPPs concentrations in studied samples were in the range <LOD-0·014 ppm with the highest value found for ethoprophos in milk powder for 6 months baby.

Residues of diazinon, chlorpyriphos and malathion at levels of 0·005 ± 0·586 mg/kg, 0·0256 and 0·11 mg/kg, respectively, have been reported in milk (El-Hoshy, 1997; El-Kohly et al. 2000; Szerletics et al. 2000). The average values of 13 OPPs measured were below established MRLs, ranging from 0·0051 to 0·0203 ppm (Salas et al. 2003). Chlorpyriphos (0·01 ± 1·35 mg/kg) was detected in 20 samples, all exceeding the MRL, out of 324 pasteurised milk samples analysed from India according to Cheema et al. (2005). In our studied samples the concentrations of OPPs are lower than the data from literature.

PAHs concentration in our studied samples are in general under the limit of detection with the highest value for B[*k*]Fl (0·37 μg/kg) found in follow-on formulae (sample 2). Rey-Salgueir et al. found B[*k*]Fl in milk powder formulae at levels of 0·10 and 0·30 μg/kg, respectively. Kishikawaa et al. have analysed three infant formulae samples, finding total levels of PAHs about 2·0 ± 0·30 μg/kg. In general the results obtained by these authors are similar to our results (Kishikawaa et al. 2003; Rey-Salgueir et al. 2009).

The food standards agency (FSA) determined 15 PAHS in 97 samples of infant formulae milk obtained from across the UK. B[a]P was detected in 39 samples in concentrations levels lower than 1·0 μg/Kg (Food Standard Agency, 2006). In this study, B[a]P, which is used as an indicator for the presence of PAHs, was not detected in selected milk powder formulae samples.

According to the Commission Regulation (EC) no 835/2011, the maximum tolerable limit for benzo[a]pyrene in baby foods has been set at the level of 1 μg/kg (Commission Regulation (EU) No 835/2011). In all studied samples contamination with benzo[a]pyrene was lower than the maximum tolerable limit for this compound.

A study performed by Ciecierska & Obiedziński (2010) observed similar profiles of PAHs to ours, both in the groups of infant formulae and follow-on formulae.

The OCP, OPPS and PAH content results (Tables 1–3) showed that the average values are not significantly different ($P > 0{\cdot}05$) between the analysed milk powder formulae samples. We can conclude that OCPs, OPPS and PAHs are detected at the same level of contamination in all samples. To verify this profile, an ANOVA design was performed.

In 2005, the European Food Safety Authority (EFSA) adopted an average ingestion rate of 0·19 kg PAHs per day for all ages of infants (EFSA, 2005). Iwegbue et al. (2014) calculated the dietary intake of the 15 PAHs investigated in this article, based on the EFSA suggested indicators of occurrence and effects of PAHs in foods. They estimated dietary exposure to PAHs by infants through consumption of these infant formulae as generally low.

The corresponding acceptable daily intake (ADI) was established by the WHO (2009) for DDT (PTDI, provisional tolerable daily intake, 10 ng/kg bw), lindane (5 ng/kg bw) and aldrin/dieldrin (PTDI, 0·1 ng/kg bw). The estimated daily intake for the sum of pesticides was found to be 8·266 ng/kg/d for children by Dos Santos et al. (2015).

According to these daily intakes, consumers should not be concerned about the levels of the compounds we selected in milk powder formulae.

Conclusions

The results of the present study indicate that the concentrations of OCPs, OPPs and PAHs in commercially available infant formulae, follow-on formulae and baby formulae were low in comparison with the EU regulations and comparable to levels reported in the literature for similar products.

We can say that the OCP, OPP and PAH levels in the investigated samples do not pose any serious concern to infant and baby health.

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