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Determination of pesticide residues, mycotoxins and benzo(a)pyrene in infant foods: quantification and reliability of results

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Abstract

A result reported as “below the limit of quantification” (LOQ) demonstrates conformity with a legal limit only to the extent that the LOQ lies below that limit. This question is examined for foods for infants and young children, to which the strictest limits apply. Official-control results are reported for 20 imported products sampled in Serbia in 2023 (nine formulae, eight milk-based desserts, three organic fruit cereals), in which an accredited laboratory determined 366 pesticide parameters by QuEChERS with GC-MS/MS and LC-MS/MS, seven mycotoxins and benzo(a)pyrene (7415 determinations). No analyte was quantified. The LOQ equalled the legal limit for 357 pesticide parameters (0.01 mg/kg for 336 and 0.003 mg/kg for the 21 parameters of the “not to be used” list) and was 13–50% of the maximum level for mycotoxins and benzo(a)pyrene. At a relative standard uncertainty of 25%, a residue exactly at such a limit would be reported as below the LOQ in half of the measurements, and only residues of at least 1.7 times the limit with 95% probability; an LOQ of about 0.6 of the limit removes this gap. A worst-case exposure assessment (residue at the LOQ, 32.5 g formula powder/kg body weight per day) shows that aldrin/dieldrin or heptachlor at 0.003 mg/kg would exhaust 98% of the provisional tolerable daily intake, whereas margins of exposure for aflatoxin M1 and benzo(a)pyrene would be about 12,000 and 11,000. The products were compliant, but for the most toxic pesticides an LOQ of 0.001 mg/kg should be targeted.

1 Introduction

Infants and young children are the most vulnerable group of consumers: they ingest considerably more food per kilogram of body weight than adults, their diet is often restricted to a single product and their detoxification systems are still immature. The European Union has therefore set a default maximum residue level (MRL) of 0.01 mg/kg for pesticides in infant and follow-on formulae [1] and in processed cereal-based foods and baby foods [2], far below the limits applying to the same commodities under Regulation (EC) No 396/2005 [3]. For five substances of high acute toxicity, even lower specific MRLs (0.004–0.008 mg/kg) have been laid down, and for eleven persistent or highly toxic pesticides such as aldrin, dieldrin, heptachlor and terbufos a "not to be used" list has been introduced, under which a product is considered compliant if the residue does not exceed 0.003 mg/kg. In 2018, EFSA showed that the default value of 0.01 mg/kg is protective for all substances whose health-based guidance value exceeds 0.0026 mg/kg body weight per day, and that lower limits are needed for more toxic substances [4]. At the same time, Regulation (EU) 2023/915 sets the lowest maximum levels for mycotoxins and polycyclic aromatic hydrocarbons precisely in foods for infants [5]. Serbia has transposed these requirements into the Rulebook on the safety of dietetic products, the Rulebook on maximum residue levels of plant protection products in food and the Rulebook on maximum levels of certain contaminants in food [6–8].

European monitoring data show that baby food is among the cleanest food categories with respect to pesticide residues: in 2021, 2022 and 2023, no quantifiable residues were found in 87.5%, 80.8% and 91.3%, respectively, of 1504–1783 samples, while the MRL was exceeded in 1.7–4.2% [9–11]. By

contrast, residues were found in 62.6% of fresh fruit samples on the Serbian market in 2016–2019, with exceedances in 4.7% [12]. Studies of infant formulae largely confirm the absence of residues above 0.01 mg/kg [13], whereas exceedances are not rare in fruit- and vegetable-based baby foods [14]. For mycotoxins the situation is more complex: aflatoxin M1 has been a recurring problem in raw milk in Serbia since 2013 [15–17] and was found in one of 21 infant formulae [18], while aflatoxins and deoxynivalenol are regularly detected in infant cereals in Europe [19, 20]. Benzo(a)pyrene exceeded the limit of 1 µg/kg in 10% of Italian infant formulae [21], whereas more recent studies using more sensitive methods found all samples below the limit of quantification [22].

Whether a measurement result demonstrates conformity with a limit depends not only on the result itself but also on its uncertainty and on the decision rule applied. ISO/IEC 17025 requires a laboratory that issues a statement of conformity to document the decision rule and to take account of the associated risk of false acceptance or false rejection [23], and guidance on the construction of such rules, including guard bands, is available [24–26]. In the official control of pesticide residues the rule is asymmetric: an MRL is regarded as exceeded only if the result reduced by the expanded uncertainty, by default 50%, still lies above the MRL [27], which protects the producer against false rejection. For results below the reporting limit no uncertainty is stated at all, and the only requirement is that the LOQ be at or below the MRL [27]. When the legal limit is set at the level of the routinely achievable LOQ, as is the case for the default MRL of 0.01 mg/kg in foods for infants, the statements “below the LOQ” and “compliant” coincide, and the risk of false acceptance carried by such a statement is seldom examined.

Results of official control in Serbia are, moreover, rarely published in the scientific literature, and the previous report on contaminants in infant foods on the Serbian market dates from 2013–2014 [18]. The aims of this work were (i) to report the results of a comprehensive analysis of pesticide residues, mycotoxins and benzo(a)pyrene in infant formulae, milk-based desserts and organic fruit cereals from the Serbian market, (ii) to compare the limits of quantification of the methods applied with the regulatory maximum levels and to quantify, for the measurement uncertainty customary in residue analysis, the probability that a residue above the limit is reported as "below the LOQ", and (iii) to establish, by a worst-case exposure assessment, how much health margin such a result actually provides.

2 Materials and methods

2.1 Samples

The study covered 20 products in original retail packaging taken within the official control of the safety of dietetic products on the Serbian market (Table 1): nine milk-based powdered formulae of one multinational manufacturer – five infant formulae for use from birth (including one food for special medical purposes against regurgitation, one hypoallergenic and one lactose-free formula), two follow-on formulae from the sixth month and two young-child formulae supplemented with *Limosilactobacillus reuteri* (packages of 400–800 g); eight milk-based desserts for infants of the same manufacturer (vanilla, semolina, banana, biscuit and cocoa flavours, for the age of 6 or 8 months); and three organic

instant fruit cereals with wholegrain flour of the private label of a retail chain (packages of 250 g). Formulae and desserts were sampled in July 2023 and the cereals in August 2023, each sample representing a separate production lot. At the request of the body that commissioned the control, the names of the manufacturers and products are not disclosed.

Table 1
Products examined

Product group	n	Intended use / age	Package	Sampling	Parameters determined
Infant formulae (including anti-regurgitation, hypoallergenic and lactose-free)	5	from birth	powder, 400–800 g	July 2023	366 pesticides, AFM1, BaP
Follow-on formulae	2	from 6 months	powder, 800 g	July 2023	366 pesticides, AFM1, BaP
Young-child formulae (with *L. reuteri*)	2	from 12 months	powder, 800 g	July 2023	366 pesticides, AFM1, BaP
Milk-based desserts for infants (vanilla, semolina, banana, biscuit, cocoa)	8	from 6 or 8 months	original retail pack	July 2023	366 pesticides, AFM1, AFB1, OTA, ZEA, DON, FB1 + FB2, BaP
Organic instant fruit cereals with wholegrain flour	3	from 6 months	250 g	August 2023	366 pesticides, AFB1, OTA, ZEA, DON, PAT, FB1 + FB2, BaP

AFM1/AFB1 – aflatoxin M1/B1; OTA – ochratoxin A; ZEA – zearalenone; DON – deoxynivalenol; PAT – patulin; FB1 + FB2 – fumonisins; BaP – benzo(a)pyrene.

2.2 Analytical methods

The analyses were carried out in July and August 2023 in the laboratory of a public health institute in Belgrade accredited to ISO/IEC 17025. Pesticide residues were determined by the multi-residue method according to SRPS EN 15662:2018 [28]: 10 g of homogenised sample was extracted with acetonitrile in the presence of triphenyl phosphate as internal standard, phase separation was achieved by addition of anhydrous $MgSO_4$, NaCl and citrate buffering salts, and the extract was cleaned up by dispersive solid-phase extraction (PSA, $MgSO_4$; C18 for fat-rich samples). Determination was performed by gas chromatography–tandem mass spectrometry (GC-MS/MS, triple quadrupole) and liquid chromatography–tandem mass spectrometry (LC-MS/MS, triple quadrupole). The scope of the method comprised 366 parameters – active substances, metabolites and isomers – including dithiocarbamates expressed as CS_2 (EN 12396-2) and glyphosate (LC-MS/MS). Validation and analytical quality control followed document SANTE/11312/2021 [27]. Aflatoxin M1 was determined in the formulae and desserts by immunoaffinity clean-up and HPLC with fluorescence detection (SRPS EN ISO 14501). In the desserts and cereals, aflatoxin B1 (HPLC-FLD with post-column derivatisation, SRPS EN 14123/EN 15851), ochratoxin A (HPLC-FLD), zearalenone (SRPS EN 15850), deoxynivalenol (SRPS EN 15791, HPLC-UV),

fumonisins B1 + B2 (LC-MS/MS) and, in the cereals, patulin (HPLC) were determined. Benzo(a)pyrene was determined by HPLC with fluorescence detection according to EPA Method 610 and AOAC 973.30. The limits of quantification (LOQ) of all parameters are given in Table 2; the redacted laboratory reports for all 20 samples, with the complete list of the 366 pesticide parameters, are provided as Online Resource 1.

Table 2
Analytical scope, limits of quantification (LOQ), regulatory maximum levels (ML) and results

Parameter (group)	No. of param.	Method	LOQ	ML	LOQ/ML	n samples	Result
Pesticides, mg/kg							
Default group	336	QuEChERS, GC-MS/MS; LC-MS/MS	0.01	0.01	1.0	20	< LOQ
"Not to be used" list[a]	21	GC-MS/MS; LC-MS/MS	0.003	0.003	1.0	20	< LOQ
Fipronil + fipronil-desulfinyl + fipronil-sulfone	3	GC-MS/MS; LC-MS/MS	0.003	0.004	0.75	20	< LOQ
Cadusafos	1	GC-MS/MS	0.003	0.006	0.50	20	< LOQ
Demeton-S-methyl (sum)	1	GC-MS/MS	0.003	0.006	0.50	20	< LOQ
Ethoprophos	1	GC-MS/MS	0.003	0.008	0.38	20	< LOQ
Carbofuran, 3-hydroxycarbofuran	2	LC-MS/MS	0.001	0.01[b]	0.10	20	< LOQ
Carbosulfan	1	LC-MS/MS	0.002	0.01	0.20	20	< LOQ
Mycotoxins, µg/kg							
Aflatoxin M1	1	IAC, HPLC-FLD	0.01	0.025	0.40	17	< LOQ
Aflatoxin B1	1	HPLC-FLD	0.05	0.10	0.50	11	< LOQ
Ochratoxin A	1	HPLC-FLD	0.25	0.50	0.50	11	< LOQ
Zearalenone	1	IAC, HPLC-FLD	5	20	0.25	11	< LOQ
Deoxynivalenol	1	IAC, HPLC-UV	25	200[c]	0.13	11	< LOQ
Fumonisins B1 + B2	1	LC-MS/MS	25	200	0.13	11	< LOQ

[a] Aldrin, dieldrin, endrin, disulfoton (+ sulfone, sulfoxide), fensulfothion (+ oxon, oxon-sulfone, sulfone), fentin, haloxyfop-2-ethoxyethyl, heptachlor (+ cis- and trans-epoxide), hexachlorobenzene, nitrofen, omethoate, terbufos (+ sulfone, sulfoxide) – the product is deemed compliant at a residue ≤ 0.003 mg/kg. [b] Reference value of the laboratory; EU legislation lists carbofuran among the substances not to be used. [c] Value in force at the time of sampling; 150 µg/kg since 1 July 2024. ML – maximum level according to the Serbian Rulebook on the safety of dietetic products, harmonised with Regulation (EU) 2016/127, Directive 2006/125/EC and Regulation (EU) 2023/915. IAC – immunoaffinity column. For the milk-based desserts the laboratory reports state no reference value for aflatoxin M1; the maximum level for infant formulae is shown.

Parameter (group)	No. of param.	Method	LOQ	ML	LOQ/ML	n samples	Result
Patulin	1	HPLC	4	10	0.40	3	< LOQ
PAH, µg/kg							
Benzo(a)pyrene	1	HPLC-FLD	0.2	1.0	0.20	20	< LOQ

[a] Aldrin, dieldrin, endrin, disulfoton (+ sulfone, sulfoxide), fensulfothion (+ oxon, oxon-sulfone, sulfone), fentin, haloxyfop-2-ethoxyethyl, heptachlor (+ cis- and trans-epoxide), hexachlorobenzene, nitrofen, omethoate, terbufos (+ sulfone, sulfoxide) – the product is deemed compliant at a residue ≤ 0.003 mg/kg. [b] Reference value of the laboratory; EU legislation lists carbofuran among the substances not to be used. [c] Value in force at the time of sampling; 150 µg/kg since 1 July 2024. ML – maximum level according to the Serbian Rulebook on the safety of dietetic products, harmonised with Regulation (EU) 2016/127, Directive 2006/125/EC and Regulation (EU) 2023/915. IAC – immunoaffinity column. For the milk-based desserts the laboratory reports state no reference value for aflatoxin M1; the maximum level for infant formulae is shown.

2.3 Compliance and exposure assessment

The results were compared with the maximum levels of the Rulebook on the safety of dietetic products [6], which are harmonised with Delegated Regulation (EU) 2016/127, Directive 2006/125/EC and Regulation (EU) 2023/915. The relation between the LOQ and the maximum level was expressed as the LOQ/ML ratio. Worst-case (upper-bound) exposure was estimated in line with the EFSA recommendations for the treatment of results below the LOQ [29], assuming that each analyte was present at the LOQ in the formula powder. Consumption was taken from the EFSA opinion on pesticides in foods for infants [4]: 260 g of reconstituted formula per kg body weight (bw) per day, corresponding to 32.5 g powder/kg bw (high consumption), and 200 mL, i.e. 25 g powder/kg bw (mean consumption). Exposure was compared with the acceptable or provisional tolerable daily intake (ADI, PTDI, TDI) from EU and JMPR evaluations [30–37]. For the genotoxic carcinogens aflatoxin M1 and benzo(a)pyrene, the margin of exposure (MOE) was calculated relative to the $BMDL_{10}$ of 0.4 µg/kg bw per day for aflatoxin B1, applying a potency factor of 0.1 for aflatoxin M1 [38], and of 0.07 mg/kg bw per day for benzo(a)pyrene [39], an MOE ≥ 10,000 indicating a low level of concern [38]. The risk of false acceptance attached to a result "below the LOQ" was evaluated for a reporting limit equal to the LOQ, assuming a normally distributed measurement result with a relative standard uncertainty of 25%, which corresponds to the default expanded uncertainty of 50% ($k = 2$) of SANTE/11312/2021 [27]. The probability that a residue with the true mass fraction c is reported as below the LOQ is then $P = \Phi[(\mathrm{LOQ} - c)/(0.25c)]$, where Φ is the standard normal distribution function. A language model (Claude, Anthropic) was used to assist with text structuring and language editing.

3 Results and discussion

3.1 Regulatory compliance

A total of 7415 determinations were performed in the 20 samples: 7320 for pesticides (366 × 20), 75 for mycotoxins and 20 for benzo(a)pyrene. No analyte was quantified in any sample; all results were below the LOQ (Table 2) and all products were, according to the prescribed criteria, safe. This outcome is consistent with the European monitoring, in which 81–91% of baby-food samples carry no quantifiable residues [9–11], the more so as 17 of the 20 samples were milk-based formulae and desserts. For milk-powder-based formulae the result is expected: in 80 formulae from the EU/EFTA market none of the toxicologically critical substances was detected [13]. The absence of aflatoxin M1 above 0.01 µg/kg in all nine formulae and eight desserts is also a favourable finding, given that aflatoxin M1 was found in 61–81% of raw milk samples in Serbia in 2013–2019, often above 0.05 µg/kg [15–17], and in one of 21 formulae on the Serbian market earlier [18]; the formulae examined here were, however, imported, so their composition does not reflect the quality of domestic milk. All three samples of organic fruit cereals were free of pesticide residues and of aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone, patulin and fumonisins above the LOQ, which compares favourably with European data on infant cereals, in which aflatoxins and deoxynivalenol are found in about 20% of samples [19, 20], bearing in mind that the number of cereal samples was small. Benzo(a)pyrene was not quantified in any sample (LOQ 0.2 µg/kg), in contrast to Italian formulae in which the limit of 1 µg/kg was exceeded in 10% of samples [21], and in line with recent findings obtained with more sensitive methods [22].

3.2 Limits of quantification in relation to the regulatory limits

The value of a result "below the LOQ" depends entirely on the relation between the LOQ and the regulatory limit (Table 2). For 336 of the 366 pesticide parameters (92%) the LOQ of 0.01 mg/kg was equal to the default MRL, and for all 21 parameters of the "not to be used" list (11 substances with their metabolites) the LOQ of 0.003 mg/kg was equal to the threshold at which the substance is deemed not to have been used; the LOQ thus equalled the limit for 357 parameters (97.5%). Only for nine parameters was the LOQ below the limit: fipronil and its two metabolites (0.003 versus 0.004 mg/kg), cadusafos and demeton-S-methyl (0.003 versus 0.006), ethoprophos (0.003 versus 0.008), carbofuran with 3-hydroxycarbofuran (0.001 versus 0.01) and carbosulfan (0.002 versus 0.01 mg/kg). Document SANTE/11312/2021 requires the LOQ to be at or below the MRL [27], which is fulfilled here, but an LOQ equal to the limit means that compliance is demonstrated without analytical margin: a residue of, say, 0.009 mg/kg would be indistinguishable from zero, and the measurement uncertainty of ± 50% customary for multi-residue methods cannot be applied around the MRL. It should be stressed, however, that the analyses were performed on the powder, whereas the MRLs for formulae refer to the product as ready for consumption: at a reconstitution of 12.5–13 g powder per 100 mL [4], a result of "< 0.01 mg/kg powder" corresponds to less than 0.0013 mg/kg in the reconstituted formula, so that the laboratory's approach for formulae is about eight times stricter than the regulation requires. For mycotoxins and benzo(a)pyrene the LOQ was 13–50% of the maximum level (Table 2), which is satisfactory; for deoxynivalenol it should be noted that the maximum level of 200 µg/kg in force at the time of sampling was lowered to 150 µg/kg by Regulation (EU) 2024/1022 from 1 July 2024 [40], which the LOQ of 25 µg/kg still covers.

The consequence of an LOQ equal to the limit can be quantified (Table 4). Under the assumptions of Sect. 2.3, a residue present exactly at the limit is reported as below the LOQ in 50% of the measurements, a residue 25% above the limit in 21% and one 50% above the limit in 9%; only a residue of at least 1.7 times the limit is reported with a probability of 95%. For a product that is in fact marginally non-compliant, the statement "below the LOQ" thus carries a risk of false acceptance far above the few per cent usually tolerated in conformity assessment [24, 25], and it does so in addition to the allowance of 50% that the enforcement rule already grants above the MRL. Conversely, for a residue at the limit to be reported with 95% probability the LOQ must not exceed 0.59 of the limit, i.e. about 0.006 mg/kg for the default MRL and 0.002 mg/kg for the "not to be used" list. In the present data set this condition is met for cadusafos, demeton-S-methyl, ethoprophos, carbofuran and carbosulfan and for all mycotoxins and benzo(a)pyrene (LOQ/ML ≤ 0.50), but not for fipronil (0.75) or the 357 parameters with LOQ/ML = 1.0. For the formulae the dilution on reconstitution provides the missing margin implicitly; for the other products this cannot be assumed. The calculation is a simplification: a signal between the limit of detection and the LOQ may be noticed by the analyst, but such information does not appear in the test report on which the statement of conformity rests.

Table 4
Probability that a residue with a true mass fraction *c* is reported as “below the LOQ” when the reporting limit equals the LOQ and the relative standard uncertainty of the measurement is 25% (normal distribution), and the LOQ required for a residue at the limit to be reported with 95% probability

c/LOQ	c at LOQ = 0.01 mg/kg	c at LOQ = 0.003 mg/kg	P (reported as < LOQ), %
1.00	0.0100	0.0030	50
1.25	0.0125	0.0038	21
1.50	0.0150	0.0045	9
1.70	0.0170	0.0051	5
2.00	0.0200	0.0060	2
3.00	0.0300	0.0090	0.4
LOQ required for *P* ≤ 5% at *c* equal to the limit: 0.59 × limit, i.e. 0.0059 mg/kg for the default MRL of 0.01 mg/kg and 0.0018 mg/kg for the threshold of 0.003 mg/kg.			

3.3 Worst-case exposure

The exposure assessment under the assumption that each analyte is present exactly at the LOQ is given in Table 3. At high consumption (32.5 g powder/kg bw per day), a residue of 0.01 mg/kg in the powder corresponds to an intake of 0.325 µg/kg bw per day, which is precisely the value EFSA identifies as the threshold below which the default MRL is no longer protective for substances with low health-based guidance values [4]. For substances with the default LOQ and an ADI in the order of mg/kg bw, such as glyphosate, acetamiprid or chlorantraniliprole, a residue at the LOQ would consume less than 2% of the ADI; for aldicarb, with an ADI of 3 µg/kg bw, about 11%. For the substances on the "not to be used" list the picture is different: a residue of 0.003 mg/kg of aldrin and dieldrin or of heptachlor at the LOQ would

correspond to 75–98% of the provisional tolerable daily intake of 0.1 µg/kg bw [37], of hexachlorobenzene to 44–57% of the tolerable daily intake [36], of endrin and fipronil to 38–49%, of disulfoton and demeton-S-methyl to 25–33%, and of cadusafos, ethoprophos, fentin and terbufos to 13–24% (Table 3). For carbofuran, for which the laboratory achieved an LOQ of 0.001 mg/kg, a residue at the LOQ corresponds to 17–22% of the ADI of 0.15 µg/kg bw [31]. For omethoate and chlorpyrifos the EU currently has no valid health-based guidance values because genotoxicity could not be excluded [41, 42], so any lower LOQ is desirable for them. These values do not represent actual exposure – none of these pesticides was detected and actual residues, if any, may be far lower than the LOQ – but show how much protection a result "below the LOQ" guarantees. For the persistent organochlorine insecticides, which were placed on the "not to be used" list precisely because of their bioaccumulation in milk fat, a limit of 0.003 mg/kg with an equal LOQ guarantees only that the PTDI is not exceeded; an LOQ of 0.001 mg/kg, as achieved here for carbofuran and as routinely reached by modern GC-MS/MS methods for organochlorine pesticides, would triple that margin. The scenario for desserts and cereals is less severe: a daily portion of 100 g of milk dessert or 25 g of dry cereal for a 5-kg infant gives a 1.6–6.5 times lower intake per kg body weight than formula, and these products do not constitute the sole diet.

Table 3
Worst-case exposure at a residue equal to the LOQ in formula powder (mean consumption 25 g, high consumption 32.5 g powder/kg bw per day) relative to toxicological reference values

Substance	LOQ, mg/kg	Reference value, µg/kg bw/day	Intake at LOQ, mean / high, µg/kg bw/day	% of reference value, mean	% of reference value, high	Source of reference value
Aldrin + dieldrin	0.003	0.1 (PTDI)	0.075 / 0.098	75	98	JMPR 1994 [37]
Heptachlor (sum)	0.003	0.1 (PTDI)	0.075 / 0.098	75	98	JMPR 1994 [37]
Hexachlorobenzene	0.003	0.17 (TDI)	0.075 / 0.098	44	57	[36]
Endrin	0.003	0.2 (PTDI)	0.075 / 0.098	38	49	JMPR 1994 [37]
Fipronil (sum)	0.003	0.2 (ADI)	0.075 / 0.098	38	49	[30]
Disulfoton (sum)	0.003	0.3 (ADI)	0.075 / 0.098	25	33	JMPR 1996 [37]
Demeton-S-methyl (sum)	0.003	0.3 (ADI)	0.075 / 0.098	25	33	JMPR 1989 [37]
Cadusafos	0.003	0.4 (ADI)	0.075 / 0.098	19	24	[32]
Ethoprophos	0.003	0.4 (ADI)	0.075 / 0.098	19	24	[33]
Fentin	0.003	0.5 (ADI)	0.075 / 0.098	15	20	JMPR 1991 [37]
Terbufos (sum)	0.003	0.6 (ADI)	0.075 / 0.098	13	16	JMPR 2003 [37]
Carbofuran (sum)	0.001	0.15 (ADI)	0.025 / 0.033	17	22	[31]
Aldicarb (sum)	0.01	3 (ADI)	0.25 / 0.33	8	11	JMPR 1992 [37]
Acetamiprid	0.01	25 (ADI)[a]	0.25 / 0.33	1.0	1.3	[35]

PTDI – provisional tolerable daily intake; TDI – tolerable daily intake; ADI – acceptable daily intake; MOE – margin of exposure ($BMDL_{10}$/intake); bw – body weight. [a] In 2024 EFSA proposed lowering the ADI to 5 µg/kg bw, at which an intake at the LOQ would account for 5–6.5%. [b] Applying a potency factor of 0.1 for aflatoxin M1 relative to aflatoxin B1.

Substance	LOQ, mg/kg	Reference value, µg/kg bw/day	Intake at LOQ, mean / high, µg/kg bw/day	% of reference value, mean	% of reference value, high	Source of reference value
Glyphosate	0.01	500 (ADI)	0.25 / 0.33	0.05	0.07	[34]
Omethoate; chlorpyrifos	0.003; 0.01	no valid value	–	–	–	[41, 42]
Aflatoxin M1, µg/kg	0.01	$BMDL_{10}$ 0.4 µg/kg bw (AFB1)[b]	0.25 / 0.33 ng/kg bw	MOE 16,000	MOE 12,300	[38]
Benzo(a)pyrene, µg/kg	0.2	$BMDL_{10}$ 70 µg/kg bw	5.0 / 6.5 ng/kg bw	MOE 14,000	MOE 10,800	[39]

PTDI – provisional tolerable daily intake; TDI – tolerable daily intake; ADI – acceptable daily intake; MOE – margin of exposure ($BMDL_{10}$/intake); bw – body weight. [a] In 2024 EFSA proposed lowering the ADI to 5 µg/kg bw, at which an intake at the LOQ would account for 5–6.5%. [b] Applying a potency factor of 0.1 for aflatoxin M1 relative to aflatoxin B1.

For the genotoxic carcinogens the result is more favourable. Aflatoxin M1 at the LOQ of 0.01 µg/kg would give an intake of 0.33 ng/kg bw per day, i.e. 0.033 ng/kg bw of aflatoxin B1 equivalents, which relative to the $BMDL_{10}$ of 0.4 µg/kg bw yields an MOE of 12,000–16,000 [38]; for comparison, the actual exposure of children aged 1–3 years in Serbia was estimated at 0.16–0.19 ng/kg bw per day [17]. Benzo(a)pyrene at the LOQ of 0.2 µg/kg would give 5.0–6.5 ng/kg bw per day and an MOE of 11,000–14,000 relative to the $BMDL_{10}$ of 70 µg/kg bw [39]. Both margins are above 10,000, but only just, meaning that the LOQ for these contaminants is exactly as low as is needed for a result "below the LOQ" to rule out concern – any more sensitive method [22] increases that margin.

3.4 Limitations

The sample set is small (20 products, two manufacturers, one sampling campaign), originates from routine official control and each parameter was determined once; the results therefore describe the market in summer 2023 rather than continuous monitoring. Since all results were below the LOQ, actual exposure could not be estimated, only its upper bound. Its strength lies in the breadth of the analytical scope and in placing official-control results, which are as a rule not published, in the context of regulatory limits and toxicological reference values.

4 Conclusions

In 20 products for infants and young children sampled within official control in Serbia – formulae, milk-based desserts and organic fruit cereals – none of the 366 pesticide parameters, seven mycotoxins or benzo(a)pyrene examined was quantified, so that all products complied with national and EU regulations. The limits of quantification were equal to the regulatory limits for 357 of the 366 pesticide parameters, including all substances of the "not to be used" list, so that compliance was demonstrated without analytical margin: at a relative standard uncertainty of 25%, a residue at such a limit would be reported as below the LOQ in every second measurement, and an LOQ of about 0.6 of the limit is needed for it to be reported with 95% probability. For mycotoxins and benzo(a)pyrene the LOQ amounted to 13–50% of the maximum level. The worst-case exposure assessment showed that a residue at the LOQ would account for less than 2% of the ADI for most pesticides, but for 75–98% of the PTDI for aldrin/dieldrin and heptachlor and 38–57% of the reference value for hexachlorobenzene, endrin and fipronil; the margins of exposure for aflatoxin M1 and benzo(a)pyrene were 11,000–16,000. The results confirm the high level of safety of the products examined and at the same time indicate that, for persistent organochlorine pesticides and for omethoate and chlorpyrifos without valid reference values, official-control laboratories should aim at a limit of quantification of 0.001 mg/kg, so that a result "below the LOQ" guarantees a real health margin rather than merely formal compliance.

Declarations

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Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval Not applicable; the study involved no human participants or animals.

Data availability All results generated or analysed during this study are included in this article and its supplementary information (Online Resource 1, redacted laboratory test reports of all 20 samples).

Author contributions L. M. Babincev: conceptualisation, methodology, supervision, writing – review and editing. J. M. Kulizić and A. Ž. Sikimić: data processing, exposure assessment, literature verification, writing – original draft. All authors read and approved the final manuscript.

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