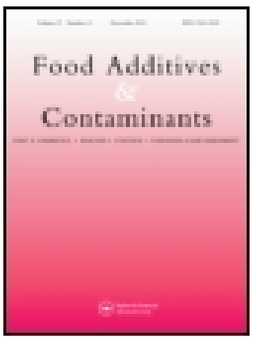

Food Additives & Contaminants: Part A

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: https://www.tandfonline.com/loi/tfac20

Presence of emerging contaminants in baby food

Maria Nobile, Francesco Arioli, Radmila Pavlovic, Federica Ceriani, Shih-Kuo Lin, Sara Panseri, Roberto Villa & Luca Maria Chiesa

To cite this article: Maria Nobile, Francesco Arioli, Radmila Pavlovic, Federica Ceriani, Shih-Kuo Lin, Sara Panseri, Roberto Villa & Luca Maria Chiesa (2019): Presence of emerging contaminants in baby food, Food Additives & Contaminants: Part A, DOI: 10.1080/19440049.2019.1682686

To link to this article: https://doi.org/10.1080/19440049.2019.1682686

Published online: 29 Oct 2019.

FOOD ADDITIVES & CONTAMINANTS: PART A
https://doi.org/10.1080/19440049.2019.1682686

Taylor & Francis
Taylor & Francis Group

Presence of emerging contaminants in baby food

Maria Nobile[a*], Francesco Arioli[a*], Radmila Pavlovic[a], Federica Ceriani[a], Shih-Kuo Lin[a,b], Sara Panseri[a], Roberto Villa[a] and Luca Maria Chiesa[a]

[a]Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy; [b]Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Taipei City, Taiwan, R.O.C

ABSTRACT

Food safety becomes imperative when it aims to protect infants. The objective of this study was to investigate the presence of emerging contaminants of which some act as endocrine-disruptors in baby food. Persistent organic pollutants (POPs), perfluoroalkyl substances (PFASs), parabens and antibiotics were analysed in 112 baby food of different categories (meat, fish, vegetables, fruit, cheese). As regard POPs, PFASs and antibiotics, no residues were detected, while one sample showed methyl-paraben (4.14 ng g^{-1}), whereas another three contained propyl-paraben (median 1.70 ng g^{-1}). Special attention must be paid on parabens metabolites, as 4-hydroxybenzoic acid, the principal parabens metabolite, was detected in all samples (median 176.7 ng g^{-1}). It may be present as a degradation product, but also, it can be released from vegetables and fruits during food processing. It is recommended to collect more data on natural vs non-natural occurrence of parabens and metabolites to evaluate the exposure of sensitive population vs ADI published by the European Food Safety Authority and European Medicines Agency.

ARTICLE HISTORY
Received 9 August 2019
Accepted 10 October 2019

KEYWORDS
Baby food; POPs; PFASs; antibiotics; parabens; food safety

Introduction

Baby food is homogenised food, packaged in sterile conditions, made from fruit, vegetables, fish, meat, or combining different of these matrices, directly ready for use. An alternative to traditional baby food is organic baby food, even if it is more expensive. In general, baby foods are produced by subjecting the selected substances to a sophisticated procedure of homogenisation that makes them digestible for infants between 4–6 months and 2 years old. Infant formulas are very useful in the first months of life, in the so-called weaning phase, when milk is gradually replaced with a practical and functional solution to ensure a complete supply of nutrients. Food safety checks are very important and challenging when the aim is to detect simultaneous residue analysis of different compounds belonging to a wide variety of different classes, in selective foodstuffs both of vegetable and animal origins (Pérez-Ortega et al. 2012), especially to protect a vulnerable and most at-risk population group, such as infants.

On the other hand, the presence of emerging contaminants and/or endocrine disruptors such as persistent organic pollutants (POPs), perfluoroalkyl substances (PFASs), parabens, human and veterinary drugs (e.g. antibiotics) has been recently reported in processed food deriving from environmental contamination and/or farming/crop practices (Chiesa et al. 2018a, 2019).

As the European Food Safety Authority (EFSA) states in its guidance on risk assessment for substances in baby food (EFSA 2017), the immune system in immediate post-natal life is particularly sensitive and exposure to immunotoxicants may result in persistent effects on the immune system that last or appear only long after exposure, but may also occur at lower doses than adult exposure. Different compounds or types of exposure may produce different severities and unpredictable alterations depending on the time of exposure during the immune system development. They may be associated with chronic immunological conditions such as immune deficiency, autoimmunity, inflammation and allergic reactions.

CONTACT Sara Panseri sara.panseri@unimi.it Via Celoria, 10, Milan, MI 20133, Italy
*These authors contributed equally to this work.

Although the guidance addresses specifically the risk assessment of infants less than 16 weeks of age, the matters affects infants and young children above 16 weeks of age.

To ensure appropriate nutritional composition and safety of foods for infants and young children, the European Commission has defined specific rules for such foodstuffs.

The Directive 99/39/EC encompasses the specific rules on the presence of pesticide residues in processed cereal-based baby foods and baby foods and requires that this type of food contains no detectable levels of pesticide residues, meaning not more than 0.01 mg kg^{-1}, as consumed. In addition, the Directive prohibits the use of certain very toxic pesticides in the production of processed cereal-based baby foods and baby foods and establishes levels lower than the general maximum level of 0.01 mg kg^{-1} for a few other very toxic pesticides.

In addition, the Directive 2006/125/EC, indicates that cereal-based foods and baby foods must also comply with other specific provisions laid down in the relevant measures of EU law on hygiene, on the use of food additives, on the presence of contaminants and on the use of materials intended to come into contact with the products.

As well known, food is considered as a cumulative daily source of parabens and other legislation was established to ensure consumers' safety. A risk assessment of parabens was recommended by the EFSA (2004) and was set an acceptable daily intake (ADI) of 10 mg kg^{-1} body weight (bw)/day for methyl paraben (MeP) and ethyl paraben (EtP), but for a long time safety evaluations have not been defined for other parabens. In recent years, special attention has been paid to propyl-paraben (PrP) and ADI of 1.25 mg kg^{-1} bw was established just a few years ago (European Medicines Agency 2015). The levels of residues that might occur following its utilisation in veterinary products are expected to be too low to impact on industrial food processing and therefore maximum residual limits (MRL) were not set-up, as was declared in EU regulations (European Commission 2015).

The question about paraben presence in processed foods is even more complicated when the parabens transformation products, namely 4-hydroxybenzoic acid (*p*-hydroxybenzoic acid, *p*-HBA), 3,4-dihydroxybenzoic acid (protocatechuic acid, 3,4-DHB), methyl-protocatechuate (OH-MeP) and ethyl-protocatechuate (OH-EtP), are taken into consideration (Xue et al. 2015, 2017). Those (di) hydroxybenzoic acids have been recognised as metabolites of parabens and thus might serve as potential markers of parabens incidence (Wang et al. 2018; Chiesa et al. 2018e). Nevertheless, the parabens are not their unique, exclusive source: *p*-HBA and 3,4-DHB are also naturally present in many plants and vegetables (Tomás-Barberán and Clifford 2000; Kakkar and Bais 2014). Also, both *p*-HBA and 3,4-DHB appear as intermediates in several industrial processes with potential biotechnological applications in food production (Wang et al. 2018), and if not managed properly they could represent a risk for baby food, as well. Additionally, OH-MeP and OH-EtP are recognised as hydroxylation products of MeP and EtP, respectively, and generally, they are produced by biotic and abiotic transformation of many xenobiotics (Xue et al. 2017). There is no available literature data about their origin, level and risk assessment in the baby food.

Salicylic acid, a structural isomer of *p*-HBA, is a compound that is naturally present in foods can cause adverse reactions to persons who are intolerant. Salicylate sensitivity is not as common as other type of food intolerance, but it should be taken into consideration especially when its quantity in baby food is concerned. Studies on the salicylic content of foods are sparse and have produced distinctly different results, giving rise to controversy (Malakar et al. 2017).

As regards veterinary drugs or other class of substances, there is not any current legislation for MRL in baby food, so a zero-tolerance policy is applied establishing that the presence of these compounds is illegal at any level (Aguilera-Luiz et al. 2012).

As regards PFASs, EFSA recommended the analysis of this class of compounds in different food items to assess a reliable risk evaluation, and this appears essential when the highest chronic dietary exposure to perfluorooctanesulfonic acid (PFOS) was estimated for the youngest population groups (EFSA 2018b).

Therefore, in the light of these considerations, the application of these preventive policies require

the development of sensitive analytical methods to determine the presence of these compounds and of their metabolites, useful as markers, at very low concentrations to protect infant health.

There are few works in literature on the multi-residue analysis of emerging contaminants and endocrine-disrupting chemicals in baby food, and those deal with single or only a few classes of compounds, as reported in Table 1, a summary table on the state of art on this topic.

In this regard, our aim was to analyse different baby food on the basis of the matrix type (meat, fish, cheese, vegetables and fruit) for the detection of POPs, PFASs, antibiotics and parabens evaluating the possible direct or indirect contamination of residues, relative to the different breeding/crop practices or environmental contamination, to evaluate infant health risk.

Material and methods

Chemicals and reagents

All solvents were purchased from Merck and water was purified by a Milli-Q system (Merck KGaA, Darmstadt, Germany). SupelTM QuE Citrate (EN) tubes and SupelTM QuE-ZSEP (EN) tubes were from Supelco (Sigma Aldrich, St. Louis, MO, USA). The Oasis HLB 3 mL, 60 mg and Oasis WAX 3 mL, 60 mg cartridges were from Waters (Milford, MA, USA). Non-dioxin like-polychlorinated biphenyls (NDL-PCB) (PCB 28; −52; −101; −138; −153 and −180) [congener 209 as internal standard (IS)] and PBDEs (PBDE 28; −33; −47; −99; −100; −153 and −154) [3-fluoro-2,2,4,4,6- pentabromodiphenyl ether (FBDE) as IS] were from AccuStandard (New Haven, USA). Organochlorine pesticides (OCs) (aldrin; α-hexachlorocyclohexane (α-HCH); β-hexachlorocyclohexane (β-BHC); hexachlorobenzene (HCB); dichlorodiphenyldichloroethylene (DDE); dichlorodiphenyltrichloroethane (DDT); dichlorodiphenyldichloroethane (DDD); endosulphan I; endosulphan II; endosulphan sulphate; endrin; heptachlor; heptachlor epoxide; lindane and trans chlordane) were from Restek (Bellefonte, PA, USA). Organophosphorus pesticides (OP): chlorpyriphos diazinon, disulphoton, ethoprophos, mevinphos and phorate, and 4-nonylphenol (IS for OCs and OPs) were from Sigma-Aldrich. The four PAHs: chrysene, benz(a)anthracene, benzo(b)fluoranthene and benzo-(a)pyrene were from Restek (Bellefonte, PA, USA).

PFASs: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorobutane sulphonic acid (PFBS), perfluoroheptanoic acid (PFHpA), PFOA, perfluorohexane sulphonate (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), PFOS, perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUnDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), and perfluorooctadecanoic acid (PFODA) were from Chemical Research 2000 Srl (Rome, Italy) and ISs perfluoro-[1,2,3,4,5-^{13}C5]nonanoic acid (MPFNA) and perfluoro-[1,2,3,4-^{13}C4]octanesulfonic acid (MPFOS).

Antimicrobial agents: amoxycillin, ampicillin, benzylpenicillin, cefquinome, ceftiofur, cefalexin, ciprofloxacin, chloramphenicol, chlortetracycline, cloxacillin, danofloxacin, dicloxacillin, dimetridazole, doxycycline, enrofloxacin, florfenicol, florfenicol amine, flumequine, furaltadone, furazolidone, lincomycin, lomefloxacin, marbofloxacin, nalidixic acid, nitrofurazone, oxolinic acid, oxytetracycline, ronidazole, spiramycin, sulphadiazine, sulphathiazole, sulfadimethoxine, sulphadimidine, sulfamerazine, tetracycline, thiamphenicol, tiamulin, tilmicosine, tinidazole, trimethoprim, tylosin and enrofloxacin-d5 (IS) were from Merck.

Parabens: MeP, EtP, propyl-(PrP), butyl-(BuP) and benzylparaben (BzP), 4-hydroxybenzoic acid (pHBA), 3,4-DHB, OH-MeP and OH-EtP including 4-fluorobenzoic acid (4-FB) used as IS, were from Merck (KGaA, Darmstadt, Germany).

Standard solutions

For stock and working solutions, kept at −20°C, hexane was used as solvent for GC-MS/MS and methanol for HPLC-HRMS analyses.

Sample collection

The total number of collected samples was 112. In detail: 45 meat (veal, swine, horse, lamb, rabbit, chicken, turkey), 13 fish (plaice, salmon, sea bream, hake, trout, bass, cod), 47 fruit (apple, pear, plum, blueberry, apricot, peach, mixed

Table 1. Literature summary on emerging contaminants and endocrine-disrupting chemicals detected in baby food.

Reference	Compounds	Baby food typologies	Extraction Technique	Detection techniques	LOD/LOQ CCα/CCβ (ng g^{-1})	Min and Max Conc. detected (Application) (ng g^{-1})
Antibiotics						
Gentili et al. (2004)	Sulphonamides	Bovine (veal and beef), Porcine (pig and ham), Poultry meat (chicken and turkey),	ASE	LC-ESI-MS/MS	LOD: 0.4–1.7 LOQ: 1.2–5.1	<LOQ- 3.5
Díaz-Alvarez (2009)	Quinolones Fluoroquinolones	Chicken meat and vegetables	Ultrasound-assisted extraction + solid-phase extraction	HPLC-UV	LOD: 30–110 LOQ: 100–350	No application
Rodriguez et al. (2011)	Fluoroquinolones	Baby food purées ham, chicken, turkey, lamb, beef, sole, hake	MISPE (molecularly imprinted solid phase extraction)	LC-FLD (liquid chromatography with fluorescence detection)	CC_{α}:11–19 CC_{β}:18–32	n.d.-3
Aguilera-Luiz et al. (2012)	Multiresidue veterinary drugs	Meat-based baby food and powdered milk-based infant formulae	QuEChERS	UHPLC-MS/MS	CCα:0.5–16.2 CCβ:1.4–22.4	<5–25.2
Jia et al. (2014)	MULTI-RESIDUES (333 veterinary drugs and pesticides included antibiotics, OCs and OPs)	Baby food (93 including VBF, MBF, CBF, FBF and PMBIF)	QuEChERS	UHPLC-Q-Orbitrap	CCα:0.01–5.35 CCβ:0.01–9.27	1.45–22.34
Nasr et al. (2014)	Macrolides (Tylosin and josamycin)	(chicken muscles, chicken liver, bovine muscles, liver, milk and eggs) Chicken-based baby food and baby formulae	Liquid–liquid extraction	MLC–monolithic method with UV	LOD: 1100–3000 LOQ:3600–9900	No application
Nebot et al. (2014)	Tetracyclines	Meat/vegetables	Liquid–liquid extraction	HPLC-MS/MS	LOQ: 5.0	5.0–9.0
Vakh et al. (2018)	Fluoroquinolones	Chicken, beef or turkey	Automated magnetic dispersive micro-solid phase extraction	HPLC-FLD	LOD: 1.5–3.0 LOQ: 5.0–10.0	No application
Persistent Organic Pollutants – POPs (PBDEs, PCBs, OCs, PAHs, OPs)						
Pandelova et al. (2011)	PCBs OCs	Fruits, vegetables, meat, fish,	ASE for PCBs Soxhlet extraction for OCs	HRGC/HRMS	LOD: 0.0005 LOQ: 0.0035	0.001–0.04
Jeong et al. (2014)	PBDEs	Homemade baby food	Soxhlet extraction	HRGC/HRMS	LOQ: 0.0001–0.01	0.245–6.00
Jeong et al. (2014)	OCs PCBs	Homemade baby food	Soxhlet extraction	HRGC/HRMS	LOD: 0.00012–0.0015 LOQ: 0.0004–0.005	0.00028–3.338
Liu et al. (2014)	PBDEs	Baby food (formula, cereal, and puree)	ASE	GC/MS	-	n.d.- 0.94
Schecter et al. (2010)	PBDEs	Meat based baby food (Ham/veal, beef)	Soxhlet extraction	HRGC-HRMS GC–ECD	LOD: 0.0002–0.1	0.102–3.156
Notardonato et al. (2018)	OPs	Freeze-dried products (chicken, rabbit, turkey) and soft baby foods (chicken, rabbit, sea bream, plaice)	Ultrasound–vortex-assisted DLLME (liquid–liquid microextraction)	GC–IT/MS	LOD: 0.2–4.7 LOQ: 2.3–8.5	<LOQ
Toms et al. (2016)	PBDEs OCPs PCBs	Fruit-, vegetable-, meat-, fish- and dairy-based baby foods	ASE	GC/MS	LOD: 0.0001–0.0005	<LOD–0.095
Lorán et al. (2010)	PCBs	Processed cereal baby food, meat (chicken, beef and lamb), fish (sole and hake)	Soxhlet extraction	HRGC coupled to Ion Trap MS/MS	0.1–0.5	0.03–0.29
Leandro et al. (2005)	OCs and OPs	Fruit and rice, fish and pasta, potato and pork	QuEChERS	Large volume injections LVI–GC–MS/MS LC-MS GC-MS	0.5–10	No application
Fontcuberta et al. (2008)	OCs	Not specified	Liquid extraction	GC/MS	LOQ: 5–10	n.d
Dobrinas et al. (2011)	OCs	Fruit, vegetable, meat–vegetable + fish–vegetable based purée	Soxhlet extraction	GC-ECD	-	<LOD – 304
Radford et al. (2014)	Ops	Vegetable and fruit	Solid phase extraction	HPLC-MS/MS	LOD: 0.18–2.7	0.08–1.43

(*Continued*)

Table 1. (Continued).

Reference	Compounds	Baby food typologies	Extraction Technique	Detection techniques	LOD/LOQ CCα/CCβ (ng g^{-1})	Min and Max Conc. detected (Application) (ng g^{-1})
Al-Zahraa et al. (2016)	OCPs, OPPs	Fruit-vegetables and rice cereal-based baby foods	QuEChERS	GC/MS	LOD:0.0001–0.0191	n.d.- 13.97
Santonicola et al. (2017)	PAH	Meat (chicken, turkey, calf, pig, lamb, horse) and fish (trout, flounder, salmon, hake, sea bass, gilthead bream)	Liquid extraction	HPLC-FD	LOD: 0.005–0.11	n.d – 72.88
Perfluoroalkyl Substances – PFASs						
Ullah et al. (2012)	PFASs	Vegetables, meat, and fish	Liquid extraction +SPE C18 + SPEC8	HPLC/HRMS (qTOF)	LOD 0.0018–0.2 LOQ 0.006–0.066	n.d.-1.84
Lorenzo et al. (2016)	PFASs	Meat, poultry, fish, offal, vegetables and fruit	Liquid extraction +SPE Strata X	UHPLC-MS/MS.	LOD 0.75–4.5 LOQ 3.75–15.00	0.017–5.013
Parabens						
Chiesa et al. (2018e)	Methyl- (MeP), ethyl-, propyl-, butyl-, benzylparaben, 4-hydroxybenzoic acid (pHBA)	Fish and fish products (including baby food)	Simple liquid-liquid extraction	LC-HRMS	LOD 0.65–3.50 LOQ 2.15–11.70	pHBA 27.40–94.00

Abbreviations: Accelerated Solvent Extraction (ASE); cereal-based food (CBF); liquid–liquid equilibrium (LLE); meat-based food (MBF); magnetic dispersive solid phase extraction (MDSPE); powdered milk-based infant formulae (PMBIF); Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS); (non-fatty based on fruit (FBF); Solid Phase Extraction (SPE); vegetable baby food (VBF).

fruit) and vegetable (legumes, zucchini, carrots, potatoes, sweet potato, tomato, broccoli, peas, spinach, mixed vegetables) and 7 cheese baby food. They were from different commercial Italian brands, present in the international market, and bought in different Italian supermarkets. Moreover, 11 samples of different matrices were bought in some supermarkets of Serbia, to extend the international scope. The sample details are specified in Table 2.

Sample treatment protocol for PoPs

Two gram samples were extracted by the QuEChERS protocol described in Chiesa et al. (2018a).

Sample treatment protocol for PFASs

Two gram samples were extracted as in our previous works (Chiesa et al. 2018b).

Sample treatment protocol for antibiotics

One gram samples were extracted as described by Chiesa et al. (2017), (2018c) and (2018d).

Sample treatment protocol for parabens and metabolites

The sample procedure performed for parabens is reported by Chiesa et al. (2018e).

GC-MS/MS analyses for POPs

The instrument was a GC Trace 1310 chromatograph coupled to a TSQ8000 triple quadrupole mass detector (Thermo Fisher Scientific, Palo Alto, CA, USA) with electronic impact (EI) mode set in selected reaction monitoring mode (SRM). The column was a fused-silica capillary Rt-5MS Crossbond-5% diphenyl 95% dimethylpolysiloxane (35 m x 0.25 mm i.d., 0.25 µm film thickness, Restek, Bellefonte, PA, USA). The oven temperature program and all operation parameters were the same as for our previous work (Chiesa et al. 2018a). Xcalibur software was used to control instrument and Trace Finder 3.0 for data processing (Thermo Fisher Scientific).

Table 2. Sample collection details according to food categories.

Meat	Fish	Fruit/vegetables	Cheese
Veal	Plait	Apple	Cheese (bovine milk)
Swine	Hake	Plum	
Horse	Plait and potatoes*	Pear	
Lamb	Trout and vegetables*	Pear and blueberry	
Rabbit	Bream and vegetables*	Apple and blueberry	
Chicken	Bream and potatoes*	Apple and banana	
Turkey	Bass and vegetables*	Apple and peach	
Veal and ham	Cod and potatoes*	Apple and apricot	
Chicken and carrots*	Cod and vegetables*	Banana and kiwi	
Chicken with green beans and zucchini*	Salmon and vegetables*	Mixed fruit	
Veal and vegetables*		Carrot and apple	
Veal and carrots*		Legumes	
Veal and potatoes*		Zucchini	
Veal, broccoli and carrots*		Broccoli	
Veal, potatoes and mushrooms*		Carrots, potatoes and zucchini	
Turkey, corn and potatoes*		Sweet potato and carrots	
		Tomato and vegetables	
		Peas and spinach	
		Mixed vegetables	
Total n = 45	**n = 13**	**n = 47**	**n = 7**

*for mixed categories, meat and fish represented the major component as declared in the label.

LC-HRMS orbitrap analyses for PFASs, antibiotics, and parabens

A Q-Exactive Orbitrap equipped with a heated electrospray ionisation source (HESI) was used. The HPLC system was a Surveyor MS quaternary pump (Thermo Fisher Scientific, San Jose, CA, USA) with a Synergi Hydro-RP reverse-phase HPLC column (150 × 2.0 mm, i.d. 4 µm) and a C18 guard column (4 × 3.0 mm; Phenomenex, Torrance, CA, USA). The mobile phase used for PFASs was a gradient of aqueous NH4COOH (20 mM) and MeOH; for antibiotics and parabens separation a binary mixture of aqueous HCOOH (0.1%) and MeOH was used. All the parameters are described in our previous works (Chiesa et al. 2018a, 2018d, 2018e).

For each analytical method, we combined a full scan (FS) with a data-independent acquisition (DIA), providing the MS^2 spectra for confirmatory analysis.

Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA) acquired and elaborated data.

Validation parameters

Antibiotic validation was assessed following the Commission Decision guidelines 657/2002/CE, while for the other analytes SANTE/11813/2017 guidelines were followed. All the validation parameters are described in our previous works (Chiesa et al. 2018a, 2018d). Regarding parabens, our analytical procedure published earlier (Chiesa et al. 2018e) was followed strictly, including also the determination of validation parameters for 3,4-DHB, OH-MeP and OH-EtP that were not previously elaborated.

Statistical evaluation

Preliminary statistical evaluation (Shapiro-Wilk Test) revealed that data were not normally distributed. Therefore, non-parametric Kruskal-Wallis One Way analysis followed by all pairwise multiple comparison processes (Dunn's method) were used to check the differences between the medians of the datasets. Statistical analyses were performed using Sigma Stat (Statistical Analysis System, version 12.5) software (Jandel Scientific GmbH, Erkrath, Germany). A *P*-value of 0.05 was set as statistically significant.

Results and discussion

No POPs were found in samples analysed. In literature, one of the compounds detected with highest frequency were PCBs, where concentrations ranged up to 95 pg g^{-1} (Toms et al. 2016), 0.03 ng g^{-1} and 0.29 ng g^{-1} for fish and gluten-free cereals products (Lorán et al. 2010), 7.78–270 pg g^{-1} (Jeong et al. 2014) while negligible PCB levels were detected in another study, in line with our results (Table 1). Literature results showed PBDEs were found with

median concentrations at 21 pg g^{-1} in United States samples and 36 pg g^{-1} in Chinese samples (Liu et al. 2014). In one study, conducted on homemade Korea samples, PBDEs were found with highest frequency in 90% of samples at concentrations from 24.5 to 6000 pg g^{-1}(Jeong et al. 2014), higher than those found in commercial formulae from the United States where median concentration were 1725 pg g^{-1} for meat samples, 283 pg g^{-1} fish, 31.5 pg g^{-1} in dairy products (Schecter et al. 2004). The lower levels were found in European products, with whom our results are in line suggesting a safety of the products. Moreover, according to European Community in 2006 (European Commission 2006), baby food should be free of pesticides residues and EFSA panel set a Maximum Residue Level of 0.01 mg kg^{-1} in food for infant, as consumed (EFSA 2018a). In one study conducted in Spain (Fontcuberta et al. 2008), the authors observed a gradual disappearance of regulated chlorinated organic pesticides from 1989–2000 period and 2001–2006 period, suggesting that this could reflect an improvement of worldwide regulation (Fontcuberta et al. 2008). In our study, no pesticides residues were found and this reflects what has been reported in other studies (Fontcuberta et al. 2008) on the progressive lower detection of pesticides as a consequence of the improvement of industrial processes and regulation. So, on the base of our results, a growing enhancement of regulation could be linked to an improvement of product safety and therefore an absence of contaminants (EFSA 2018a).

As regard PFASs, none were detected, demonstrating that this kind of contamination in the different baby food analysed may currently not be of concern. In particular if we compare our results to the few studies present in literature, in that of Ullah et al. (2012) the detection frequency (percentage detects) for the 13 investigated PFASs was 77% in fish, 64% in meat, 49% in vegetables at concentrations below the respective minimum detectable level of 7 to 20 pg g^{-1}, and could thus only be estimated semi-quantitatively. Quantifiable concentrations of several PFASs were found in pig liver and fish and the highest level of PFOS (1.8 ng g^{-1}) was quantified in fish from The Netherlands, if compared to 13 pg g^{-1} found in those from Bangladesh. In the study of Lorenzo et al. (2016), PFBA and PFOA were detected in 100% of analysed samples with concentrations up to 5013 ng g^{-1}, followed by PFDA (83%) up to 387 ng g^{-1} and PFOS detected only in 17% of the samples and they stated they can derive from the production chain since many parts of the equipment were made of perfluoroalkylated materials.

As regard antibiotics, also in this case we found no residues in any analysed baby food. In the study of Gentili et al. (2004), among 30 analysed infant foods for sulphonamides, one, whose formulation was based on veal meat, was positive to sulfamethizole (1.4 ng g^{-1}) and other two samples were <LOQ. In the work of Aguilera-Luiz et al. (2012) only one meat baby food sample out of 21 showed the presence of levamisole at 9.5 ng g^{-1}. In the work of Nebot et al. (2014) 31 baby food samples containing between 15% and 20% beef analysed for tetracyclines, only 3 samples showed doxycycline with concentrations between 5 and 9 ng g^{-1}, one tetracycline (5.4 ng g^{-1}) and another chlortetracycline (7.2 ng g^{-1}). In the other few works reported in Table 1, no compounds were present.

Parabens affect reproductive or endocrine endpoints at high concentrations in both male and female immature experimental animals, and with exposure, both boys and girls may be at risk of endocrine disruption. Oestrogenic effects in boys may increase the risk for incomplete masculinisation resulting in decreased sperm quality. In girls, an increased oestrogenic load may increase the risk of early puberty, and premature mammary development (Boberg et al. 2010). The great majority of samples enrolled in this study did not reveal measurable levels of parabens (Table 3), except one plum preparation that contained 4.14 ng g^{-1} of MeP and one apple, one pear and one turkey sample that contained PrP at the concentrations of 1.2, 3.4 and 1.33 ng g^{-1}, respectively. Although having such low incidence, this kind of contamination should not be underestimated as it is not clear what might be the origin of those two parabens discovered randomly in 4 out of 112 samples (<3.5%). The range of concentration detected herein corresponds to the daily intake which is 2–3 orders of magnitude (about 1000 times) below ADI recommended by European Medicines Agency (2015) which was set at 1.25 mg kg^{-1}.

Table 3. Concentration levels (ng g^{-1}) of parabens and their analogues/possible metabolites in all baby food sample analysed.

	MeP[a]	EtP	PrP	BtP	BzP	p-HBA	3,4-DHB	OH-MeP	OH-EtP
Positive (%)	1 (0.9%)	n.d.	3 (2.7%)	n.d.	n.d.	112 (100%)	86 (76%)	10 (8,9%)	3 (2.7%)
Mean	4.14	n.d.	1.70	n.d.	n.d.	321.7	162.2	3.7	7.5
Median	/	n.d.	1.33	n.d.	n.d.	176.6	10.1	2.1	7.3
Min	/	n.d.	1.20	n.d.	n.d.	14.4	2.1	0.8	7.2
Max	/	n.d.	3.24	n.d.	n.d.	2149	3638	14.4	8.2
Percentile 25% (Q1)	/	n.d.	1.33	n.d.	n.d.	93.9	3.3	1.1	7.2
Percentile 75% (Q3)	/	n.d.	3.24	n.d.	n.d.	455.9	52.6	4.6	8.2

[a] Refer to text (materials and methods section) for full names of the abbreviated compounds.
*n.d. = not detected.

Special attention needs to be directed towards PrP because legislation concerning this compound has been rather confusing in the past and an ADI has been recommended recently (European Medicines Agency 2015). PrP is an antimicrobial preservative used in veterinary medicinal products, and it was previously classified as additive E216. As a result of EFSA's re-evaluation (2004) of parabens with E numbers E214-E219, the E classification of PrP (and its sodium salt) were successively suspended. This decision was based on the scientific data indicating that administration of PrP to male rats resulted in adverse effects on the hormonal system and male reproductive functions. It is therefore recommended to collect more occurrence data for parabens and transformation products to conduct a thorough exposure and safety assessment.

Unfortunately, the literature data regarding parabens' occurrence in processed food intended for infants' diet is rather limited, apart from the preliminary results reported by our group that concerns exclusively baby food containing fish (Chiesa et al. 2018e) where no parabens were detected.

p-HBA was found in all samples which is why results obtained here in regard to different type of infant food preparation were obtained. It is well-established that *p*-HBA does not exclusively derive as degradation product and potential indicator of parabens treatment, but it is also naturally present in many vegetables (Tomás-Barberan and Clifford, 2000). Indeed, when samples from four food groups were taken into consideration there were evident differences in the *p*-HBA level (Figure 1). The vegetable samples possessed an extremely high amount of *p*-HBA most probably due to the endogenous origin of *p*-HBA, with preparations based on carrot and plum showing the highest levels. However, the reason why samples that consisted of meat only, contained a substantial amount of this metabolite is uncertain (n = 36, median = 89,3, 25–75 ng g^{-1} percentile = 50.9–99.1 ng g^{-1}). One possible explanation might lay down in the fact that those samples were subjected to more elaborate technological processes (such as cooking) including the addition of water treatment that might be source of parabens, as well. Also, it remains to be defined what would be the safe levels of *p*-HBA because regardless of its origin it has been reported (independently from other parabens) to exhibit oestrogenic activity (Boberg et al. 2010). Actually, *p*-HBA is used as a flavouring additive, with no safety concern declared at current levels of intake.

Regarding 3,4-DHB, recent studies indicate its potential to act as a protective antioxidant polyphenolic compound against various diseases including neoplasms (Xie et al. 2018) while the findings about the positive correlation between its urinary concentration and childhood obesity call for caution (Xue et al. 2015). The differences between infant food based on meat or vegetables/fruit is also apparent when the amount and distribution of 3,4-DHB is concerned (Table 3, Figure 2): the median level (with 25^{th}-27^{th} percentile) in 22 meat/meat+veg samples was 3.4 ng g^{-1} (1.8–4.6 ng g^{-1}) vs 38 ng g^{-1} (5.2–177.8 ng g^{-1}) for all 46 fruit and vegetables preparations. Considerable variability within each class and limited number of fish/fish+veg samples disabled any statistical confirmation regarding the fact that fish/fish+veg samples contained notably lower levels when compared with veg/fruit samples. Cheese samples did not reveal any measurable amount of 3,4-DHB. Extremely high contents of 3,4-DHB were found in all three pure plum specimens (2148, 2471 and 3638 ng g^{-1}). A high concentration of 3,4-DHB was found in one sample

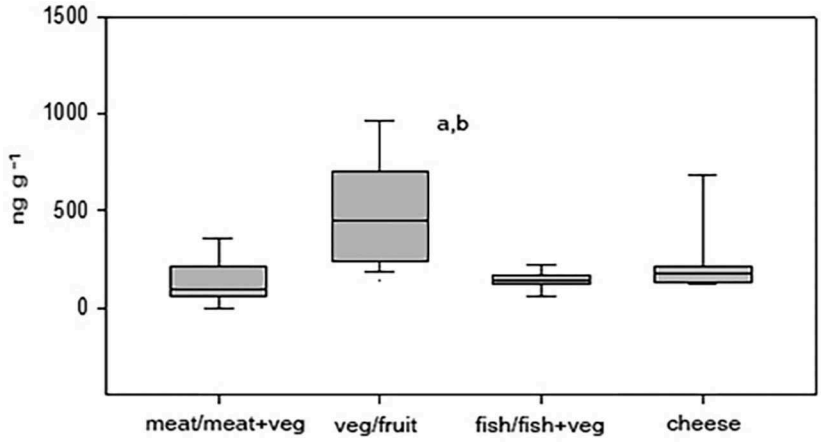

Figure 1. Distribution of *p*-HBA according to baby food category: animal, vegetable/fruit, cheese and mixed matrix for which meat or fish represented the major component as declared on the label. Data are reported as median with 25th–75th percentile range. Comparison was done using Kruskal-Wallis One Way Analysis of Variance on Ranks, followed by Dunn's test for pairwise multiple comparison procedures: a – stands for $p < .001$ when meat/meat +vegetables samples were compared with vegetables/fruit preparation; b – stands for $p < .001$ when fish/fish +vegetables samples were compared with vegetables/fruit preparation.

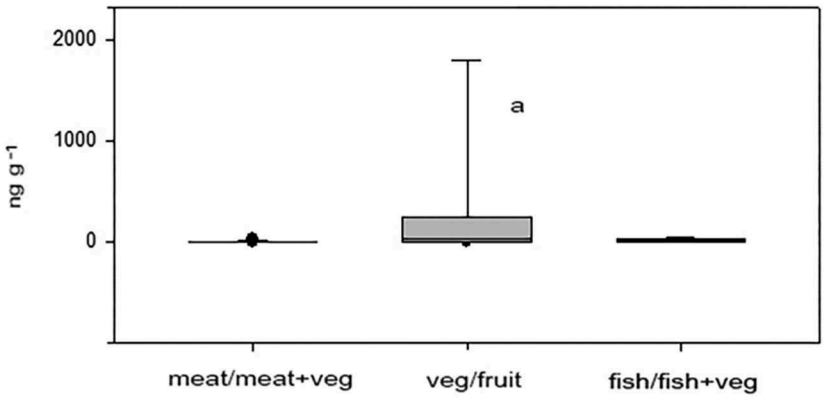

Figure 2. Distribution of 3,4-DHB in the samples where it was detected. N (meat/meat+veg) = 22; N (veg/fruit) = 46; N (fish/fish+veg) = 9. Data are reported as median with 25^{th}–75^{th} percentile range. Comparison was done using Kruskal-Wallis One Way Analysis of Variance on Ranks that revealed statistical significance (a stands for $p < .001$ vs meat/meat+veg group).

that was plum-apple homogenate (943.2 ng g^{-1}). The endogenous origin of 3,4-DHB in those samples is apparent, as plum has been shown to contains a substantial amount of polyphenolic compounds, 3,4-DHB included (Kakkar and Bais 2014). The same samples contained OH-EtP and also here their natural origin as part of polyphenolic pertinence is more plausible. Random occurrence of OH-MeP in meat and vegetable also points towards its endogenous origin. On the other hand, a very important finding concerning OH-MeP is highlighted by its frequent appearance in preparations that contained fish as a main constituent: 7 of 13 fish samples showed OH-MeP presence. Considering that OH-MeP is the main hydroxylated MeP derivate in aquatic biota (Xue et al. 2017) the content of OH-MeP especially in infant food preparation based on fish (without any other ingredient) might be a reliable indicator of parabens contamination.

The analysis conducted for parabens confirmed the presence of salicylic acid in all infant food samples and its distribution is presented in Figure 3. This is due to the addition of ingredients rich in salicylates, such as vegetables where salicylates are naturally present in high quantities (Malakar et al. 2017). Plant salicylates have an important role against pathogens, herbivores, and abiotic stresses,

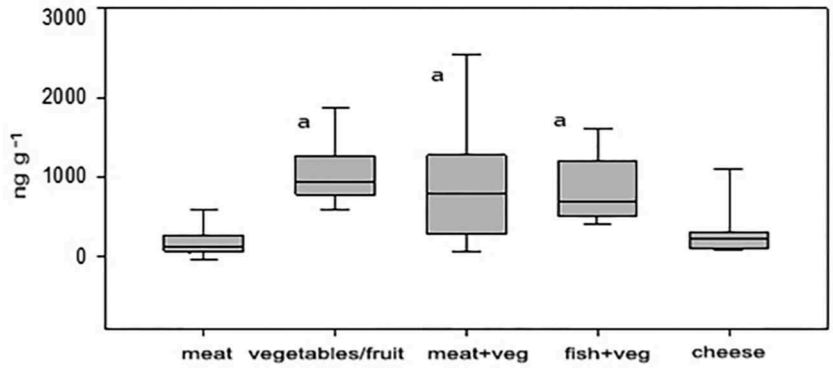

Figure 3. Distribution of salicylic acid according to baby food category: meat, vegetable/fruit, mixed meat + vegetables, mixed fish + vegetable and cheese. Data are reported as median with 25th–75th percentile range. Comparison was done using Kruskal-Wallis One Way Analysis of Variance on Ranks that revealed statistical significance (a stands for $p < .001$ vs meat group).

mediating physiological and biochemical processes. Several studies reported the beneficial action of the salicylates on the human health, due to the anti-inflammatory and antioxidative activities (Malakar et al. 2017). However, the concentration of salicylic acid is species-dependent and different plants could produce high amounts of these substances that could be a potential health risk (Cunningham 2010), especially for infants as particularly vulnerable category. In this regard, infants are a matter of concern because some of them may have adverse reactions to even a small quantity of salicylates. Salicylates are well-known food additives and therefore an analytical strategy that would distinguish between naturally occurring and industrially introduced salicylic acid is needed for further investigation. This is especially because of increased incidence of allergic reaction to salicylate. Our data regarding the salicylic acid concentration in food items for infant diet are the first of this kind; therefore it was not possible to make a comparison with similar studies. Our results indicate the much lower content compared to fresh food items as recently was reported by Kęszycka et al. (2017). Therefore, it remains to be elucidated whether the concentration found in the samples enrolled in this study represents a safety risk for some paediatric categories and in which extend food processing influences its final quantity.

Conclusions

POPs, PFASs or antibiotics were not detected and all samples were compliant with European legislation. Confirmation of negative data is also important, particularly for the indications and needs dictated by EFSA and other competent authorities in expanding a database on residual analyses of emerging contaminants in different types of food for a reliable risk assessment. On the other hand, some parabens and their metabolites, which are classified as endocrine disruptors, were detected at trace levels and significantly below the ADI recommended by EFSA and the European Medicines Agency. This study shows the importance of collecting more data on the occurrence of parabens and transformation products to assess exposure and possible health impact for sensitive populations such as infants and young children.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Research Support Plan (PSR) 2018 of the University of Milan.

References

Aguilera-Luiz MM, Vidal JM, Romero-González R, Frenich AG. 2012. Multiclass method for fast determination of veterinary drug residues in baby food by ultra-high -performance liquid chromatography–tandem mass spectrometry. Food Chem. 132:2171–2180.

Al-Zahraa MD, Soumia MD, Fathy EE. 2016. Analysis of organochlorine and organophosphorus pesticide residues in dairy products and baby foods from Egyptian Markets. J Env Anal Toxi. 6:1–5.

Boberg J, Taxvig C, Christiansen S, Hass U. 2010. Possible endocrine disrupting effects of parabens and their metabolites. Reprod Toxicol. 30:301–312.

Chiesa LM, Nobile M, Ceriani F, Malandra R, Arioli F, Panseri S. 2019. Risk characterisation from the presence of environmental contaminants and antibiotic residues in wild and farmed salmon from different FAO zones. Food Addit Contam Part A. 36:152–162.

Chiesa LM, Nobile M, Malandra R, Panseri S, Arioli F. 2018d. Occurrence of antibiotics in mussels and clams from various FAO areas. Food Chem. 240:16–23.

Chiesa LM, Nobile M, Malandra R, Pessina D, Panseri S, Labella GF, Arioli F. 2018a. Food safety traits of mussels and clams: distribution of PCBs, PBDEs, OCPs, PAHs and PFASs in sample from different areas using HRMS-Orbitrap® and modified QuEChERS extraction followed by GC-MS/MS. Food Addit Contam Part A. 35:959–971.

Chiesa LM, Nobile M, Panseri S, Arioli F. 2017. Antibiotic use in heavy pigs: comparison between urine and muscle samples from food chain animals analysed by HPLC-MS/MS. Food Chem. 235:111–118.

Chiesa LM, Nobile M, Panseri S, Arioli F. 2018c. Suitability of feathers as control matrix for antimicrobial treatments detection compared to muscle and liver of broilers. Food Control. 91:268–275. doi:10.1016/j.foodcont.2018.04.002

Chiesa LM, Nobile M, Pasquale E, Balzaretti C, Cagnardi P, Tedesco D, Arioli F. 2018b. Detection of perfluoroalkyl acids and sulphonates in Italian eel samples by HPLC-HRMS Orbitrap. Chemosphere. 193:358–364.

Chiesa LM, Pavlovic R, Panseri S, Arioli F. 2018e. Evaluation of parabens and their metabolites in fish and fish products: a comprehensive analytical approach using LC-HRMS. Food Addit Contam Part A. 35:2400–2413.

Cunningham E. 2010. Are there foods that should be avoided if a patient is sensitive to salicylates? J Am Diet Assoc. 110:976.

Díaz-Alvarez M, Turiel E, Martín-Esteban A. 2009. Selective sample preparation for the analysis of (fluoro) quinolones in baby food: molecularly imprinted polymers versus anion-exchange resins. Anal Bioanal Chem. 393:899–905.

Dobrinas S, Stanciu G, Soceanu A, Culea A. 2011. Analysis of organochlorine and pyrethroid pesticide residues in baby food samples. Ovidius Univ Ann Chem. 22:107–112.

(EFSA) European Food Safety Authority. 2004. EFSA advises on the safety of paraben usage in food. European Union. https://www.efsa.europa.eu/en/press/news/040929.

(EFSA) European Food Safety Authority. 2017. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. doi: 10.2903/j.efsa.2017.4849.

(EFSA) European Food Safety Authority. 2018a. Scientific opinion on pesticides in foods for infants and young children. EFSA J. 16(5286):1–75. doi:10.2903/j.efsa.2018.5286.

(EFSA) European Food Safety Authority. 2018b. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA J. 16:5194. doi:10.2903/j.efsa.2018.5194

European Commission. EC Council Directive 2006/125/EC on processed cereal-based foods and baby foods for infants and young children. OJ. 2006;L339:5. http://data.europa.eu/eli/dir/2006/125/oj.

European Commission. 2015. Commission Implementing Regulation (EU) No 2015/1080, O.J. L 175, of 03 July 2015 amending Regulation (EU) No 37/2010 as regards the substance propyl 4-hydroxybenzoate and its sodium salt. http://data.europa.eu/eli/reg_impl/2015/1080/oj.

European Medicines Agency. 2015. European Public MRL assessment report (EPMAR) Propyl 4-hydroxybenzoate and its sodium salt (all food producing species) MA/CVMP/632934/2014.

Fontcuberta M, Arqués JF, Villalbi JR, Martinez M, Centrich F, Serrahima E, Pineda L, Duran J, Casas C. 2008. Chlorinated organic pesticides in marketed food: barcelona, 2001–06. Sci Total Env. 389:52–57. doi:10.1016/j.scitotenv.2007.08.043

Gentili A, Perret D, Marchese S, Sergi M, Olmi C, Curini R. 2004. Accelerated solvent extraction and confirmatory analysis of sulfonamide residues in raw meat and infant foods by liquid chromatography electrospray tandem mass spectrometry. J Agric Food Chem. 52:4614–4624.

Jeong Y, Lee S, Kim S, Choi SD, Park J, Kim HJ, Kim SY. 2014. Occurrence and exposure assessment of polychlorinated biphenyls and organochlorine pesticides from homemade baby food in Korea. Sci Total Environ. 470:1370–1375.

Jia W, Chu X, Ling Y, Huang J, Chang J. 2014. High-throughput screening of pesticide and veterinary drug residues in baby food by liquid chromatography coupled to quadrupole Orbitrap mass spectrometry. J Chromatogr A. 1347:122–128.

Kakkar S, Bais S. 2014. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol. 2014:1–9.

Kęszycka PK, Szkop M, Gajewska D. 2017. Overall content of salicylic acid and salicylates in food available on the European market. J Agric Food Chem. 65:11085–11091.

Leandro CC, Fussell RJ, Keely BJ. 2005. Determination of priority pesticides in baby foods by gas chromatography tandem quadrupole mass spectrometry. J Chromatog A. 1085:207–212.

Liu LY, Salamova A, Hites RA. 2014. Halogenated flame retardants in baby food from the United States and from China and the estimated dietary intakes by infants. Environ Sci Technol. 48:9812–9818.

Lorán S, Bayarri S, Conchello P, Herrera A. 2010. Risk assessment of PCDD/PCDFs and indicator PCBs contamination in Spanish commercial baby food. Food Chem Toxicol. 48:145–151.

Lorenzo M, Farré M, Blasco C, Onghena M, Picó Y, Barceló D. 2016. Perfluoroalkyl substances in breast milk, infant formula and baby food from Valencian Community (Spain). Environ Nanotechnol Monit. 6:108–115.

Malakar S, Gibson PR, Barrett JS, Muir JG. 2017. Naturally occurring dietary salicylates: A closer look at common Australian foods. J Food Compos Anal. 57:31–39.

Nasr JJ, Shalan S, Belal F. 2014. Simultaneous determination of tylosin and josamycin residues in muscles, liver, eggs

and milk by MLC with a monolithic column and time-programmed UV detection: application to baby food and formulae. Chem Cent J. 8:37.

Nebot C, Guarddon M, Seco F, Iglesias A, Miranda JM, Franco CM, Cepeda A. 2014. Monitoring the presence of residues of tetracyclines in baby food samples by HPLC-MS/MS. Food Control. 46:495–501.

Notardonato I, Salimei E, Russo MV, Avino P. 2018. Simultaneous determination of organophosphorus pesticides and phthalates in baby food samples by ultrasound–vortex-assisted liquid–liquid microextraction and GC–IT/MS. Anal Bioanal Chem. 410:3285–3296.

Pandelova M, Piccinelli R, Lopez WL, Henkelmann B, Molina-Molina JM, Arrebola JP, Schramm KW. 2011. Assessment of PCDD/F, PCB, OCP and BPA dietary exposure of non-breast-fed European infants. Food Addit Contam Part A. 28:1110–1122.

Pérez-Ortega P, Lara-Ortega FJ, García-Reyes JF, Gilbert-López B, Trojanowicz M, Matamoros V, Calderón-Preciado D, Domínguez C, Bayona JM. 2012. Analytical procedures for the determination of emerging organic contaminants in plant material: a review. Anal Chim Acta. 722:8–20.

Radford SA, Panuwet P, Hunter RE Jr, Barr DB, Ryan PB. 2014. HPLC-MS/MS method for the measurement of insecticide degradates in baby food. J Agr Food Chem. 62:7085–7091.

Rodriguez E, Moreno-Bondi MC, Marazuela MD. 2011. Multiresidue determination of fluoroquinolone antimicrobials in baby foods by liquid chromatography. Food Chem. 127:1354–1360.

Santonicola S, Albrizio S, Murru N, Ferrante MC, Mercogliano R. 2017. Study on the occurrence of polycyclic aromatic hydrocarbons in milk and meat/fish based baby food available in Italy. Chemosphere. 184:467–472.

Schecter A, Colacino J, Patel K, Kannan K, Yun SH, Haffner D, Harris TR, Birnbaum L. 2010. Polybrominated diphenyl ether levels in foodstuffs collected from three locations from the United States. Toxicol Appl Pharm. 243:217–224.

Schecter A, Päpke O, Tung KC, Staskal D, Birnbaum L. 2004. Polybrominated diphenyl ethers contamination of United States food. Environ Sci Technol. 38:5306–5311.

Tomás-Barberán FA, Clifford MN. 2000. Dietary hydroxybenzoic acid derivatives–nature, occurrence and dietary burden. J Sci Food Agric. 80:1024–1032.

Toms LML, Hearn L, Mueller JF, Harden FA. 2016. Assessing infant exposure to persistent organic pollutants via dietary intake in Australia. Food Chem Toxicol. 87:166–171.

Ullah S, Alsberg T, Vestergren R, Berger U. 2012. Determination of perfluoroalkyl carboxylic, sulfonic, and phosphonic acids in food. Anal Bioanal Chem. 404:2193–2201.

Vakh C, Alaboud M, Lebedinets S, Korolev D, Postnov V, Moskvin L, Osmolovskaya O, Bulatov A. 2018. An automated magnetic dispersive micro-solid phase extraction in a fluidized reactor for the determination of fluoroquinolones in baby food samples. Anal Chimica Acta. 1001:59–69.

Wang S, Bilal M, Hu H, Wang W, Zhang X. 2018. 4-Hydroxybenzoic acid—a versatile platform intermediate for value-added compounds. Appl Microbiol Biotechnol. 102:3561–3571.

Xie Z, Guo Z, Wang Y, Lei J, Yu J. 2018. Protocatechuic acid inhibits the growth of ovarian cancer cells by inducing apoptosis and autophagy. Phytother Res. 32:2256–2263.

Xue J, Sasaki N, Elangovan M, Diamond G, Kannan K. 2015. Elevated accumulation of parabens and their metabolites in marine mammals from the United States coastal waters. Environ Sci Technol. 49:12071–12079.

Xue X, Xue J, Liu W, Adams DH, Kannan K. 2017. Trophic magnification of parabens and their metabolites in a subtropical marine food web. Environ Sci Technol. 51:780–789.