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Endocrine-disrupting pesticides in infant formulas marketed in Brazil: Interference-free GC–MS analysis and early-life dietary exposure assessment

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ABSTRACT

Endocrine-disrupting compounds (EDCs) include ubiquitous and persistent environmental contaminants that interfere with the endocrine system's functions. Many of these compounds are used as acaricides, fungicides, herbicides, and insecticides in agricultural fields worldwide. Considering the serious implications of exposure to EDCs in the first months of life and the few works on pesticide residues in infant formulas, the present research focused exclusively on endocrine-disrupting pesticides in infant formulas intended for babies below 1 year old available in the Brazilian market. An accurate, sensitive, and selective gas chromatography–mass spectrometry (GC–MS) method was successfully validated, and then, applied to infant formula samples. The limits of detection and quantification were low enough to meet the maximum residue level (MRL) of 10.0 μg/kg established for infant formula. Recoveries varied from 86.3 to 119.8 % and precision values, under repeatability and within-laboratory reproducibility, were ≤ 19.7 %. Another unique feature of the study was the detection and strategies to remove a potential matrix-interfering compound, which shared the same ions monitored for malathion in GC–MS analysis, thus preventing false positives. Among the 60 infant formula samples analysed, dimethoate, an organophosphate insecticide, was detected in five samples, with one soy-based infant formula exceeding the MRL. Based on a deterministic approach, the estimated daily intakes were within the acceptable daily intake (ADI) values and below the acute reference dose (ARfD), indicating no major health concerns.

1. Introduction

Endocrine-disrupting compounds (EDCs) comprise "exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes" (Kavlock et al., 1996). Several chemical compounds have been recognised as EDCs, including certain pesticides typically applied in agricultural fields worldwide. Given the stability and persistence of some of them in the environment, associated with the capacity for bioaccumulation and biomagnification in trophic chains, humans may be exposed to EDCs through distinct sources whose effects on health could be additive or synergistic even if individual compounds occur at safe levels (Flaws et al., 2020; Petrarca et al., 2022; Stockholm Convention on Persistent Organic Pollutants (POPs), 2024).

The consequences of exposure to EDCs depend, predominantly, on age, compound mixtures, circadian rhythms, latency from exposure, life stage, and sex (Diamanti-Kandarakis et al., 2009; La Merrill et al., 2020). These chemicals may act on the endocrine system by many mechanisms, they can (i.) interact with or activate hormone receptors, (ii.) antagonise hormone receptors, (iii.) alter hormone receptor expression, (iv.) alter signal transduction in hormone-responsive cells, (v.) induce epigenetic modifications in hormone-producing or hormone-responsive cells, (vi.) alter hormone synthesis, (vii.) alter hormone transport across cell membranes, (viii.) alter hormones distribution or circulating levels of hormones, (ix.) alter hormone metabolism or clearance, and (x.) alter the fate of hormone-producing or hormone-responsive cells (La Merrill et al., 2020). There is evidence that the health risks increase when the exposure window corresponds to the phases of development, formation or differentiation of organs and systems, periods of highest sensitivity (La Merrill et al., 2020). Therefore, early-life exposure to EDCs may have consequences different from that observed in adults, which may only be apparent in later stages (Diamanti-Kandarakis et al., 2009). In humans, exposure to EDCs has been linked with obesity and metabolic

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dysfunctions such as type 2 diabetes and thyroid and lipid metabolism-related disorders; alterations in the reproductive system including abnormal timing of puberty, genital malformations, endometriosis, polycystic ovarian syndrome, reduced testosterone production, poor semen quality, and hormone-sensitive cancers such as breast, endometrial, ovarian, testicular and prostate; and neuro-developmental disorders in children including attention-deficit hyperactivity, autism spectrum disorder, and decreased IQ (Flaws et al., 2020; Kahn, Philippat, Nakayama, Slama, & Trasande, 2020).

It is presumed that infants (0–1 year of age) and young children (1–3 years of age) are more vulnerable to chemical contaminants occurring in the diet because they have lower metabolic capacities and higher daily food intake based on a body weight compared to adults (Scientific Committee et al., 2017). Furthermore, infants below 16 weeks of age represent a particular subpopulation with unique features, such as inefficient mechanisms of enzymatic detoxifying, reduced plasmatic protein levels to bind chemicals, deficient function of excretory organs, and immaturity of physiological barriers such as blood-brain barrier (Scientific Committee et al., 2017). Additionally, when breastfeeding is not feasible, commercial infant formula has been the exclusive source of nutrition for non-breastfed infants in the first months of life (Scientific Committee et al., 2017). All these particularities attract special attention to investigate the exposure to endocrine-disrupting pesticides in early childhood through infant formula consumption.

Pesticides constitute the largest group of EDCs (Vieira, Farias, Spaolonzi, Silva, & Vieira, 2020). Scientific literature reviews have consolidated relevant findings, *in vivo* or *in vitro*, on the potential of certain pesticides to interfere with endocrine system functions (Combarnous, 2017; Encarnação, Pais, Campos, & Burrows, 2019; European Food Safety Authority, 2015; Hrouzková & Matisová, 2012; Kiyama & Wada-Kiyama, 2015; Mckinlay, Plant, Bell, & Vouvoulis, 2008; Mnif et al., 2011; Stoker & Kavlock, 2010; Vieira et al., 2020). Many of these pesticides are approved for use and/or present maximum residue levels (MRL) set in foodstuffs typically used as ingredients in infant formulas, such as soybeans, cereals and vegetable oils (acetochlor, alachlor, atrazine, λ-cyhalothrin, diuron, malathion, procymidone, thiabendazole, trifluralin, and others), and milk (diazinon, dimethoate, simazine, triadmefon, and others), by the European Commission and Brazilian legislation for example (Agência Nacional de Vigilância Sanitária, 2024; European Commission, 2024). Other endocrine-disrupting pesticides, such as aldrin, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, and lindane (γ-HCH), are banned in some countries and listed as persistent organic pollutants (POPs) in the Stockholm Convention (Stockholm Convention on Persistent Organic Pollutants (POPs), 2024). In this way, restrictions, or even prohibitions, on the use of pesticides in food commodities, intended for the manufacture of foods destined for infants and young children, do not guarantee the absence of residues in the final product considering their persistence and environmental contamination. Thus, residues should be the lowest achievable possible to safeguard this vulnerable consumer group (European Commission, 2016). Particularly, a default MRL of 10.0 μg/kg was established by the European Commission for pesticides in infant formula (European Commission, 2016; European Commission, 2021).

Early-life exposure to EDCs through infant formula consumption has been assessed, mainly, for bisphenols, parabens, phthalates, poly- and perfluoroalkyl substances and certain POPs (Hatzidaki et al., 2023; Isci, 2023; Martín-Carrasco, Carbonero-Aguilar, Dahiri, Moreno, & Hinojosa, 2023; Nuti et al., 2024; Petrarca, Perez, & Tfouni, 2022; Souza et al., 2023; van Beijsterveldt et al., 2022; Yesildemir & Akdevelioglu, 2021). Very few studies on pesticide residues in infant formulas are available (Table SM1 of Supplementary Material). Furthermore, studies exclusively focused on exposure to endocrine-disrupting pesticides through infant formula consumption were not reported to date. Even though few works are available, pesticide residues, specifically, organochlorines, organophosphates, pyrethroids, and triazines, have been reported in infant formula worldwide. In most cases, at least one of the target pesticides was detected in at least one infant formula sample analysed (Table SM1). Moreover, residues exceeding the MRL were found for the insecticides ethoprophos in infant formulas from Romania (Dobrinas, Soceanu, Popescu, & Coatu, 2016); α-endosulfan, malathion, and β-HCH in samples from India (Mishra, Johnson, & Vankar, 2002); and the herbicide glyphosate in Brazilian infant formulas (Rodrigues & Souza, 2018; Souza, Ferreira, Pagliarini, & Rodrigues, 2021). These data are of great concern since ultra-processed foods like infant formulas are not commonly inserted in pesticide monitoring programs. High-sensitive analytical methods have been required to monitor pesticide residues in infant formula at their strict MRLs. Gas chromatography (GC), coupled with mass spectrometry (MS) or electron-capture detector, is among the main analytical techniques employed. In addition, liquid chromatography interfaced with MS or fluorescence detector has been applied (Table SM1). Regarding the sample preparation methods, large volumes of organic solvents and considerable amounts of sample and chemicals, including pressurised liquid extraction or solid-phase extraction (SPE) on cartridges or columns, have been used in some cases, suggesting the need for more eco-friendly and less costly approaches for the monitoring of pesticide residues in infant formula (Table SM1).

Given the scarcity of works on pesticide residues in infant formulas in the last five years (Table SM1) and the serious consequences of exposure to EDCs in the first months of life, the present research focused exclusively on endocrine-disrupting pesticides (Fig. 1) in infant formulas intended for babies below 1 year old available in the Brazilian market. The study also included: (i) validation and application of an accurate, sensitive, and selective GC–MS method for multi-residues of endocrine-disrupting pesticides in infant formulas; (ii) appropriate means and strategies to overcome a potential matrix-interfering compound in GC–MS analysis; (iii) assessment of dietary exposure to EDCs through infant formula consumption; and (iv) risk characterisation addressed for formula-fed babies below 16 weeks of age.

2. Material and methods

2.1. Standards and chemicals

Analytical standards of aldrin (98.8 % of purity), *p,p'*-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, 88.5 %), diazinon (92.8 %), hexachlorobenzene (99.5 %), procymidone (98.0 %), simazine (98.9 %), thiabendazole (98.0 %), triadimefon (99.1 %), and the internal standard triphenyl phosphate (TPP; 99.3 %) were supplied by Sigma-Aldrich (Saint Louis, MO, USA); alachlor (99.2 %), atrazine (98.0 %), lindane (99.5 %), malathion (98.0 %), and trifluralin (99.5 %) by Fluka (Neu-Ulm, Germany); acetochlor (98.0 %), dieldrin (98.8 %), diuron (98.5 %), endrin (99.0 %), heptachlor (99.2 %), and lambda-cyhalothrin (99.1 %) by Dr. Ehrenstorfer GmbH (Augsburg, Germany); and dimethoate (99.4 %) by Riedel-de-Haën (Seelze, Germany).

Individual standard solutions were prepared in acetonitrile at concentrations between 1000.0 and 2000.0 μg/mL. Specifically for simazine, a standard solution at 500.0 μg/mL was prepared in methanol, and for dieldrin and hexachlorobenzene, individual solutions at 1000.0 μg/mL were prepared in a mixture of acetonitrile: toluene (1:1, *v*/v). A multi-analyte solution at 100.0 μg/mL in acetonitrile was obtained from the individual stock solutions, and intermediate multi-analyte solutions at 10.0 and 5.0 μg/mL were obtained from appropriate dilutions. Working multi-analyte solutions at 1.0, 0.5, and 0.025 μg/mL were freshly prepared in acetonitrile before use from intermediate solutions. All standard solutions were kept at −20 °C in amber glass flasks.

HPLC-grade solvents, including acetonitrile and methanol, were purchased from J.T. Baker® (Avantor Performance Materials, Inc. C.V. Xalostoc, Mexico), and toluene (Chromasolv®, 99.9 %) was supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Deionised water was obtained from a Milli-Q purifier system (Direct 8, Millipore, Bedford, MO, USA).

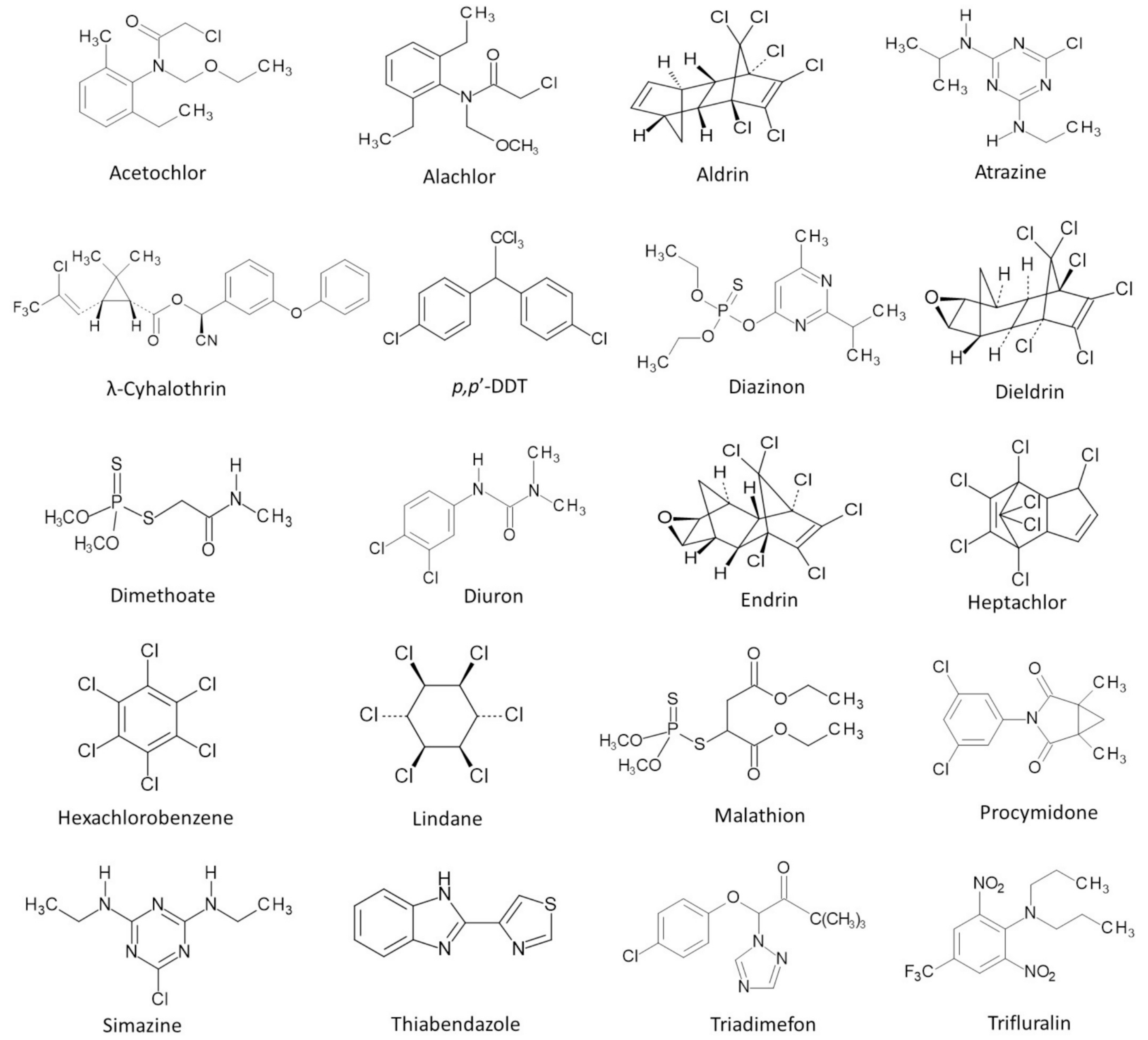


Fig. 1. Chemical structures of the endocrine-disrupting pesticides investigated in infant formulas. The chemical structures were drawn using the Accelrys Draw 4.2 software.

The clean-up sorbents Bondesil-C18 bulk sorbent (particle size of 40.0 μm) and primary-secondary amine (PSA) were purchased from Agilent (Santa Clara, CA, USA) and Supelco (Bellefonte, PA, USA), respectively.

2.2. *Commercial infant formulas*

A total of 60 units of infant formula was acquired from drugstores and supermarkets in the city of Campinas, SP, in southeastern Brazil. The sample collection included the following criteria: infant formulas intended for babies up to 12 months of age; products containing soy-based ingredients (protein isolate and/or lecithin emulsifying) and/or milk-based ingredients (skimmed milk and/or whey) in their composition; brands and manufacturing companies from different countries; varied commercially available sizes (350.0, 400.0, or 800.0 g); different manufacture date and batch number within a same brand; and retail stores located in distinct regions of the city. Infant formulas addressed for special diets were not included in the study. Before the analysis, all samples were kept light-protected at 20 °C in their original packaging.

2.3. *Determination of pesticide residues by GC–MS*

An analytical method based on dispersive microextraction techniques and GC–MS analysis, optimised for multi-contaminants in infant formula (Petrarca, Vicente, & Tfouni, 2024), was applied with slight modifications.

2.3.1. *Sample preparation method*

Two grams of infant formula powder, previously homogenised, were weighed into a 15.0 mL glass centrifuge tube, followed by 40.0 μL of triphenyl phosphate (TPP) internal standard solution at 1.0 μg/mL. In sequence, 5.0 mL of acetonitrile was added, and the mixture was vigorously vortexed for 1 min and then centrifuged at 4000 x*g* for 10 min at 20 °C (Centrifuge 5804R, Eppendorf, Hamburg, Germany). An aliquot of the supernatant was collected for subsequent dispersive solid-phase extraction (d-SPE) clean-up, in which 2.5 mL of extract was quickly shaken by hand with 25.0 mg of PSA sorbent and then centrifuged at 4000 xg for 2 min at 20 °C. Afterwards, the cleaned-up extract was submitted to the dispersive liquid-liquid microextraction (DLLME) pre-concentration step, where 2.0 mL of acetonitrile extract (as a disperser solvent) and 200.0 μL of toluene (extraction solvent) were mixed and transferred to a 15.0 mL glass centrifuge tube containing 6.0 mL of deionised water, followed by vigorous vortex agitation for 10 s. The toluene was dispersed as very fine droplets throughout the all-water-acetonitrile phase, resulting in a cloudy solution. The mixture was then centrifuged at 4000 xg for 2 min at 20 °C, and 100.0 μL of the upper phase (toluene) was transferred to a glass insert placed into a glass vial for GC–MS analysis.

2.3.2. GC–MS analysis

The chromatographic analyses were carried out in an Agilent 7890 A gas chromatography system coupled to a 7693 autosampler and 5975C inert MSD single quadrupole mass analyser with electron ionisation (EI) at 70 eV. The GC–MS system was operated under the ChemStation platform and the data acquisition was in selected ion monitoring (SIM) mode. The quadrupole, EI source, and transfer line were maintained at 180 °C, 300 °C, and 320 °C, respectively. An HP-1MS ultra-inert capillary column (30 m × 0.25 mm × 1.0 μm; Agilent Technologies, Santa Clara, CA, USA) was used and 2.0 μL of sample extract was injected in pulsed splitless mode at 300 °C with a pulse pressure of 50 psi until 0.5 min. The initial oven temperature was fixed at 85 °C, and then, it was ramped to 330 °C at a 12 °C/min rate and finally kept for 2 min. Carrier gas was the ultra-high purity helium (99.999 %) at a 1.2 mL/min flow. The total run time for each injection was 22.4 min with a solvent delay of 7 min.

2.3.3. Identification requirements and quantification of analytes

The identification and confirmation of the target analytes in the samples followed the requirements set in Document N° SANTE/11312/2021 v2 for pesticide residues analysis in food and feed, including (i) a minimum of 3 ions for each analyte being the most characteristic and/or abundant ion for quantification and other two for qualification purpose (Table 1); (ii) retention time equal to that observed in spiked samples with a maximum tolerance of ±0.1 min; and (iii) ion ratio (calculated as an intensity ratio of the less intense ion to that of the more intense ion) within a tolerance of ±30 % in comparison with calibration curves (EU Reference Laboratories for Residues of Pesticides, 2024). To compensate for matrix effects, matrix-matched calibration curves containing at least five concentration levels each were employed for analyte quantification, which were obtained by plotting the ratio of the analyte area/ internal standard (IS) area against the concentration level (μg/kg). The retention time of the IS triphenyl phosphate was 18.54 min, the *m/z* 326 was used as the quantifier ion and *m/z* 233 and 215 were qualifier ions. The samples were extracted and analysed in triplicate, and the results obtained were not corrected for the recovery.

2.4. Method validation

A commercial soy-based infant formula, previously checked for the absence of the target analytes, was employed as a representative matrix for the validation procedure. The limits of detection (LOD) and quantification (LOQ) were established as the lowest concentration of analyte in infant formula extract that provided a signal-to-noise ratio of 3 and 10, respectively. The linear range was evaluated through matrix-matched calibration curves prepared in blank matrix extracts, including the LOQ as the first level. The accuracy was verified through recovery and precision experiments at four fortification levels (1.0; 5.0; 10.0, and 50.0 μg/kg). For this, 2.0 g of infant formula was weighed into a 15.0 mL glass centrifuge tube and spiked with a working multi-analyte solution followed by vigorous shaking by hand, then the mixture was kept at room temperature for at least 1 h before the extraction. The mean recovery (%) was obtained from 5 independent replicates of spiked matrices at each concentration level. Precision, expressed as relative standard deviation (RSD %), was evaluated under repeatability conditions by analysing 5 independent replicates at each level on the same day, and under within-laboratory reproducibility conditions through 10 independent replicates at each level analysed on two different days. Document N° SANTE/11312/2021 v2 was used for the method performance criteria (EU Reference Laboratories for Residues of Pesticides, 2024).

2.5. Early-life dietary exposure assessment

Dietary exposure to selected endocrine-disrupting pesticides was based on a deterministic/point estimate approach, combining the pesticide concentration detected in the samples with the daily infant formula consumption by babies, according to the recommendations of

Table 1

Characteristics and parameters for GC–MS analysis of the endocrine-disrupting pesticides studied in infant formula.

Analyte	Chemical class	Use type	Molecular weight (g/mol) [a]	log k_{ow} [a]	GC–MS parameters	
					Retention time (min)	Selected ion monitoring (SIM), *m/z* [b]
Acetochlor	Organochlorine	Herbicide	269.8	4.1	14.6	**146**; 162; 223
Alachlor	Organochlorine	Herbicide	269.8	3.5	14.8	**160**; 188; 237
Aldrin	Organochlorine	Insecticide	364.9	6.5	15.8	**263**; 293; 329
Atrazine	Triazine	Herbicide	215.7	2.6	13.2	**200**; 173; 215
λ-Cyhalothrin	Pyrethroid	Acaricide and insecticide	449.8	7.0	19.8	141; **181**; 197
p,p'-DDT	Organochlorine	Insecticide	354.5	6.9	18.5	165; 235; **237**
Diazinon	Organophosphate	Insecticide	304.3	3.8	13.7	**137**; 179; 304
Dieldrin	Organochlorine	Insecticide	380.9	5.4	17.4	**79**; 263; 277
Dimethoate	Organophosphate	Acaricide and insecticide	229.3	0.8	13.0	**87**; 93; 125
Diuron	Urea	Herbicide	233.1	2.7	8.8	124; 159; **187**
Endrin	Organochlorine	Insecticide	380.9	5.2	17.8	245; **263**; 317
Heptachlor	Organochlorine	Insecticide	373.3	6.1	15.2	237; **272**; 337
Hexachlorobenzene	Organochlorine	Fungicide	284.8	5.7	13.4	214; 249; **284**
Lindane	Organochlorine	Insecticide	290.8	3.7	13.6	147; **181**; 219
Malathion	Organophosphate	Acaricide and insecticide	330.4	2.4	15.2	**125**; 158; 173
Procymidone	Dicarboximide	Fungicide	284.1	3.0	16.3	96; 255; **283**
Simazine	Triazine	Herbicide	201.7	2.2	13.0	173; 186; **201**
Thiabendazole	Benzimidazole	Fungicide	201.2	2.5	16.1	129; 174; **201**
Triadimefon	Triazole	Fungicide	293.7	2.8	15.6	57; 181; **208**
Trifluralin	Dinitroaniline	Herbicide	335.3	5.3	12.6	264; 290; **306**

[a] Source: https://pubchem.ncbi.nlm.nih.gov.

[b] Ions used for quantification are highlighted in bold.

WHO and FAO (2009). The exposure assessment was addressed to infants below 16 weeks of age because infant formula constitutes the exclusive nutrition source for this infant group in cases where breastfeeding is not a viable option (Scientific Committee et al., 2017).

The estimated daily intake (EDI) was calculated using Eq. (1:

$$EDI\ (\mu g/kg\ bw\ per\ day) = C\ x\ F \tag{1}$$

where: C is the concentration of pesticide quantified in the sample (μg/g) and F is the daily infant formula consumption per kilogram of body weight (g/kg bw). Mean, median, and 75th, 90th, and 95th percentiles of the measured concentrations of pesticide in infant formula were used in the EDI calculation. Three distinct scenarios were considered regarding the non-detected and non-quantified values (left-censored data – LCD): scenario 1 (lower-bound exposure, where the LCD was assumed as zero), scenario 2 (middle-bound exposure, where the LCD was replaced with one-half of LOD), and scenario 3 (upper-bound exposure, where LOD substituted the LCD) (European Food Safety Authority, 2010; WHO & FAO, 2009). An average (200.0 mL/kg bw per day) and high (260.0 mL/kg bw per day) consumption of reconstituted infant formula was assumed for babies below 16 weeks of age (Scientific Committee et al., 2017), which corresponds to a daily amount of 26.0 g/kg bw and 34.0 g/kg bw of powdered infant formula, respectively, considering the preparation instructions fixed in the label of samples analysed.

The risk characterisation was established by the comparison of the obtained EDI with health-based guidance values available for the investigated pesticides (WHO & FAO, 2009), such as acceptable daily intake (ADI) set as "the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health" (EFSA, 2024), and acute reference dose (ARfD) set as "the amount of the substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 hours or less, without appreciable risk to the health of the consumer, on the basis of all the known facts at the time of the evaluation" (World Health Organization, 2017).

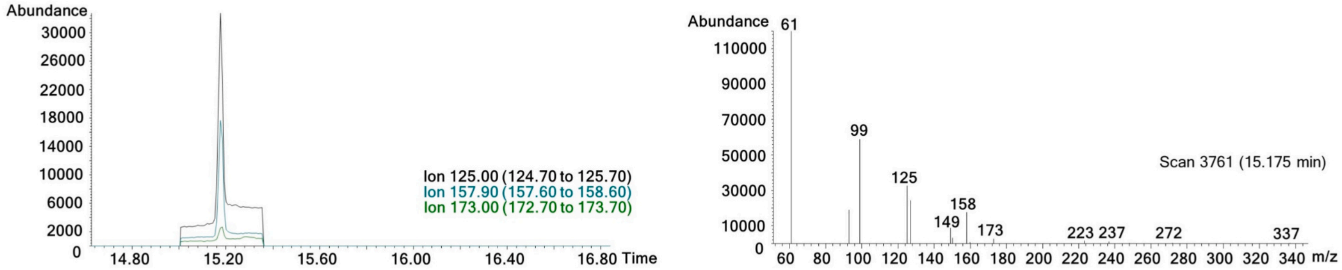
3. Results and discussion

3.1. Overcoming a potential matrix interference in GC–MS analysis

In the trace analysis of complex matrices, analytical selectivity is fully as important as sensitivity. Although the mass spectrometry technique contributes greatly to the method's selectivity, particular matrix interferences may not be completely avoided as shown in Fig. 2. Under the optimised GC–MS conditions, a potential interfering compound was observed in several infant formula samples co-eluting at the retention time (15.2 min) of malathion (Fig. 2A). This interfering peak shared the same ions monitored for the identification and quantification of malathion, specifically, m/z 125, 158, and 173 (Fig. 2B). However, the EI mass spectrum of interfering compound showed a fragmentation profile with notable differences in the abundance of ions compared with the malathion mass spectrum (Fig. 2A and B). Moreover, the ion ratio calculated for the interfering compound in the samples (0.06) was conclusively much lower than that observed for malathion in matrix-matched calibration curves (0.77), with a relative difference (RD) of −92 %, calculated using the equation: $RD\% = [(ion\ ratio_{sample} - ion\ ratio_{reference})/\ ion\ ratio_{reference}]\ x\ 100$ (University of Tartu, 2024). Thus, an equivocal identification of the target pesticide in the samples was avoided. Then, the efforts were concentrated on a strategic sample preparation approach to eliminate this matrix-interfering compound and minimise the chances of false positives.

Octadecylsilane (C18) and primary-secondary amine (PSA), two well-known sorbents used in the QuEChERS procedure, were tested separately in the d-SPE clean-up at a concentration of 10.0 mg of sorbent per mL of extract. Distinctively, PSA provided a complete removal of the interfering compound (Fig. 3B) without compromising the extraction of malathion. In parallel, the use of C18 sorbent was inefficient in the elimination of the matrix co-eluting component, thus hampering the identification and quantification of malathion (Fig. 3A). The efficiency of PSA sorbent has been attributed to its polar and anionic exchanger characteristics, providing the retention of acidic matrix compounds such

(A) Interfering peak at 15.18 min in infant formula extract without clean-up and its respective mass spectrum



(B) Malathion at 15.15 min in infant formula extract and its respective mass spectrum

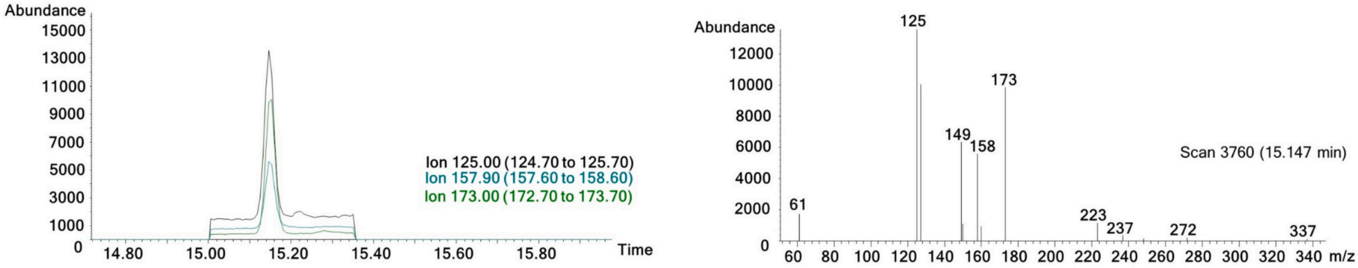
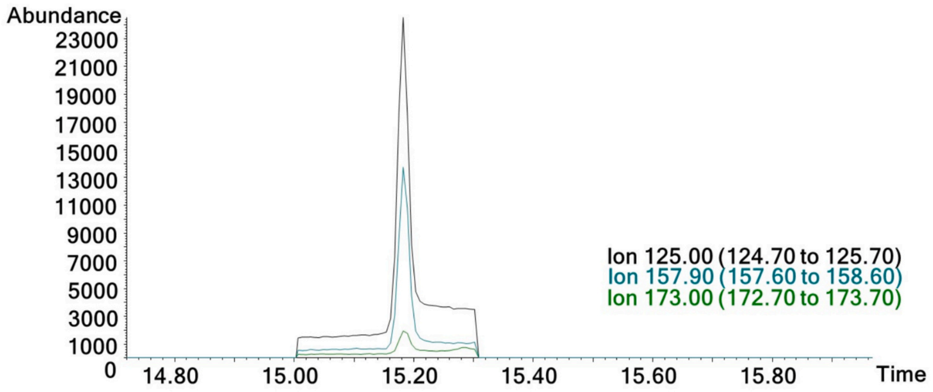


Fig. 2. GC–MS extracted ion chromatograms of (**A**) interfering peak occurring at 15.18 min in an infant formula extract without d-SPE clean-up and its respective mass spectrum, and (**B**) malathion (20 μg/kg) occurring at 15.15 min in an infant formula extract obtained after d-SPE clean-up with PSA sorbent and its respective mass spectrum.

(A) Interfering peak at 15.18 min in infant formula extract after d-SPE clean-up with C18 sorbent



(B) Removal of interfering peak after d-SPE clean-up with PSA sorbent

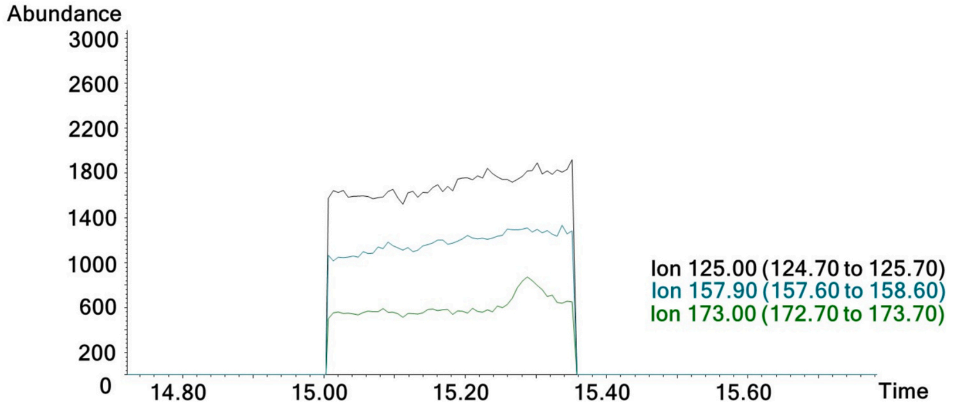


Fig. 3. GC–MS extracted ion chromatograms of (**A**) interfering peak occurring at 15.18 min in an infant formula extract obtained after d-SPE clean-up with C18 sorbent, and (**B**) infant formula extract obtained after d-SPE clean-up with PSA sorbent indicating the complete removal of the interfering peak.

as free fatty acids, organic acids, free amino acids, sugars, and certain pigments. Whereas C18 has been indicated mainly to remove lipophilic matrix co-extractives as lipids due to its hydrophobic nature (Rutkowska, Lozowicka, & Kaczynski, 2018).

The occurrence of matrix-interfering components at the retention time of malathion was also reported in other works. In multi-residues analysis of tropical fruits by LC-MS/MS, an interfering compound presented the same SRM transitions monitored for malathion in pitahaya (m/z 331 → 99) and tamarillo (m/z 331 → 127) extracts, thus imposing the selection of a third SRM transition (m/z 331 → 125) for an unequivocal identification and quantification of the pesticide (Botero-Coy, Marín, Ibáñez, Sancho, & Hernández, 2012). Additionally, the interfering isobaric compound was confirmed in tamarillo extracts by high-resolution mass spectrometry with an accurate mass of 331.1023 Da, however, the malathion presented an accurate mass of 331.0439 Da, indicating a mass error of 58.4 mDa (Botero-Coy et al., 2012). In another study involving the determination of organophosphates and pyrethroids in several foodstuffs by GC with electron-capture detection, recoveries above 120.0 % were observed for malathion in most of the samples, which was attributed to a possible co-elution at the retention time of the pesticide, even after a clean-up step using Supelclean™ ENVI-Carb™ II/PSA SPE cartridges (Hunter Jr., Riederer, & Ryan, 2010). Moreover, matrix-interfering components co-eluted with malathion in broccoli-, carrot- and cheese-based baby food extracts analysed by GC–MS in SIM mode, resulting in recoveries higher than 100.0 % for the pesticide (Chuang et al., 2001). In this case, distinct elution solvents were applied to Supelclean™ ENVI-Carb™ SPE cartridges to obtain the pesticide in a fraction (30.0 mL of acetonitrile) different from that containing matrix-interfering compounds (60.0 mL of 20.0 % dichloromethane in acetonitrile) (Chuang et al., 2001).

In comparison with previous sample preparation approaches reported to overcome the matrix interferences, the d-SPE procedure stands out for its simplicity, speed, and low cost, as well as it avoids disposable plastic SPE cartridges, thus saving time and organic solvents for SPE conditioning and eluting steps. Although the interfering compound shared the same ions monitored for malathion, the d-SPE step did not affect the recovery and precision of malathion (Table 2). Moreover, after the application of the d-SPE clean-up with PSA sorbent, the interfering compound was not observed in any sample, including soy- and milk-based infant formulas ($n = 60$).

3.2. *Performance characteristics of the analytical method*

In addition to the ability of the analytical method to accurately identify and quantify the target analytes without interference from other matrix co-extractives of similar behaviour (Fig. 2), suitable limits of detection (LOD) and quantification (LOQ), and accuracy were also observed for all pesticides (Table 2). The LOD and LOQ were sufficiently low to monitor and quantify the target analytes at the maximum residue limit (MRL) of 10.0 μg/kg set for pesticide residues in infant foods

Table 2

Performance characteristics of the analytical method obtained from analysis of a representative soy-based infant formula matrix [a].

Analyte	LOD µg/kg	LOQ µg/kg	Linearity		Recovery [b] (RSD repeatability [b]; RSD within-laboratory reproducibility [c]) %			
			Range, µg/kg (R^2)	Regression equation	1.0 µg/kg	5.0 µg/kg	10.0 µg/kg	50.0 µg/kg
Acetochlor	0.5	1.0	1.0–100.0 (0.9986)	y = 0.0144× + 0.0019	115.9 (5.7; 8.1)	113.6 (2.8; 4.2)	104.6 (1.9; 2.9)	102.3 (1.7; 2.2)
Alachlor	0.5	1.0	1.0–100.0 (0.9987)	y = 0.0209× + 0.0287	105.4 (7.0; 10.9)	111.1 (3.0; 8.3)	107.4 (3.8; 5.7)	106.9 (0.5; 0.8)
Aldrin	2.5	5.0	5.0–100.0 (0.9985)	y = 0.0127× − 0.0089	n.a.	116.0 (2.9; 4.6)	102.6 (2.2; 3.5)	100.2 (1.2; 1.9)
Atrazine	0.5	1.0	1.0–100.0 (0.9982)	y = 0.0216× − 0.0153	116.4 (5.1; 7.8)	111.5 (0.8; 4.3)	101.0 (3.2; 5.4)	100.9 (1.3; 2.0)
λ-Cyhalothrin	2.5	5.0	5.0–100.0 (0.998)	y = 0.0266× − 0.0147	n.a.	101.7 (4.6; 7.8)	96.7 (3.2; 5.5)	97.5 (2.9; 5.1)
p,p'-DDT	0.5	1.0	1.0–100.0 (0.997)	y = 0.0247× − 0.0363	100.9 (8.5; 13.2)	109.8 (3.4; 4.6)	98.3 (2.4; 6.4)	102.2 (0.5; 4.6)
Diazinon	2.5	5.0	5.0–100.0 (0.9985)	y = 0.0247× − 0.0094	n.a.	113.8 (5.7; 7.4)	109.0 (2.5; 3.6)	102.4 (0.9; 1.4)
Dieldrin	5.0	10.0	10.0–100.0 (0.9961)	y = 0.0156× + 0.0069	n.a.	n.a.	96.2 (1.4; 4.0)	99.8 (2.5; 3.8)
Dimethoate	5.0	10.0	10.0–100.0 (0.992)	y = 0.0057× − 0.0217	n.a.	n.a.	107.8 (2.0; 7.5)	108.0 (2.9; 4.0)
Diuron	0.5	1.0	1.0–100.0 (0.9985)	y = 0.0213× − 0.012	86.3 (17.1; 19.7)	97.7 (2.3; 4.1)	99.6 (2.4; 3.5)	98.7 (1.6; 2.5)
Endrin	2.5	5.0	5.0–100.0 (0.9993)	y = 0.0081× + 0.0099	n.a.	114.9 (2.3; 6.2)	102.4 (2.4; 6.2)	103.3 (1.4; 2.2)
Heptachlor	0.5	1.0	1.0–100.0 (0.9979)	y = 0.0164× − 0.0078	114.8 (2.4; 8.9)	111.1 (2.3; 5.4)	101.3 (3.9; 6.8)	100.7 (1.9; 3.2)
Hexachlorobenzene	2.5	5.0	5.0–100.0 (0.9992)	y = 0.0472× + 0.0542	n.a.	106.3 (3.4; 4.6)	97.5 (2.8; 4.6)	95.2 (0.8; 1.2)
Lindane	2.5	5.0	5.0–100.0 (0.9988)	y = 0.0154× + 0.008	n.a.	112.6 (7.8; 12.9)	101.5 (6.1; 15.9)	99.9 (1.9; 2.4)
Malathion	2.5	5.0	5.0–100.0 (0.9987)	y = 0.0249× + 0.0233	n.a.	103.8 (3.4; 4.7)	98.3 (2.9; 3.7)	99.3 (1.7; 2.5)
Procymidone	5.0	10.0	10.0–100.0 (0.9986)	y = 0.0191× − 0.0271	n.a.	n.a.	108.9 (5.9; 9.7)	106.2 (2.3; 3.9)
Simazine	2.5	5.0	5.0–100.0 (0.9981)	y = 0.0114× − 0.0105	n.a.	119.8 (1.4; 4.3)	101.9 (3.5; 5.4)	99.5 (1.8; 2.9)
Thiabendazole	5.0	10.0	10.0–100.0 (0.9925)	y = 0.011× − 0.0149	n.a.	n.a.	97.9 (2.4; 10.2)	98.6 (2.5; 3.7)
Triadimefon	2.5	5.0	5.0–100.0 (0.9992)	y = 0.019× + 0.0206	n.a.	90.6 (6.4; 15.8)	103.9 (2.6; 4.4)	101.2 (1.3; 2.2)
Trifluralin	0.5	1.0	1.0–100.0 (0.9991)	y = 0.0259× + 0.0092	115.3 (1.5; 4.2)	112.1 (3.7; 5.3)	98.6 (0.9; 2.8)	99.0 (1.0; 1.6)

LOD: limit of detection; LOQ: limit of quantification; R^2: coefficient of determination; RSD: relative standard deviation; n.a.: not applicable because the spiked level is lower than the LOQ fixed for the analyte.

[a] Commercial sample of powdered infant formula based on soy-protein isolates, maltodextrin, vegetable oils, soy lecithin, and other ingredients, containing 55 % carbohydrates, 26 % fats, and 14 % proteins, according to the label information. [b] $n = 5$. [c] $n = 10$.

(European Commission, 2016; European Commission, 2021). The minimum concentration of each analyte detected in matrix extracts, adopting a signal-to-noise ratio of 3, was fixed as the LOD, with values ranging between 0.5 and 5.0 µg/kg. Whereas, the lowest concentration of the analyte that can be detected and quantified with acceptable trueness (recoveries within the range 70.0–120.0 %) and precision (RSD values ≤20.0 %) was set as the LOQ, whose values varied from 1.0 to 10.0 µg/kg (Table 2). In addition, the criteria adopted for identification and quantification purposes described in section 2.3.3 were also considered in establishing the LOQs.

The LOQs were fixed as the first concentration level of the calibration curves, which included at least five concentration levels for each analyte. The matrix-matched calibration curves were obtained by adding proper aliquots of standard solutions to blank matrix extracts to achieve 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 75.0, and 100.0 µg/kg of standard equivalent in the sample, and 20.0 µg/kg of the internal standard TPP. Adequate linearity was achieved for all target pesticides with coefficients of determination (R^2) ≥ 0.992 in different linear ranges: 1.0–100.0 µg/kg (acetochlor, alachlor, atrazine, *p,p'*-DDT, diuron, heptachlor, and trifluralin), 5.0–100.0 µg/kg (aldrin, λ-cyhalothrin, diazinon, endrin, hexachlorobenzene, lindane, malathion, simazine, and triadmefon), and 10.0–100.0 µg/kg (dieldrin, dimethoate, procymidone, and thiabendazole) (Table 2). The matrix-matched calibration curves were prepared daily for each set of samples injected into GC–MS.

Since no reference material is available for the target analytes in infant formula, the recovery (%) and precision, expressed as relative standard deviation (RSD %), were determined using a representative matrix of infant formula spiked at levels 1.0, 5.0, 10.0, and 50.0 µg/kg (Table 2). All the values obtained were consistent with the criteria required for pesticide residue analysis in food and feed set in Document N° SANTE/11312/2021 v2 (EU Reference Laboratories for Residues of Pesticides, 2024), including recoveries between 86.3 % (for diuron at 1.0 µg/kg) and 119.8 % (for simazine at 5.0 µg/kg), and RSD values under repeatability conditions ($n = 5$) varying from 0.5 % (for alachlor and *p,p'*-DDT at 50.0 µg/kg) to 17.1 % (for diuron at 1.0 µg/kg). Under within-laboratory reproducibility conditions, the RSD values were between 0.8 % (for alachlor at 50.0 µg/kg) and 19.7 % (for diuron at 1.0 µg/kg) (Table 2).

3.3. *Residues of pesticides in infant formulas*

The target pesticides were investigated in 60 infant formula samples available in the Brazilian market. Many of these samples were manufactured in other countries such as Argentine (11), United States of America (9), Mexico (6), Netherlands (4), Singapore (3), Germany (2), Ireland (2), England (1), and Spain (1), according to the label information. Residues of dimethoate, an organophosphorus insecticide, were detected in five samples of infant formula, including one sample with a mean concentration of 54.8 ± 2.2 µg/kg (Fig. SM1 of Supplementary Material) and four samples with concentrations lower than the LOQ (10.0 µg/kg). Specifically, this sample with quantifiable content comprises a soy-based infant formula produced in Brazil, intended for babies up to 12 months of age, containing soy-protein isolates and soy lecithin emulsifier in the composition. Maximum residue levels (MRL) of pesticides in infant formulas have not been established in Brazil. However, the dimethoate content found in the sample largely exceeded the MRL of 10.0 µg/kg fixed by the European Commission for pesticides in infant formulas (European Commission, 2016; European Commission, 2021). Among the other pesticides investigated, residues equal to or higher than the LOD were not found in any infant formula sample.

Particularly in Brazil, dimethoate is approved for use as an acaricide and/or insecticide in several food commodities but does not include soybeans (Agência Nacional de Vigilância Sanitária, 2024). Besides, results obtained from the Brazilian Governmental Monitoring Program on Pesticide Residues Analysis (PARA 2017–2022), in which residues of up to 311 pesticides were investigated in 5068 samples, revealed that dimethoate was among the pesticides most frequently detected, occurring mainly in fruits and vegetables in which the pesticide is non-authorized for use, indicating the irregular use for specific food crops (Agência Nacional de Vigilância Sanitária, 2024). Furthermore, dimethoate was also identified as one of the main pesticides reported in the 2020 European Union Report on Pesticide Residues in Food, in some cases exceeding the MRL (EFSA et al., 2022). Recently, in a study on children's exposure to pesticides in Brazil involving various foodstuffs consumed in childhood, dimethoate was also reported in oranges (Oliveira, Bastos, Cardoso, & Nóbrega, 2022).

Concerning the occurrence of dimethoate, as well as other pesticides, in infant formulas commercialised worldwide, 60 % of the samples collected from the Indian market presented residues of dimethoate at levels between 0.7 and 9.6 μg/kg (Mishra et al., 2002). In general, organochlorides (aldrin, *o,p'*-DDT, *p,p'*-dicofol, endosulfan, heptachlor, hexachlorobenzene, and hexachlorocyclohexane isomers), organophosphates (chlorpyrifos, ethoprophos, malathion, and parathion-methyl), the pyrethroid cypermethrin, and the glyphosate were the compounds with the largest frequency of occurrence (≥ 70.0 %) in infant formulas (Table SM1 of Supplementary Material). Concentration levels above the MRL of 10.0 μg/kg were observed for the insecticide ethoprophos (14.0 μg/kg) in one sample from Romania (Dobrinas et al., 2016); for the herbicide glyphosate (< 20.0–1080.0 μg/kg) in soy-based infant formulas from Brazil (Rodrigues & Souza, 2018; Souza et al., 2021); and for the insecticides α-endosulfan (21.7 μg/kg), malathion (26.8 μg/kg), and β-HCH (335.9 μg/kg) in samples from India (Mishra et al., 2002) (Table SM1). Additionally, metabolites of endrin (endrin aldehyde), glyphosate (aminomethylphosphonic acid – AMPA), and heptachlor (heptachlor epoxide) were also identified in some infant formulas (Table SM1).

The presence of pesticide residues in infant formula could be associated with contaminated ingredients used in the formula's production, such as vegetable oils and milk- and soy-derived products. The physical-chemical properties of certain pesticides, including hydrophobic and lipophilic nature, environmental stability and persistence, and capacity for bioaccumulation and biomagnification in trophic chains, also contribute to the occurrence of pesticides in these ingredients, which present a considerable lipid content. Furtheremore, the thermal spray drying process used in infant formula manufacturing could also be promising for the concentration of chemical contaminants in the final product.

3.4. Estimated daily intake and risk characterisation

From birth to the first 16 weeks of life, infants are expected to be exclusively fed on breast milk or infant formula (Scientific Committee et al., 2017). Furthermore, this life stage is recognised by the highest relative intake on a body weight (bw) basis, having infant formula as the only source of nutrition for non-breastfed babies (Scientific Committee et al., 2017). Therefore, based on the occurrence of dimethoate in some infant formula samples, early-life dietary exposure to the organophosphorus compound was estimated considering an average (200.0 mL/kg bw per day) and high (260.0 mL/kg bw per day) consumption of infant formula by babies below 16 weeks of age (Scientific Committee et al., 2017). A comparison of the estimated daily intake (EDI) at different exposure scenarios is presented in Table 3.

Scenarios 1, 2, and 3 represent the lower-, middle-, and upper-bound dietary exposures, respectively, based on managing the left-censured data (LCD), namely, non-detected and non-quantified results (Table 3). In cases where there is a large proportion of LCD (> 80.0 %), it is recommended that these values be assumed as zero (lower-bound scenario) and equal to LOD (upper-bound scenario) (European Food Safety Authority, 2010). In addition, the substitution of LCD with one-half of the LOD value (middle-bound scenario) has also been highly recommended (WHO & FAO, 2009). The upper-bond scenario provides a more conservative assessment which tends to overestimate the exposure since pesticide residues are expected at the LOD level in the unquantified samples, while the lower-bound scenario results in underestimates because it is assumed that these samples are completely free of pesticides (EFSA Panel on Plant Protection Products and their Residues (PRP) et al., 2018).

Table 3
Estimated daily intake (EDI) of dimethoate for infants below 16 weeks of age fed exclusively on infant formula.

Infant formula consumption	Scenario	EDI (μg/kg bw per day)				
		Mean	Percentiles			
			50th	75th	90th	95th
Average (200.0 mL/kg bw per day)	1 (LCD = 0)	0.02	0.00	0.00	0.00	0.00
	2 (LCD = LOD/2)	0.09	0.07	0.07	0.07	0.07
	3 (LCD = LOD)	0.15	0.13	0.13	0.13	0.13
High (260.0 mL/kg bw per day)	1 (LCD = 0)	0.03	0.00	0.00	0.00	0.00
	2 (LCD = LOD/2)	0.12	0.09	0.09	0.09	0.09
	3 (LCD = LOD)	0.20	0.17	0.17	0.17	0.17

LCD: left-censored data; LOD: limit of detection (5.0 μg/kg); bw: body weight. Scenario 1: lower-bound dietary exposure; Scenario 2: middle-bound dietary exposure; Scenario 3: upper-bound dietary exposure.

Considering all the scenarios studied, the EDI values varied from 0 to 0.17 μg/kg bw per day of dimethoate for infants below 16 weeks of age (Table 3). Particularly, the EDIs obtained from the 50th percentile (median) pesticide content varied between 0 and 0.13 μg/kg bw for average consumers, including a middle-bound scenario of 0.07 μg/kg bw; and from 0 to 0.17 μg/kg bw for high consumers, with a middle-bound exposure of 0.09 μg/kg bw (Table 3). As can be observed, the attribution of different values to the LCD greatly influences the exposure estimates, which are strictly linked to the sensitivity of the applied analytical method. In this case, the LOD of the dimethoate corresponds to one-half of the default MRL of 10.0 μg/kg established for pesticide residues in infant formula. Furthermore, when a large percentage of samples are below the LOD or LOQ, the median (50th percentile) has been suggested as a more appropriate measure for analyte concentration purposes (WHO & FAO, 2009).

As far as we know, the present work comprises the first dietary exposure assessment of formula-fed babies to the dimethoate insecticide, thus limiting its comparison with previous studies. For example, in a French infant total diet study, mean dietary exposure of 6.6×10^{-6} and 0.2 μg/kg bw per day of dimethoate was estimated for infants of 1 to 4 months considering the lower- and upper-bound scenarios, respectively (Nougadère et al., 2020). Moreover, mean dietary exposures from 4.7×10^{-5} to 9.5×10^{-6} μg/kg bw (lower-bound) and between 0.172 and 0.097 μg/kg bw (upper-bound) of dimethoate per day were estimated for infants of 5 to 36 months (Nougadère et al., 2020). It is relevant to point out that the EFSA Panel on Plant Protection Products and their Residues, in its scientific opinion on pesticides in foods for infants and young children, estimated a potential pesticide residue intake of 2.6 μg/kg bw per day for infants below 16 weeks of age assuming the default MRL of 10.0 μg/kg for pesticide residues in infant formula and consumption of 260.0 mL/kg bw per day, suggesting that acceptable exposure of infants is expected for any pesticide with health-based guidance values (HBGVs) equal to or higher than 2.6 μg/kg bw per day (EFSA Panel on Plant Protection Products and their Residues (PRP) et al., 2018).

The risk characterisation was based on the comparison of the obtained EDI with HBGVs, such as acceptable daily intake (ADI) and acute reference dose (ARfD) (WHO & FAO, 2009). ADI from 0 to 1.0 μg/kg bw was set for dimethoate by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) considering a non-observed adverse effect level (NOAEL) of 0.1 mg/kg bw for inhibition of erythrocyte acetylcholinesterase activity in rats and a standard uncertainty factor (UF) of 100, accounting for inter- and intraspecies differences (FAO & WHO, 2020). In addition, an ARfD of 20.0 μg/kg bw was established for dimethoate based on a NOAEL of 2.0 mg/kg bw for acetylcholinesterase activity in an acute neurotoxicity study in rats and a standard UF of 100 (FAO & WHO, 2020). Therefore, compared with the HBGVs, the EDIs obtained from the 50th percentile (median) were at least 5.9 and 117.6 times lower than the ADI and ARfD, respectively (Table 4).

However, considering the particularities of infants below 16 weeks of age, an additional UF of 3 has been recommended as a conservative option to account for the immaturity of absorption, distribution, metabolism and excretion system of this infant subpopulation, which may be

Table 4
Risk characterisation associated with the exposure to dimethoate through infant formula consumption by infants below 16 weeks of age. [*]

Infant formula consumption	Scenario [**]	% of ADI [a]	% of ADI [b]	% of ARfD [c]	% of ARfD [d]
Average (200.0 mL/kg bw per day)	1 (LCD = 0)	0.0	0.0	0.0	0.0
	2 (LCD = LOD/2)	7.0	23.3	0.35	1.0
	3 (LCD = LOD)	13.0	43.3	0.65	1.9
High (260.0 mL/kg bw per day)	1 (LCD = 0)	0.0	0.0	0.0	0.0
	2 (LCD = LOD/2)	9.0	30.0	0.45	1.3
	3 (LCD = LOD)	17.0	56.7	0.85	2.5

ADI: acceptable daily intake; ARfD: acute reference dose; LCD: left-censored data; LOD: limit of detection (5.0 μg/kg); bw: body weight.

[*] Expressed as a percentage of the estimated daily intake (obtained from the 50th percentile) to the acceptable daily intake (ADI) or acute reference dose (ARfD).

[**] Scenario 1: lower-bound dietary exposure; Scenario 2: middle-bound dietary exposure; Scenario 3: upper-bound dietary exposure.

[a] ADI = 1.0 μg/kg bw, considering a standard uncertainty factor of 100 (FAO & WHO, 2020).

[b] ADI = 0.3 μg/kg bw, recalculated using an additional uncertainty factor of 3.

[c] ARfD = 20.0 μg/kg bw, considering a standard uncertainty factor of 100 (FAO & WHO, 2020).

[d] ARfD = 6.7 μg/kg bw, recalculated using an additional uncertainty factor of 3.

at risk at doses smaller than older infant groups (Scientific Committee et al., 2017; EFSA Panel on Plant Protection Products and their Residues (PRP) et al., 2018). Therefore, the ADI and ARfD established for dimethoate by the JMRP (FAO & WHO, 2020), discussed previously, were recalculated here considering an additional UF of 3, whose values obtained were 0.3 and 6.7 μg/kg bw per day, respectively. In this case, the EDIs (obtained from the 50th percentile) were at least 1.8 and 39.4 times lower than the recalculated ADI and ARfD, respectively, confirming that there is no health concern at the estimated exposure levels taking into account the high and average consumption of infant formula by infants below 16 weeks of age (Table 4).

4. Conclusions

This work contributes to advancing the knowledge of early-life exposure to pesticide residues through infant formula consumption in the first months of life. Multi-residues of pesticides, exclusively endocrine-disrupting compounds, were investigated in infant formulas from the Brazilian market, including a risk characterisation for formula-fed babies below 16 weeks of age. Another distinguished attribute of the work was the detection and efficient removal of a matrix-interfering compound using a fast and simple dispersive solid-phase extraction without compromising the determination of the target analytes, avoiding false positives in GC–MS analysis. Furthermore, the selective GC–MS method achieved adequate performance characteristics for pesticide monitoring at the maximum residue limit (MRL) of 10.0 μg/kg established for infant formula. The occurrence of dimethoate insecticide in five soy-based infant formulas, exceeding the MRL in one of them, reinforces the need for safest food control systems. Although the estimated daily intake values are within the acceptable daily intake (ADI) values and below the acute reference dose (ARfD) set for dimethoate, permanent monitoring of pesticide residues, particularly those identified as persistent organic pollutants (POPs) as well as pesticides with MRL set in milk or authorized for use in soybeans and other crops destined for the production of infant formula ingredients, should be strengthened.

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CRediT authorship contribution statement

Mateus Henrique Petrarca: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Silvia Amelia Verdiani Tfouni:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2025.116172.

Data availability

Data will be made available on request.

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