EURL-SRM - Analytical Observations Report

concerning the following…

- **Compound(s)**: 3-Hydroxycarbofuran, Abamectin, Amitrole, Cotinine, Diclofop, Diquat, Emamectin, Fentin, Gamma-Cyhalothrin, Haloxyfop, Nicotine, **,** PTU, Topramezone,
- **Additional compounds:** Chlorate, Cyanuric acid, Ethoxyquin dimer, Melamine, Paraquat, Perchlorate, Phosphonic acid Thiocyanate, Triazole acetic acid, Triazole lactic acid, Triazole alanine and Trifluoroacetic acid
- **Commodities**: Infant formulae of various types and milk
- **Extraction Method(s)**: Citrate buffered QuEChERS (EN 15662), QuPPe
- **Instrumental analysis**: LC-MS/MS, IC-MS/MS

Analysis of Toxicologically Critical SRM Compounds in Infant Formulae and Milk- Part 1: Analytical Aspects

Version 2 (last update: 10.03.2021)

1. Background information:

Following a request by DG-SANTE, EFSA prepared a scientific opinion in May 2018[1], in which it was concluded that for infant food for children up to 16 weeks of age, the default MRL of 0.01 mg/kg currently applying for infant formulae (Reg. 141/2006/EC)[2], may not be sufficiently protective for pesticides with ADI values below a health-based guidance value (HBGV) of 0.0026 mg/kg bw per day.

Based on this evaluation, DG-SANTE identified a number of compounds, with ADI values <0.0026 mg/kg bw per day, and calculated the highest possible MRLs for reconstituted infant formulae, that would be still considered safe for children up to 16 weeks of age. Thereafter, the EURLs were asked to comment on the technical feasibility of monitoring these compounds at or below the MRLs considered safe for infant food. The EURLs were further asked to develop and validate methods for a number of compounds and to conduct a pilot monitoring study for infant formulae of various types and origins. In addition, milk should be analysed. The LOQs of these methods should be equal or lower than the levels considered safe.

The selected, toxicologically critical compounds were divided into the following groups in collaboration with the EURL-AO: a) MRM (amenable to multiresidue methods), b) MRM/SRM (requiring modified MRM methods or where markers can be first screened by an MRM-method triggering re-analysis by a SRM in case of positive findings); c) SRM (compounds not amenable to

[1] Scientific opinion on pesticides in foods for infants and young children; https://www.efsa.europa.eu/de/efsajournal/pub/5286

[2] Regulation 2006/141/EC referring to infant formulae and follow-on formulae repealed by Regulation 609/2013/EU

multiresidue methods). Compounds classified as SRM are listed in Table 1 and those classified as MRM/SRM in Table 2.

At a meeting with DG-SANTE and MSs in Brussels it was agreed to exclude certain of the initially selected compounds from the pilot monitoring survey for various reasons, including analytical difficulties and the likelihood of encountering a compound in infant formulae. These reasons for excluding those compounds are compiled in Table 1 and Table 2. The compounds that were agreed to skip in this pilot monitoring are marked in grey.

It was further agreed to start with the analysis of the collected milk samples and then continue with the infant formulae. Amongst the infant formulae it was decided to start with “normal” products and continue with products for special groups.

The focus of the project was to examine the residue situation of toxicologically critical compounds in infant food formula. Still, it was decided to include to the scope, certain additional compounds known to be ubiquitous in the environment and therefore frequently encountered in various food commodities, such as nicotine, trifluoroacetic acid, chlorate and phosphonic acid (see

Table **3**). Some of these compounds are of toxicological concern (e.g. chlorate) and others are suspected of potentially exceeding the default MRL of 0.01 mg/kg applying to infant food formulae and dietary food.

Table 1: Compilation of SRM-compounds with critical toxicology. Compounds agreed to be excluded from the pilot monitoring are marked in grey

Compound	Residue Definition	ADI (mg/kg bw per day)	Max. MRL/LOQ for reconst. products (mg/kg)	Max. LOQ for infant formula powder [3] mg/kg)	Appr.	Notes and reasons for exclusion from pilot monitoring
Abamectin	Avermectin B1a	0.0025/0.00125[4]	**0.0096 / 0.0048**[4]	0.072 /0.036[4]	✓	**Toxicological endpoints were modified in Aug 2020**
Emamectin	Emamectin benzoate B1a, expressed as emamectin	0.005	**0.0019**	0.0143	✓	
Fentin	Fentin (fentin including its salts, expressed as triphenyltin cation)	0.0004	**0.0015**	0.0113	×	
Amitrole	Amitrole	0.001	**0.0038**	0.0285	×	NL listed in pesticide database
Nicotine	Nicotine	0.0008	**0.0031**	0.0233	×	Natural occurrence and potential for cross contaminations through contact with hands, soil and air. The inclusion of the animal metabolite cotinine was considered useful
PTU	Regulated in current infant food regulation (MRL of 0.003 mg/kg)	0.0003	**0.0012**	0.015	×	**M**ax. period of grace for propineb June 2019. PTU is a byproduct in propineb formulations, it is also a degradant of propineb in the field and during food processing. It is not regulated in 396/2005/EC but it has its proper MRLs established in the baby food regulation (0.003 mg/kg)
Diquat	Diquat	0.002	**0.0076**	0.057	×	Max period of grace: 4 February 2020
Topramezone	Topramezone (BAS 670H)	0.001	**0.0038**	0.0285	P	**Pending** Approval (provisional authorizations in EL, NL)
Chloropicrin	Chloropicrin	0.001	**0,0038**	0.0285	×	It was agreed to skip this fumigant from the pilot monitoring, as there were some analytical difficulties due to degradation of the compound during analysis.
1-Methyl-cyclopropene (1-MCP)	1-Methyl-cyclopropene	Formerly 0.0009, now 0.02	0,0035 (old) 0.076 (new)	0.57 (new)	✓	Given the shift in the toxicological threshold and the very low likelihood of relevant residues in milk, It was agreed to exclude this compound from the pilot monitoring
Metam	Dazomet (Methylisothiocyanate resulting from the use of dazomet and metam)	Metam: 0.001 MITC: 0.004	**0,0038 (Metam) 0.015 (MITC)**	0.0285 (metam) 0.11 (MITC)	✓	Due to the fast degradation of Metam (and Dazomet) in soil, MITC is the marker compound for the residue definition. The ADI of MITC (methyl isothiocyanate) = 0,004, exceeds the threshold 0f 0.0026 mg/kg bw. It was thus agreed to remove this compound from the scope of the pilot monitoring.
Methyl bromide	Bromide ion	0.001 for (intact MeBr)	**0,0038**	0.0285	×	The toxicity threshold refers to MeBr, which is unlikely to be found in intact form in products of animal origin, as it degrades to bromide ion. Levels of intact MeBr decrease rapidly during aeration. Analyzing residues of bromide ion seems superfluous as it does not pose any toxicological concerns and as natural levels of bromide ion in milk are quite high anyway (intake through feed).
Cyanamide	Cyanamide	0.002	**0,0076**	0.057	×	**Not approved** (as pesticide)**.** According to an EFSA report, cyanamide degrades rapidly and incorporates into natural plant products. In animals it is intensively metabolised with the major metabolite being N-acetylcyanamide[5]. Cyanamide is **widely used as a fertilizer**, with the most common cyanamide-containing product (Perlka) containing 45% of cyanamide and being typically employed at 200 and 450 kg/ha. Considerable exposure of farm workers, neighbouring residents and animals is thus expected. Cyanamide use is also used for the control of certain pests such as snails. It is also reported to control salmonella in liquid sewage[6]. Cyanamide has been, furthermore, in use for many years as a deterrent to alcohol consumption at doses (>20 mg/person/ day). These medicinal doses clearly exceed the ADI. A reassessment of the risks associated with cyanamide exposure seems indicated. Analytically, cyanamide poses immense difficulties and the EURL has no routinely applicable method at the time being.

[3] Based on a conversion factor of 7.5

[4] New ADI value introduced after finalizing the analyses of the present study (published in Peer review of the pesticide risk assessment of the active substance abamectin; EFSA Journal 2020;18(8):6227; Link: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6227

[5] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.1873

[6] (https://ec.europa.eu/health/sites/health/files/scientific_committees/environmental_risks/docs/scher_o_169.pdf)

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Table 2: Compilation of MRM/SRM-Compounds with critical toxicology. Compounds decided to exclude from the pilot monitoring are marked in grey

Compound	Residue Definition	ADI (mg/kg bw per day)	Max. MRL/LOQ for reconst. products (mg/kg)	Max. LOQ for infant formula powder (mg/kg)	EU Approval	Notes and reasons for exclusion from pilot monitoring
Diclofop	Diclofop (sum diclofop-methyl and diclofop acid expressed as diclofop-methyl)	0.001	**0.0038**	0.0285	✓	**Approved**: It was decided that for the pilot monitoring it would be acceptable to focus on free diclofop and to reanalyse by a method including the methyl ester and possibly also conjugates, in case of a positive finding of diclofop free acid. The methyl ester of diclofop is MRM amenable, but according to literature, it is rapidly hydrolyzed in plants, thus no residues in food of animal origin are actually expected.
Dicofol	Dicofol (sum of p, p´ and o,p´ isomers)	0.002	**0.0076**	0.057	✗	**Not approved.** Given the instability of dicofol in GC-applications, it was agreed to analyse for dicofol (sum) after a positive screening result by a multiresidue method focusing on the degradant p,p´dichlorobenzophenone and dicofol**.** Within the pilot monitoring, this screening was agreed to be done by the EURL-AO
Gamma-Cyhalothrin	Lambda-cyhalothrin (includes gamma-cyhalothrin) (sum of R,S and S,R isomers) (F)	Gamma 0.0012 Lamda 0.0025	**gamma 0.0046, lambda 0,0095**	gamma 0.035 Lambda 0.071	✓	**Approved** (both gamma and lambda) It was decided that for the pilot monitoring it would be acceptable to focus on the analysis of cyhalothrin by a multiresidue method and, in case of a positive result, to reanalyse by a method allowing enantiomeric separation of the two constituent isomers of lambda cyhalothrin and the quantification of the gamma-isomer which is the most toxic. Within the pilot monitoring, the analysis of lambda cyhalothrin was agreed to be done by the EURL-AO
3-Hydroxycarbofuran	3-OH-carbofuran (free and conjugated) expressed as carbofuran	3-OH-CF 0.00015 Related comp. CF: 0.00015 BF: 0.0035 FT: 0.0035 CS: 0.005	**0.0006**	0.0045	✗	**Not approved** (None of the relevant products carbofuran, benfuracarb, furathiocarb, carbosulfan) In the pilot monitoring iot was decided that it would be acceptable to **focus on residues of free 3-OH carbofuran as a marker** and to perform re-analyses by a method involving de-conjugation in case of a positive screening result. Within the pilot monitoring, the analysis of carbofuran was agreed to be done by the EURL-AO
Haloxyfop	Sum of haloxyfop, its salts and conjugates expressed as haloxyfop (sum of the R- and S- isomers at any ratio)	0.00065	**0.0025**	0.01875	✓	**Approved**. It was decided that for the pilot monitoring it would be acceptable to focus on free haloxyfop and to re-analyse by a method entailing alkaline hydrolysis only in case of a positive finding of haloxyfop free acid.
Sulcotrione	CMBA (2-chloro-4-(methylsulfonyl)benzoic acid)	0.0004 (parent)	**0.0015 (parent)**	0,0114 (parent)	✓	**Approved**: The RD for food of AO only contains CMBA. According to EFSA, CMBA is the major constituent of the residue in maize forage and grains. Furthermore EFSA notes that CMBA exhibits a much lower toxicity than its parent sulcotrione (ca 100 times lower toxicological burden). Concerning residues in Milk: EFSA notes that residues of CMBA are very low (<0.005 mg/kg at an exposure rate of the animals 3 times higher than the critical dietary burden for ruminants). Based on the above facts it was decided to exclude sulcotrione/CMBA from the present study

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Table 3: Compounds that were additionally analysed during the project to supplement the scope regarding compounds that are likely to be encountered in infant foods or their ingredients

Compound	ADI (mg/kg bw per day)	Notes
Ethoxyquin-Dimer (EQDM)	0.005	Ethoxyquin is used as antioxidant agent in fish feed and in dried cereals. Degrades to a multitude of metabolites of which Ethoxyquin-Dimer (EQDM) is the most prominent in salmon. EQDM is also more stable than EQ. In infant food formulae fish oil is a frequent ingredient as it is rich in polyunsaturated fatty acids
Trifluoro acetic acid (TFA)	0.05	TFA is a pesticides metabolite of fluorine containing active substances. Moreover, it is generated during decomposition of coolants. It was detected in drinking and surface water and can be classified as environmental contaminant.
Chlorate	0.01	Former herbicide and biocide. Currently not approved as active substance. A by-product of disinfection of drinking water and therefore of wash, process and irrigation water which is coming in contact to food and food contact materials. It is often found as residue in milk. Temporarily inhibits the intake of iodine in the thyroid gland.
Perchlorate	0.0003 (TDI)	Persistent and ubiquitous environmental contaminant. Mainly originating from fertilizers, may be also formed as a byproduct of disinfection of drinking water. Temporarily inhibits the intake of iodine in the thyroid gland. Regulated by Reg. 1881/2006/EC as a contaminant
Phosphonic acid	2.25	Fungicide. Additional input from fertilizers. Plants presumably accumulate phosphonates; also after transition periods of several years with no application, residues are detected in fruits.
Triazole derivative metabolites: • **1,2,4-Triazole-acetic acid (TAA)** • **1,2,4-Triazole-lactic acid (TLA)** • **1,2,4-Triazol-1-yl-alanine (TA)**	TAA: 1* TLA: 0.3* TA: 0.3*	Triazole derivative metabolites result from the use of pesticides belonging to the group of triazoles, which contain a triazole moiety in their structure. 1,2,4-Triazole is also used as a nitrification inhibitor in fertilizers and may convert to TAA, TLA and TA within the plants.
Thiocyanate	?	Not approved active substance and naturally occurring in foodstuff especially in brassicaceae. Brassicaceae especially rape is potential feedingstuff for cows. Rape seed oil is a common ingredient in infant food formulae. Temporarily inhibits the intake of iodine in the thyroid gland.
Paraquat	0.004	Not approved active substance and herbicide.
Melamine	0.2	Metabolite of the insecticide Cyromazine (not approved). In relation to a food fraud scandal in 2008 it was revealed that melamine was used to adulterate infant formulae simulating high milk protein contents by the presence of nitrogen. Formed through trimerization of cyanamide fertilizers. Also Metabolite of cyromazine (pesticide and vet. drug). May also originate from cyanamide-based fertilizers (trimerization of cyanamide) as well as urea-based fertilizers (through trimerisation of urea and elimination of ammonia and carbon dioxide, Non-cyclic dimer (biuret) and trimer (triuret) are also formed). Melamine hydrolyzes to cyanuric acid via ammeline and ammelide. Melamine is widely used in the synthesis of melamine-formaldehyde resins used in synthetic surfaces of furniture and textiles, kitchenware, moulding, packaging materials. Also used as a fire-retardant. Regulated by Reg. 1881/2006/EC as a contaminant
Cyanuric acid	1.5 (TDI by WHO 2008)	Non-regulated metabolite and hydrolysis product of various pesticides. Compound originating from multiple sources, e.g.: **Triazine pesticides** (incl. the herbicides terbuthylazine, atrazine, cyanazine, the fungicide; anilazine and the insecticide cyromazine). From the above only terbuthylazine and cyromazine are currently in use within the EU, with the latter having lost approval. **Cyanamide-based fertilizers.** Cyanamide contained in fertilizers may convert to melamine through trimerization, which can further hydrolyze to cyanuric acid. **Urea-based fertilizers or feed:** Especially at high temperatures, urea loses ammonia converting to to isocyanic acid (HNCO), which trimerizes to cyanuric acid. **Mono-, Di- and Trichloroisocyanurates:** Used as disinfectants, algaecides and bactericides. They are used in sanitation liquids and bleaching agents as well as in swimming pools (pool-tabs) to retard the loss of chlorine in chlorinated water. In water, they gradually convert to cyanuric acid. **Natural formation** of cyanuric acid has also been reported (e.g. in humus).

*Peer review EFSA

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Some facts on infant formulae

Infant formula, also known as baby formula, is mostly based on skimmed cows' milk or whey, which is mixed with vegetable fats, oils, emulsifiers, vitamins, minerals and stabilizing agents. The mixture is pasteurized and then dried into a powder. Infant food for babies below 16 weeks of age can be classified into the following groups:

a) 'Normal' infant formula
b) Lactose-free infant formula (containing whey, in which lactose was hydrolysed to glucose and galactose)
c) Hypoallergenic infant formula (containing extensively hydrolysed milk proteins)
d) Anti-reflux infant formula (containing thickening agents)
e) "Comfort formula" for Infants with digestive problems such as colic and constipation (contains partly hydrolysed proteins)
f) Plant-based infant formula (based on e.g. soy or rice)

Any of these formulae can be certified as organic. Infant formula products are usually sold as powders, which have to be made up (reconstituted) with water to a liquid product. Some of the products are also offered on the market in reconstituted form, i.e. ready-to-feed formulae.

Infant formulae contain large numbers of ingredients with various chemical characteristics generating complex matrices. Individual compositions of infant food formulae largely dependent on the respective group, e.g. hypoallergenic infant formula contains extensively hydrolysed milk proteins for infants that suffer from milk protein allergies. Furthermore, compositions can also differ between products from the same group. Table 4 gives an insight in compositions of infant food formula products from different groups. Ingredients that are regularly added regardless of group and feature are for example vegetable oils thereof mainly palm, rape and sunflower seed oil and in addition fish oil for the alimentation with polyunsaturated fatty acids.

Most of the milk-based products are based on cow's milk derivatives but there are also formulae on the market that are based on milk of other animals, such as goats.

As cow's milk is a major ingredient and initial product of mostly all kinds of infant formula, it was decided to analyse milk samples in addition to the samples of infant food formula. The developed methods were therefore validated and used in cow's milk as well.

The consumption figures of infant formulae refer to reconstituted (ready-to-feed) products, so the "safe MRLs" and the maximum LOQs that would need to be reached for the various compounds were calculated on reconstituted products. For calculating the maximum LOQs for non-reconstituted powders the preparation recipes were taken into account. The recipes of the various products were largely similar, with conversion factors from powder to reconstituted product ranging between 7.52 and 7.98 (7.87 on average), see Table 5. Finally, it was decided to multiply the maximum MRL in the ready-to-use infant formula (IFRTU) by the factor 7.5, resulting into the most conservative MRLs in the infant formula powder (IFP).

*Table 4: Exemplary compositions of infant formulae (only major ingredients are shown); the columns refer to individual samples, the numbers refer to the **rank** in the ingredients lists*

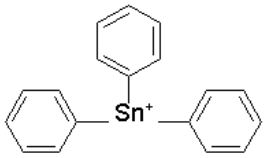
Ingredient	Group a)				Group b)		Group c)		Group d)		Group e)		Group f)	
				Goat										
Skimmed milk	1	1	1						1					
Full fat milk powder (in this case goat)				1										
Whey powder	2	2							2		1	4		
Hydrol. whey powder							3	3						
Milk Casseins					3									
Sol. Milk proteins						3								
Plant protein (soy or rice)													3	3
Vegetable Oil (Palm, rape, sunflower)	3	3	3	4	2	2	2	2	3	4	2	1	2	2
Milk fat														
Mid Chain TGs					4									
Fish oil	✓	✓	✓	-	✓	✓	-	-	✓	-	✓	✓	-	-
Lactose	5	4	5	3			1	1	5	1	6	3		
Other Oligosaccharides (mainly fructose-based)		5					4	4			5	6		
Maltodextrin	4	6	2	2		4			4			2	1	
Glucose sirup					1	1					3			1
Starch (Potatoes, Maize)			4							2	4	5	4	
Thickener (Carob flour)									6					

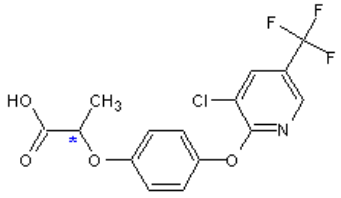
Table 5: Exemplary calculations of conversion factors of products belonging to categories a) to e) based on the preparation instructions on the package. The most conservative factor is highlighted in yellow

Category	Brands	Powder (g)	Water (ml)	Reconstituded Product		MRL Factor powder vs reconst.
				Weight (g)	Moisture (%)	
a) Normal	DM	13.0	90	103	87.4	7.92
	Holle	13.2	90	103.2	87.2	7.82
	Nestle	12.9	90	102.9	87.5	7.98
	Novalac	12.9	90	102.9	87.5	7.98
	Nestle	13.1	90	103.1	87.3	7.87
	Average	**13.02**	**90**	**103.0**	**87.4**	**7.91**
b) Lactose-free	Aptamil	12.9	90	102.9	87.5	7.98
	Guigoz	13.25	90	103.2	87.2	7.82
	NAN	12.9	90	102.9	87.5	7.98
	Average	**13.0**	**90**	**103.0**	**87.4**	**7.92**
c) Hypo-allergenic	Humana	13.2	90	103.2	87.2	7.82
	Guigoz	13.2	90	103.2	87.2	7.82
	Aptamil	13.8	90	103.8	86.7	**7.52**
	NAN	13.1	90	103.1	87.3	7.87
	Beba	13.1	90	103.1	87.3	7.87
	Average	**13.28**	**90**	**103.3**	**87.1**	**7.78**
d) Anti-Reflux	SMA	12.9	90	102.9	87.5	7.98
	Aptamil	13.2	90	103.2	87.2	7.82
	Hipp	12.9	90	102.9	87.5	7.98
	Average	**13.0**	**90**	**103.0**	**87.4**	**7.92**
e) Anti-Colic	Hipp	13.2	90	103.2	87.2	7.82
Total	**Average**	**13.1**	**90**	**103.1**	**87.3**	**7.87**

2. Compound details:

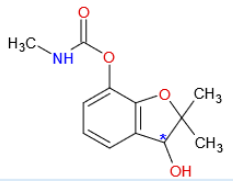
Table 6: General information on substances amenable to the QuEChERS extraction method.

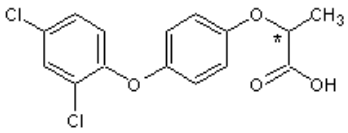
Name: Fentin (CAS: 668-34-8) **IUPAC: Triphenyltin**		
Parameter	**Value**	
Molecular Mass	350.0 g/mol	
Formula	$C_{18}H_{15}Sn$	
Exact mass	351.01957 g/mol	
pKa	-	
LogKow	3,43 (for fentin hydroxid)[7]	
Residue definition EU	Fentin (fentin including its salts, expressed as triphenyltin cation) (F)	
Fentin is approved in…	Not Approved	
ADI / ARfD	ADI: 0.0004 mg/kg bw per day / ARfD: 0.001 mg/kg bw	
There are two types of fentin salts on the market: acetate and hydroxide. The counter ion dissociates in aqueous environment		

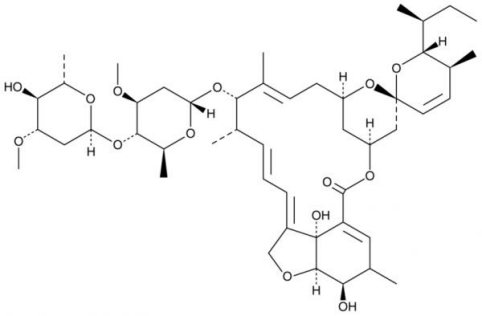
Name: Haloxyfop (CAS: 69806-34-4) **IUPAC: (RS)-2-{4-[3-chloro-5-(trifluoromethyl)-2-pyridyloxy]phenoxy}propionic acid**			
Parameter	**Value**		
Molecular Mass	361.7 g/mol		
Formula	$C_{15}H_{11}ClF_3NO_4$		
Exact mass	361.03287 g/mol		
pKa	4,27	FAO[8]	
LogD	2.8 @ pH 4 0.27 @ pH 7 0.21 @ pH 10		
Residue definition EU	**PO**: Haloxyfop (Sum of haloxyfop, its esters, salts and conjugates expressed as haloxyfop (sum of the R- and S- isomers at any ratio)) (F) **AO**: Sum of haloxyfop, its salts and conjugates expressed as haloxyfop (sum of the R- and S-isomers at any ratio)		
Haloxyfop is approved in…	AT, BE, CZ, DE, HU, LU, NL, PL, RO, SK		
ADI / ARfD	ADI: 0.00065 mg/kg bw per day / ARfD: 0.075 mg/kg bw		

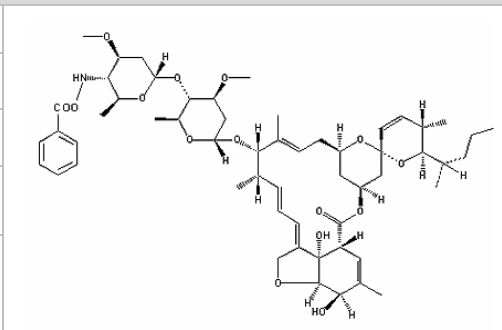
[7] Tomlin, C.D.S. (ed.). The Pesticide Manual - World Compendium, 11 th ed., British Crop Protection Council, Surrey, England 1997, p. 534

[8] http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation09/Haloxyfop.pdf

Name: 3-Hydroxycarbofuran (CAS: 16655-82-6) IUPAC: 1-[(3-hydroxy-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)oxy]-N-methylmethanimidic acid		
Parameter	**Value**	
Molecular Mass	237.3 g/mol	
Formula	$C_{12}H_{15}NO_4$	
Exact mass	237.10010 g/mol	
pKa	13 (OH-group, very weak acid)	
LogKow/logP	1.13 (pH 0-11; Chemicalize)	
Residue definition EU	**AO**: 3-OH-carbofuran (free and conjugated) expressed as carbofuran **PO**:Carbofuran (sum of carbofuran (including any carbofuran generated from carbosulfan, benfuracarb or furathiocarb) and 3-OH carbofuran expressed as carbofuran)	
Approved in…	None of the possible parent compounds is approved	
ADI / ARfD	ADI: 0.00015 mg/kg bw per day / ARfD; 0.00015 mg/kg bw EFSA: concluded that the toxicological values proposed for carbofuran are also applicable to its main metabolites; 3-OH-carbofuran and 3-keto-carbofuran.	

Name: Diclofop (CAS: 40843-25-2) IUPAC: (RS)-2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid		
Parameter	**Value**	
Molecular Mass	327.2 g/mol	
Formula	$C_{15}H_{12}Cl_2O_4$	
Exact mass	326.01126 g/mol	
pKa	3.43 (carboxy group, weak acid)	
LogKow/logD	3.5 (pH4); 2.5 (pH5); 1.6 (pH6)	
Residue definition EU	Diclofop (sum diclofop-methyl and diclofop acid expressed as diclofop-methyl)	
Approved in…	EL, ES, IT, PT	
ADI / ARfD	ADI: 0.001 mg/kg bw per day	

Name: Avermectin B1a (CAS: 65195-55-3) IUPAC: (1'R,2S,4'S,5S,6R,8'R,10'E,12'S,13'S,14'E,16'E,20'R,21'R,24'S)-6-[(2S)-butan-2-yl]-21',24'-dihydroxy-12'-{[(2R,4S,5S,6S)-5-{[(2S,4S,5S,6S)-5-hydroxy-4-methoxy-6-methyloxan-2-yl]oxy}-4-methoxy-6-methyloxan-2-yl]oxy}-5,11',13',22'-tetramethyl-5,6-dihydro-3',7',19'-trioxaspiro[pyran-2,6'-tetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosane]-10',14',16',22'-tetraen-2'-one		
Parameter	**Value**	
Molecular Mass	873.1 g/mol	
Formula	$C_{48}H_{72}O_{14}$	
Exact mass	872.49220 g/mol	
pKa	12,5 (very weak acid)	
LogKow/logD	5,8 (at any pH; Chemicalize)	
Residue definition EU	PO: Abamectin (sum of avermectin B1a, avermectin B1b and delta-8,9 isomer of avermectin B1a, expressed as avermectin B1a) (F) AO: Avermectin B1a	
Abamectin is approved in…	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK	
ADI / ARfD	ADI: 0.0025 mg/kg bw per day	

Name: Emamectin B1a (CAS: 121124-29-6) **IUPAC: (10E,14E,16E)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-6'-[(S)-sec-butyl]-21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-(3,7,19-trioxatetracyclo[15.6.1.14,8.020,24]pentacosa-10,14,16,22-tetraene)-6-spiro-2'-(5',6'-dihydro-2'H-pyran)-12-yl 2,6-dideoxy-3-O-methyl-4-O-(2,4,6-trideoxy-3-O-methyl-4-methylamino-α-L-lyxo-hexapyranosyl)-α-L-arabino-hexapyranoside**		
Parameter	**Value**	
Molecular Mass	886.1 g/mol	
Formula	$C_{49}H_{75}NO_{13}$	
Exact mass	885.52384 g/mol	
pKa	9,34 (methylamine group, basic; computed by Chemicalize)	
LogKow/logD	2.9 (pH range 0-4); 3,0 (pH5); 3.2 (pH6); 3.9 (pH7); 4,8 (pH8); 5,7 (pH9)	
Residue definition EU	Emamectin benzoate B1a, expressed as emamectin	
Approved in…	BE, BG, CY, EL, ES, FR, HR, HU, IT, NL, PL, PT, RO, SI, SK; CZ in progres	
ADI / ARfD	ADI: 0.005 mg/kg bw per day / ARfD: 0.01 mg/kg bw	

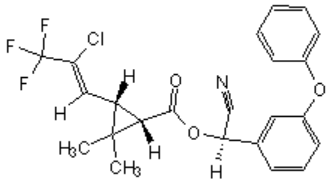
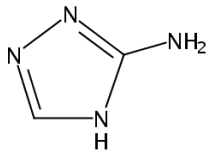
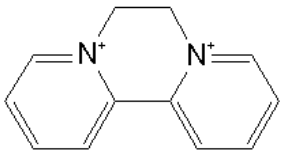
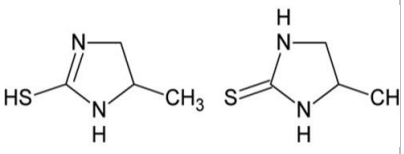
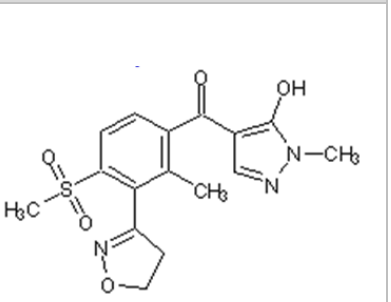
Name: Gamma-Cyhalothrin (CAS: 767-03-62-3) **IUPAC: (S)-a-cyano-3-phenoxybenzyl (1R,3R)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate or (S)-a-cyano-3-phenoxybenzyl (1R)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate**		
Parameter	**Value**	
Molecular Mass	449.9 g/mol	
Formula	$C_{23}H_{19}ClF_3NO_3$	
Exact mass	449.10055 g/mol	
pKa	-	
LogKow	6.8	
Residue definition EU	Lambda-cyhalothrin (includes gamma-cyhalothrin) (sum of R,S and S,R isomers) (F)	
Approved in…	Gamma: AT, BE, BG, CZ, DE, DK, FR, HR, HU, IE, LT, PL, RO, SK Lambda: AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SI, SK, UK	
ADI / ARfD	ADI: 0.0012 mg/kg bw per day / ARfD: 0.0025 mg/kg bw	

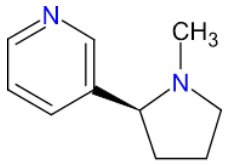
Table 7: General information on substances amenable to the QuPPe extraction method

Name: Amitrole (CAS: 61-82-5 **IUPAC: 1H-1,2,4-triazol-3-amine**		
Parameter	**Value**	
Molecular Mass	84.1 g/mol	
Formula	$C_2H_4N_4$	
Exact mass	84.04359 g/mol	
pKa	3.5 (basic) computed by Chemicalize	
LogKow/logD	-0.83 (pH2.5); -0.67 (pH3); -0.57 (pH4); -0.56 (pH.4.5 onwards)	
Residue definition EU	Amitrole	
Approved in…	Not Approved	
ADI / ARfD	ADI: 0.001 mg/kg bw per day / ARfD: 0.015 mg/kg bw	
Name: Diquat (CAS: 2764-72-9) **IUPAC:9,10-dihydro-8a,10a-diazoniaphenanthrene or 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazine-5,8-diium or 1,1'-ethylene-2,2'-bipyridyldiylium**		
Parameter	**Value**	
Molecular Mass	184.2 g/mol	
Formula	$C_{12}H_{12}N_2$	
Exact mass	184.1004 g/mol	
pKa	Estimated ~ 12[9] (slightly acidic methylene hydrogen)	
LogKow/logD	-4,6 (Tomlin "The Pesticides Manual")) -7 at any pH (computed by Chemicalize)	
Residue definition EU	Diquat	
Approved in…	CY, CZ, DK, EL, FI, MT, PL, PT, RO, SE, SK, UK Withdrawal authorisations by 4 May 2019. Max. period of grace: 4 February 2020.	
ADI / ARfD	ADI: 0.002 mg/kg bw per day / ARfD: 0.01 mg/kg bw	
Name: PTU (CAS: 2122-19-2) , degradant of probineb **IUPAC: 5-methyl-4,5-dihydro-1H-imidazole-2-thiol**		
Parameter	**Value**	
Molecular Mass	116.2 g/mol	
Formula	$C_4H_8N_2S$	
Exact mass	116.04081 g/mol	
pKa	14,2 (very weakly acidic)	
LogKow/logD	0.22 (pH-range of 1-12; computed by Chemicalize); -0.26 experimental	
Residue definition EU	Only regulated in baby food regulation	
PTU is approved in…	Parent propineb is not registered any more	
ADI / ARfD	EU Database: ADI: 0.0003 mg/kg bw per day /ARfD 0.003 mg/kg bw Peer review (2016)[10]: ADI: 0.002 mg/kg bw per day /ARfD 0.012 mg/kg bw JMPR(2004): ADI: 0.007 mg/kg bw per day /ARfD 0.1 mg/kg bw	

[9] RREL Hazardous Waste Research Symposium (1993); T.F. Speth et al.; The removal of ionic contaminants from drinking water; page 153 ff

[10] https://www.efsa.europa.eu/de/efsajournal/pub/4605

Name: Topramezone (CAS: 210631-68-8) IUPAC: [3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-o-tolyl](5-hydroxy-1-methylpyrazol-4-yl)methanone			
Parameter	**Value**		
Molecular Mass	363.4 g/mol		
Formula	$C_{16}H_{17}N_3O_5S$		
Exact mass	363.08889 g/mol		
pKa	4.06 (weakly acidic)	BASF	
	2.7 (acidic, NH of pyrazole group) 1.74 (basic, N of isoxazole moiety)	Computed by Chemicalize	
LogKow/logD	-0.81@pH4 ; -1.52@pH 7 ; -2.34 @pH 9	BASF	
	0,33 (pH1); 1,3 (pH2); 1,4 (pH2.7); 1,0 (pH3); 0,35 (pH5); 0.0 (pH >6.5)	Computed by Chemicalize	
Residue definition EU	Topramezone (BAS 670H)		
Approved in…	NL. EL		
ADI / ARfD	ADI: 0.001 mg/kg bw per day / ARfD 0.001 mg/kg bw		

Name: Nicotine (CAS: 54-11-5) IUPAC: 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine			
Parameter	**Value**		
Molecular Mass	162.2 g/mol		
Formula	$C_{10}H_{14}N_2$		
Exact mass	162.11569 g/mol		
pKa	2.7 (weakly basic at N of pyridine moiety) 8.6 (strongly basic N of pyrrolidine moiety)	All computed by Chemicalize	
LogKow/logD	-2.32 (pH 4); -2.08 (pH 5); -1.37 (pH 6); -0.42 (pH 7); 0.48 (pH 8); 1,0 (pH 9); 1.15 (pH >10),		
Residue definition EU	Nicotine	Reg. (EU) 2015/401 foresees MRLs for herbs and edible flowers 0.4 mg/kg; wild fungi 0.04 mg/kg (but dry wild mushrooms other than ceps 1.2 mg/kg; dried ceps 2.3 mg/kg); teas 0.6 mg/kg; herbal infusions 0.5 mg/kg, seed spices and fruit spices 0.3 mg/kg; bark spices, root and rhizome spices and bud spices 4 mg/kg). Reg. (EU) 2017/978 (applicable from 04/01/2018) confirms and extends Residue definition and MRLs at least up to the next re-evaluation (new data submission deadline 19 October 2021)	
Approved in…	Not approved		
ADI / ARfD	ADI: 0.0008 mg/kg bw per day /ARfD: 0.0008 mg/kg bw		

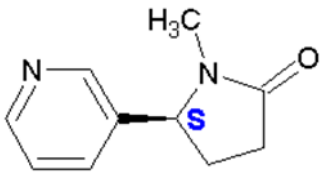
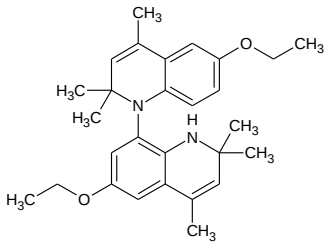
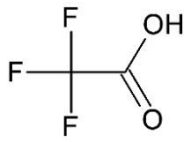
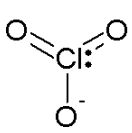
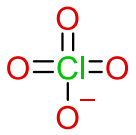
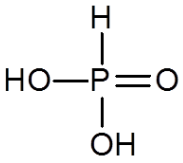
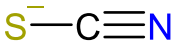
Name: Cotinine (CAS: 486-56-6) IUPAC: (5S)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one			
Parameter	**Value**		
Molecular Mass	176.2 g/mol		
Formula	$C_{10}H_{12}N_2O$		
Exact mass	176.09496 g/mol		
pKa	4.44@pH xx		
LogKow/logD	0.1@pH 5.5 0.17@pH 7.4	Computed by ACD Labs	
Residue definition EU	none		
Approved in…	Its parent nicotine is not approved		
ADI / ARfD	The values of nicotine are typically used (see above)		

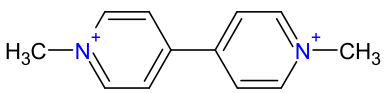
__Table 8:__ General information on substances amenable to the QuPPe extraction method that were analysed additionally and Ethoxyquin-Dimer (amenable to QuEChERS)

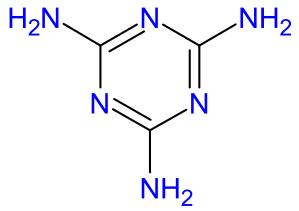
Name: Ethoxyquin-Dimer (CAS: 74681-77-9) **IUPAC: 6-ethoxy-1-(6-ethoxy-2,2,4-trimethyl-1H-quinolin-8-yl)-2,2,4-trimethylquinoline**		
Parameter	**Value**	
Molecular Mass	432.6 g/mol	
Formula	$C_{28}H_{36}N_2O_2$	
Exact mass	432.28 g/mol	
pKa	4.62 (computed by Chemicalize)	
LogP	6.22 (computed by Chemicalize)	
Residue definition EU	MRLs: Previous: Reg. (EC) No 149/2008; Applicable: Reg. (EC) No 703/2014	
Approved in…	Not approved (Ethoxyquin and metabolites)	
ADI / ARfD	ADI: 0.005 mg/kg bw per day; ARfD: 0.5 mg/kg bw per day	
Name: Trifluoroacetic acid (CAS: 76-05-1) **IUPAC: Trifluoroacetic acid**		
Parameter	**Value**	
Molecular Mass	114.02 g/mol	
Formula	$C_2HF_3O_2$	
Exact mass	113.99 g/mol	
pKa	0.95 (computed by Chemicalize)	
LogKow/logD	0.6 (pH1); -0.2(pH2); -1.1(pH3); -2 (pH4); -2.5 (pH5); -2.6 (pH6); 0.9 applies to pH<0 (computed by Chemicalize.com)	
Residue definition EU	None	
Approved in…	Metabolite and contaminant	
ADI / ARfD	ADI: 0.05 mg/kg bw per day	
Name: Chlorate (CAS: 14866-68-3) **IUPAC: Chlorate**		
Parameter	**Value**	
Molecular Mass	83.45 g/mol	
Formula	ClO_3^-	
Exact mass	82.95 g/mol	
pKa	4.62 (computed by Chemicalize.com)	
LogKow/logD	0.03@pH3; -0.05@pH4 -0.48@pH5; -1.3@pH6; 0.041 applies to pH<3 (computed by Chemicalize.com)	
Residue definition EU	Reg. (EU) 2020/749, e.g. MRLs for leaf vegetables at 0.7 mg/kg	
Approved in…	Not approved	
ADI / ARfD	ADI: 0.01 mg/kg bw per day (WHO); ARfD: 0.036 mg/kg bw per day	

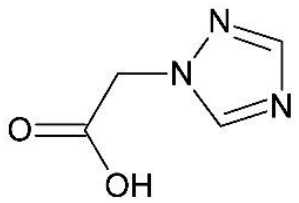
Name: Perchlorate (CAS: 14797-73-0) **IUPAC: Perchlorate**		
Parameter	**Value**	
Molecular Mass	99.45 g/mol	
Formula	ClO_4^-	
Exact mass	98.95 g/mol	
pKa	-7.06 (computed by Chemicalize.com)	
LogKow/logD	-2.47 at pH 0 – 14 (computed by Chemicalize.com)	
Residue definition EU	Contaminant Reg. 1881/2006/EC: Current consolidated version from 14/10/2020	
Approved in…	Not approved	
ADI / ARfD	TDI: 0.0003 mg/kg bw per day (EFSA CONTAM Panel)	

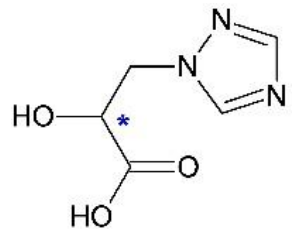
Name: Phosphonic acid (CAS: 13598-36-2) **IUPAC:** Phosphonic acid		
Parameter	**Value**	
Molecular Mass	81.99 g/mol	
Formula	H_3PO_3	
Exact mass	81.98 g/mol	
pKa	2.07; 8.54 (computed by Chemicalize.com)	
LogKow/logD	-2.4@pH3; -3.2@pH4; -3.6@pH5 (computed by Chemicalize.com)	
Residue definition EU	Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)	
Approved in…	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK	
ADI / ARfD	ADI: 2.25 mg/kg bw per day	

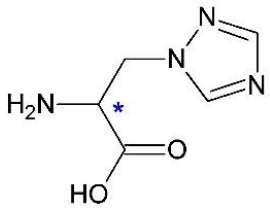
Name: Thiocyanate (CAS: 302-04-5) **IUPAC:** Cyanosulfanide		
Parameter	**Value**	
Molecular Mass	58.08 g/mol	
Formula	SCN^-	
Exact mass	57.98 g/mol	
pKa	0.5 (computed by Chemicalize.com)	
LogKow/logD	-0.5@pH2; -0.6@pH3; -0.6@pH4; (computed by Chemicalize.com)	
Residue definition EU	Potasium thiocyanate: No MRL required (Annex IV) Sodium thiocyanate: Default MRL of 0.01 mg/kg according to Art 18(1)(b) Reg 396 / 2005. (according to EU-Pesticide database)	
Approved in…	Not approved (sodium and potassium thiocyanate); ammonium thiocyanate not yet assessed at EU level	
ADI / ARfD	?	

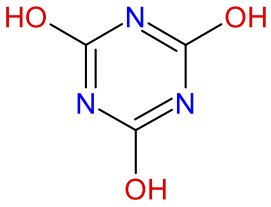
Name: Paraquat (CAS: 1910-42-5) **IUPAC: 1,1'-Dimethyl-4,4'-bipyridinium dichloride**		
Parameter	**Value**	
Molecular Mass	257.16 g/mol	
Formula	$C_{12}H_{14}Cl_2N_2$	
Exact mass	256.05 g/mol	
pKa	None	
LogKow	-6.7 at any pH (computed by Chemicalize.com)	
Residue definition EU	Paraquat (MRLs at 0.02* for most products; 0.05* for teas, spices; 0.05 for rice Applicable: Reg. (EC) No 520/2011	
Approved in…	Not approved	
ADI / ARfD	ADI: 0.004 mg/kg bw per day; ARfD: 0.005 mg/kg bw per day	

Name: Melamine (CAS: 108-78-1) IUPAC: 1,3,5-Triazine-2,4,6-triamine		
Parameter	**Value**	
Molecular Mass	126.12 g/mol	
Formula	$C_3H_6N_6$	
Exact mass	126.06 g/mol	
pKa	basic: 2.84; 9.56 (computed by Chemicalize.com)	
LogKow/logD	-3.4@pH2; -2.8@pH3; -2.6@pH4; -2.6@pH5; -1.2@pH9; (computed by Chemicalize.com)	
Residue definition EU	Metabolite of Cyromazine (not approved); Contaminant VO (EG) 1881/2006: Current consolidated version from 14/10/2020	
Approved in…	Not approved but may also originate from fertilizers	
ADI / ARfD	TDI: 0.2 mg/kg bw per day	

Name: 1,2,4-Triazole acetic acid (CAS: 28711-29-7) IUPAC: 1H-1,2,4-Triazol-1-ylacetic acid		
Parameter	**Value**	
Molecular Mass	127.10 g/mol	
Formula	$C_4H_5N_3O_2$	
Exact mass	127.04 g/mol	
pKa	acidic: 3.20 (carboxy-group) basic: 1.96 (triazole nitrogen) (computed by Chemicalize.com)	
LogKow/logD	-1.3@pH2; -1.3@pH3; -1.9@pH4; -2.8@pH5; (computed by Chemicalize.com)	
Residue definition EU	None; metabolite of triazole fungicides	
Approved in…	Not approved but several precursor compounds are approved (triazole pesticides), may also originate from various the use of 1,2,4-triazole in fertilizers.	
ADI / ARfD	ADI: 1 mg/kg bw per day	

Name: 1,2,4-Triazole lactic acid (CAS: 1450828-63-3) IUPAC: 1H-1,2,4-Triazol-1-yl-lactic acid		
Parameter	**Value**	
Molecular Mass	157.13 g/mol	
Formula	$C_5H_7N_3O_3$	
Exact mass	157.05 g/mol	
pKa	acidic: 3.14 (carboxy group); 13.7 (hydroxyl group); basic: 2.03 (nitrogen in triazole moiety) (computed by Chemicalize.com)	
LogKow/logD	-1.8@pH2; -1.8@pH3; -2.4@pH4; -3.4@pH5; (computed by Chemicalize.com)	
Residue definition EU	None; metabolite of triazole fungicides	
Approved in…	Not approved but several precursor compounds are approved (triazole pesticides), may also originate from various the use of 1,2,4-triazole in fertilizers.	
ADI / ARfD	ADI: 0.3 mg/kg bw per day	

Name: 1,2,4-Triazol-1yl-alanine (CAS: 10109-05-4) IUPAC: 3-(1H-1,2,4-Triazol-1-yl)alanine		
Parameter	**Value**	
Molecular Mass	156.143 g/mol	
Formula	$C_5H_8N_4O_2$	
Exact mass	156.06 g/mol	
pKa	acidic: 1.08; 7.90; basic: 2.44; 11.28 (computed by Chemicalize.com)	
LogKow/logD	-3.9@pH2; -3.5@pH 3 - 7 (computed by Chemicalize.com)	
Residue definition EU	None; metabolite of triazole fungicides	
Approved in…	Not approved but several precursor compounds are approved (triazole pesticides), may also originate from various the use of 1,2,4-triazole in fertilizers.	
ADI / ARfD	ADI: 0.3 mg/kg bw per day	

Name: Cyanuric acid (CAS: 108-80-5) IUPAC: 1,3,5-triazine-2,4,6-triol		
Parameter	**Value**	
Molecular Mass	129.075 g/mol	
Formula	$C_3H_3N_3O_3$	
Exact mass	129.0174 g/mol	
pKa	acidic: 5.55; 8.77; 12.27 (computed by Chemicalize.com)	
LogKow/logD	0.98 @pH<12 (computed by Chemicalize.com)	
Residue definition EU	Metabolite and hydrolysis product of melamine and chloroisocyanurates	
Approved in…	Not approved but some precursor compounds are approved (triazine pesticides), also originating from various other sources, such as from fertilizers and sanitizers	
ADI / ARfD	TDI: 1.5 mg/kg bw per day (WHO 2008)	

3. Materials

Table 9: Sources of analytical standards-updated

Substance	Purity	CAS	Sources (exemplary)
Avermectin B1a	97.76	65195-55-3	Toronto Research Chemicals
Emamectin B1a (solution; 100 µg/mL)	-	121124-29-6	HPC
3-Hydroxycarbofuran	97.16	16655-82-6	Dr. Ehrenstorfer
γ-Cyhalothrin	98.5	767-03-62-3	Dr. Ehrenstorfer
Fentin-hydroxide	99.0	76-87-9	Dr. Ehrenstorfer
Haloxyfop	99.9	69806-34-4	HPC
Diclofop	98.0	40843-25-2	Dr. Ehrenstorfer
Amitrole	98.0	61-82-5	Dr. Ehrenstorfer
Nicotine	99.5	54-11-5	Dr. Ehrenstorfer
Cotinine	98.0	486-56-6	Sigma-Aldrich
PTU	97.0	2122-19-2	Dr. Ehrenstorfer
Diquat dibromide monohydrate	100.0	6385-62-2	Sigma-Aldrich
Topramezone	100.0	210631-68-8	Dr. Ehrenstorfer
Propyzamide D_3	99.0	1219805-79-4	CDN Isotopes
Chlorpyrifos D_{10}	95.21	285138-81-0	Dr. Ehrenstorfer
Fentin D_{15}	98.0	358731-94-9	CDN Isotopes
Amitrole $^{15}N_2$ $^{13}C_2$	97.0	1346603-92-6	Toronto Research Chemicals
Nicotine D_4	99.1	350818-69-8	Dr. Ehrenstorfer
Cotinine D_3	99.9	110952-70-0	Dr. Ehrenstorfer
PTU (N,N'-(1,2-Propylene)-thiourea) D_6	98.0	-	Toronto Research Chemicals
Diquat D_8 dibromide	99 atom %D	-	CDN Isotopes
MPPA D_3	96.0	15090-23-0	Toronto Research Chemicals
Ethoxyquin-Dimer	99.2	74681-77-9	HPC
Trifluoro acetic acid	99.0	76-05-1	Fluka
Chlorate-Sodium	99	7775-09-9	Sigma-Aldrich
Perchlorate-Sodium	98	7601-89-0	Sigma-Aldrich
Phosphonic acid	99	13598-36-2	Sigma-Aldrich
Thiocyanate-Sodium	99.99	540-72-7	Sigma-Aldrich
Paraquat-dichloride hydrate	99.9	75365-73-0	HPC
Melamine	99	108-78-1	Sigma-Aldrich
1,2,4-Triazole acetic acid	97	28711-29-7	Dr. Ehrenstorfer
1,2,4-Triazole lactic acid	98	1450828-63-3	Bayer CropScience
1,2,4-Triazole-1yl-alanine	98,6	4819-36-7	Bayer CropScience
1,2,4-Triazole lactic acid (2-Hydroxy-3-(1H-1,2,4-triazol-1-yl)-propionic acid) *	-	1450828-63-3	Sigma-Aldrich
1,2,4-Triazole-1yl-alanine*	-	10109-05-4	Sigma-Aldrich
Cyanuric acid	99	108-80-5	Dr. Ehrenstorfer
Trifluoro acetic acid $^{13}C_2$	97,0	-	Toronto Research Chemicals
Chlorate $^{18}O_3$	>98%	Mixture Chlorate $^{18}O_3$/ Perchlorate $^{18}O_4$	EURL-SRM
Perchlorate $^{18}O_4$			EURL-SRM

Substance	Purity	CAS	Sources (exemplary)
Phosphonic acid $^{18}O_3$	>98%		EURL-SRM
Thiocyanate-Potassium ^{13}C ^{15}N	99,4	143673-61-4	Sigma-Aldrich
Paraquat D_8 –dichloride	100	-	Sigma-Aldrich
Melamine $^{15}N_3$	98	-	HPC
1,2,4-Triazole acetic acid $^{13}C_2$ ^{15}N	98	-	Bayer CropScience
1,2,4-Triazole lactic acid $^{13}C_2$ ^{15}N	98	-	Bayer CropScience
1,2,4-Triazole-1yl-alanine $^{13}C_2$ ^{15}N	92,2	-	Bayer CropScience
1,2,4-Triazole acetic acid D_2*	96	2409015-22-9	Reseachem
1,2,4-Triazole lactic acid D_2*	99	2409015-17-2	Reseachem
1,2,4-Triazole-1yl-alanine D_2*	95	2180306-38-9	Reseachem
Cyanuric acid $^{13}C_3$	99,8	201996-37-4	HPC

Disclaimer: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

*Recently available are also D_2 analogons.

All other materials and chemicals used as listed in EN 15662, the QuPPe-PO[11] or the QuPPE AO[12] method.

[11] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe_PO_V11_1.pdf

[12] https://www.eurl-pesticides.eu/userfiles/file/meth_QuPPe_AO_V3_2.pdf

4. Extraction and Instrument Methods

4.1. Extraction Methods

4.1.1. QuEChERS

Two methods, the QuEChERS method (EN 15662) without clean-up and the acidified-QuEChERS method (A-QuEChERS) were tested. The analytical portion used was 2 g in each case. For cow's milk and ready-to-use products the analytical portion used was 10 g. The general analytical procedure at a glance is shown in **Figure 1**.

4.1.1.1. Apparatus and Consumables

Refer to EN 15662.

4.1.1.2. QuEChERS (EN 15662)

The procedure as described in EN 15662 was followed using 2 g infant formula powder and 10 g milk or ready-to-use product as analytical portion. The first extraction step involved 15 min shaking by a mechanical shaker. No clean-up was conducted for LC-MS/MS applications.

4.1.1.3. Acidified-QuEChERS (A-QuEChERS)

The method corresponds to EN 15662, but instead of pure acetonitrile 10 mL acetonitrile containing 1% formic acid are employed for extraction. Partitioning is induced by the addition of 4 g $MgSO_4$ + 1 g NaCl (no citrate buffer salts). The first extraction step involved 15 min shaking by a mechanical shaker. No clean-up was conducted for LC-MS/MS applications.

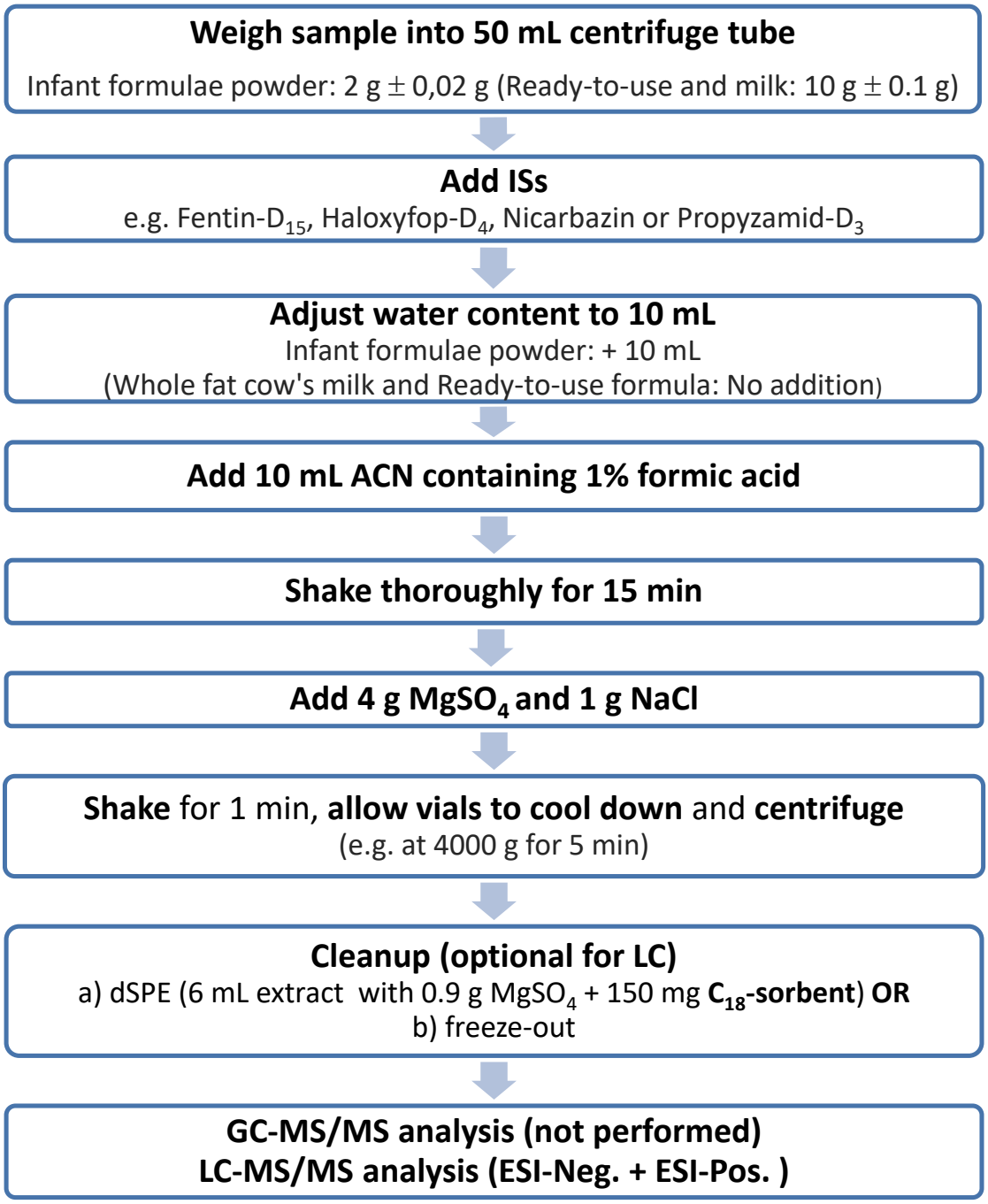


Figure 1: Method at a glance Acidified QuEChERS.

4.1.2. QuPPe AO

For the analysis of the QuPPe amenable analytes within the scope of the pilot monitoring, the QuPPe AO method is used with the analytical portion being 2 g of infant formula powder and 10 g of milk or ready-to-use products. All consumables and chemicals used are listed in QuPPe PO[13] or QuPPe AO[14] protocols. The general analytical procedure at a glance is shown in **Figure 2**.

4.1.2.1. Weighing of analytical portions

Weigh a representative analytical portion (m_a) of the sample homogenate into a 50 mL centrifuge tube. In case of infant formula powder weigh 2 g ± 0.02 g of the homogenized sample. In case of ready-to-use liquid infant formula products and cow's milk weigh 10 g ± 0.1 g.

4.1.2.2. Adjustment of water content

Add water to the vial containing the analytical portion (4.1.2.1), to reach 10 g in total. The amount of water to be added to the analytical portion is shown in Table 10**.**

Table 10: *Adjustment of water content for infant formula*

Commodity	Sample weight (m_a)	Typical natural water content in g/100 g	Water to be added	Vol. of 10% EDTA sln	Water addition may be skipped*	IS-WSln added e.g.	Extra Formic acid	Extraction Solvent
Infant formula powder	2 g	-	9 mL	1 mL	No	100 µL	100 µL	10 mL MeOH containing 1% Formic acid (FA)
Infant formula ready-to-use liquid product	10 g	85 - 87	-	1 mL	Yes	100 µL	100 µL	
Whole fat cow's milk	10 g	85	0.5 mL	1 mL	Yes	100 µL	100 µL	

* The ILIS will typically correct for volume deviations. In case no ILIS is used, volume adjustments become more important

4.1.2.3. Extraction

Add 10 mL acidified methanol and an appropriate small volume (e.g. 100 µl) of the internal standard working (IS-WSln) containing isotopically labelled analogues of the analytes of tube and shake for a few seconds to distribute the acid and allow the proteins to coagulate. Add 1 mL of 10% aqueous EDTA solution (preparation see QuPPe-document) and shake either for 1 min by hand or for 15 min by a mechanical shaker.

[13] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe_PO_V11_1.pdf
[14] https://www.eurl-pesticides.eu/userfiles/file/meth_QuPPe_AO_V3_2.pdf

4.1.2.4. Freeze-Out and Centrifugation

Depending on the available centrifugation equipment there is various options, e.g.:

(1) Centrifugation following freeze-out: Place the tubes with the extracts from 4.1.1.3 into a freezer (e.g. at ca. -80 °C for 30 min or for > 90 min at ca. -20 °C) and centrifuge them while still cold for 5 min at ≥3,000 g. Higher centrifugation forces (e.g. ≥10,000 g) and the use of a refrigerated centrifuge are preferable.

(2) Refrigerated high-speed centrifugation: Centrifuge the extracts 4.1.1.3 for ≥20 min at high centrifugation speed (e.g. >10,000 g) and low temperatures (e.g. lower than -5 °C). Centrifugation time may be reduced to 5 min if the extract is pre-frozen.

The centrifuged extracts needs **to be separated while still cold** to avoid that matrix-components, which had precipitated in the cold, will redissolve.

4.1.2.5. Removal of Lipids and Protein Precipitation

Transfer a 2 mL aliquot of the supernatant from **4.1.2.4** into a 10 mL centrifuge tube with screw cap, which already contains 2 mL of acetonitrile and 100 mg of C_{18}-sorbent and shake for 1 min. Then centrifuge for 5 minutes at >3,000 g.

4.1.2.6. Filtration

Transfer a 3 mL aliquot of the supernatant from **4.1.2.5** into an ultrafiltration unit and centrifuge at 3,000 g until enough filtrate is accumulated in the reservoir (5 min are typically enough). Transfer an aliquot of the filtrate into an autosampler vial.

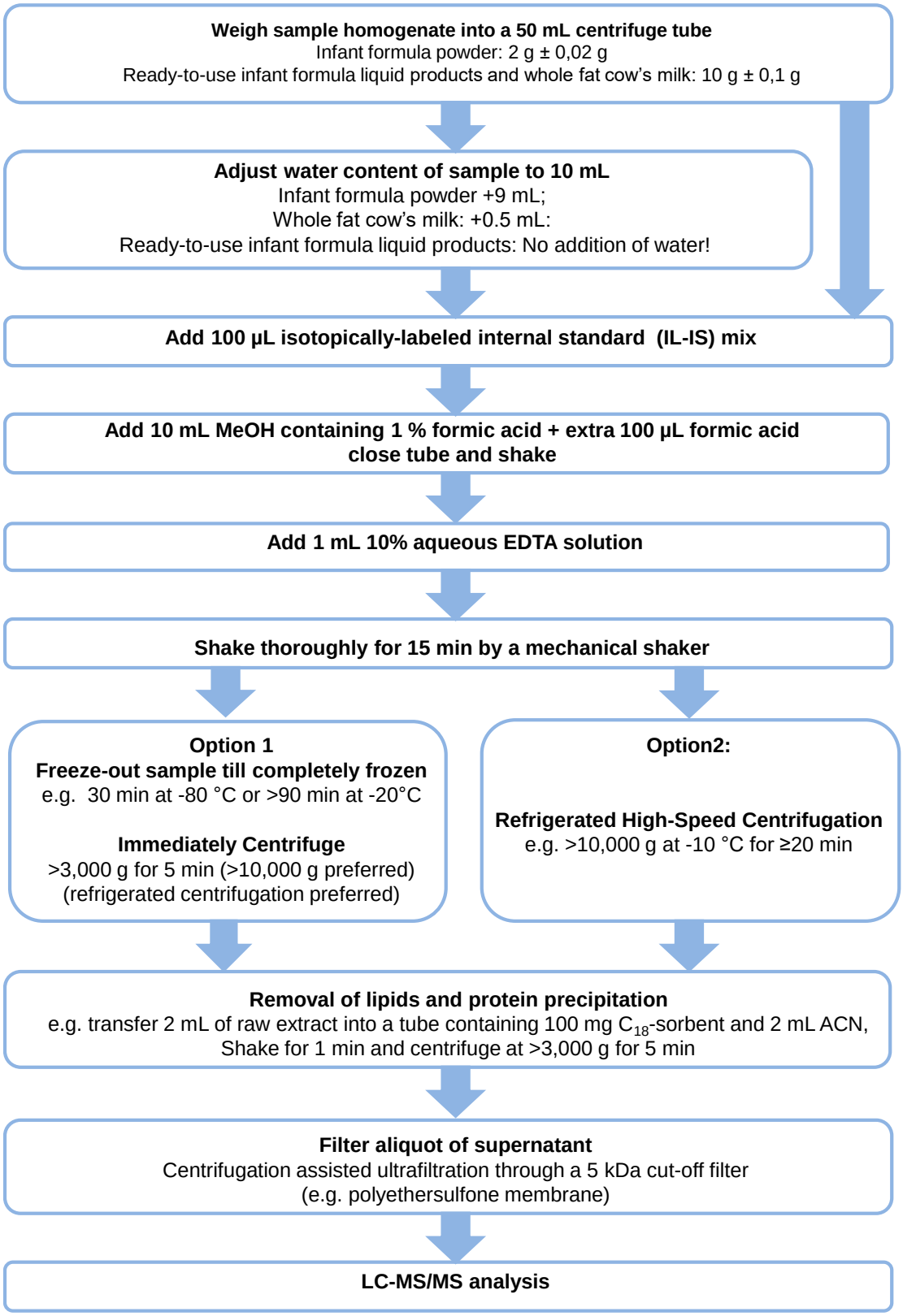


Figure 2: *Method at a glance QuPPe AO for infant formula*

4.2. Instrumentation Methods

In Table 11 an overview of the LC-MS/MS methods used for the analysis of the analytes within the scope is given. Detailed conditions are shown in the following tables.

Table 11: Overview of all analytes within the scope and the used LC-MS/MS methods

Analyte	Internal Standard	Instru-mental Method	Analytical Column	MS mode
Avermectin B1a	Propyzamide D_3	**Method 1**	Acquity UPLC BEH C_{18}	MS/MS ESI(+)
Emamectin B1a				
3-Hydroxy-carbofuran				
Ethoxyquin-Dimer				
Gamma-Cyhalothrin	Chlorpyrifos D_{10}	**Method 2**	ChiralArt Cellulose-SB	MS/MS ESI(+)
Fentin	If CEN QuEChERS is used Fentin D_{15} will help to correct for recovery. With FA-QuEChERS Propyzamide D_3 is also suitable	**Method 3**	Zorbax 3,5 µm; Eclipse XDB-C_{18}	MS/MS ESI(+)
Haloxyfop	Propyzamide D_3	**Method 4**	Acquity UPLC BEH C_{18}	MS/MS ESI(-)
Diclofop (free acid)				
Amitrole	Amitrole $^{15}N_2$ $^{13}C_2$	**Method 5[A]**	BEH Amide	MS/MS ESI(+)
Nicotine	Nicotine D_4			
Cotinine	Cotinine D_3			
PTU	PTU D_6			
Melamine	Melamine $^{15}N_3$			
Diquat	Diquat D_8	**Method 6[B]**	Obelisc R	MS/MS ESI(+)
Paraquat	Paraquat D_8			
Topramezone	MPPA D_3[H] /Propyzamide D_3	**Method 7[C]**	Waters Torus™DEA	MS/MS ESI(-)
Trifluoroacetic acid	Trifluoro acetic acid $^{13}C_2$	**Method 8[D]**	AS19	IC-MS/MS ESI(-)
Chlorate	Chlorate $^{18}O_3$	**Method 9[E]**	Hypercarb	MS/MS ESI(-)
Perchlorate	Perchlorate $^{18}O_4$			
Phosphonic acid	Phosphonic acid $^{18}O_3$			
Thiocyanate	Thiocyanate-Potassium ^{13}C ^{15}N			
Triazole acetic acid	1,2,4-Triazole acetic acid $^{13}C_2$ ^{15}N also D_2	**Method 10[F]**	Waters Torus™DEA	MS/MS ESI(+)
Triazole lactic acid	1,2,4-Triazole lactic acid $^{13}C_2$ ^{15}N also D_2			
Triazole alanine	1,2,4-Triazole-1yl-alanine $^{13}C_2$ ^{15}N also D_2			
Cyanuric acid	Cyanuric acid $^{13}C_3$	**Method 11[G]**	Hypercarb	MS/MS ESI(-)

[A] see QuPPe PO: Method 4.2 (M 4.2): "Quats & Co BEH Amide"[15]
[B] see QuPPe PO: Method 4.1 (M 4.1): "Quats & Co Obelisc R"[15]
[C] see QuPPe PO: Method 1.6 (M 1.6): "Glyphosate & Co. on Torus DEA"[15]
[D] see QuPPe PO: Method (M 11): "Anionic Pesticides with Ion Chromatography"[15]
[E] see QuPPe PO: Method (M 1.4): "PerChloPhos"[15]
[F] see QuPPe PO: Method (M 10): "Triazole derivative metabolites (TDMs) on Torus DEA"[15]
[G] see QuPPe PO: Method (M 1.3): "Glyphosate & Co.Hypercarb"[15]
[H] Please read important note on MPPA D3 under **Table 20**

[15] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Table 12: Instrumentation details on Method 1

LC	Agilent Infinity		
MS/MS	**Sciex 5500QTrap, run in ESI positive mode**		
Column	Acquity UPLC BEH C_{18} 1.7µm 2.1 x 100 mm Column		
Pre-column	Van Guard BEH C_{18} 1.7µm		
Mobile Phase	A: 5 mmol NH_4-Formiate in Water + 5 % Methanol B: 5 mmol NH_4-Formiate in Methanol		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	0	60	0.4
	10	10	0.4
	13	10	0.4
	13.1	60	0.4
	19	60	0.4
Injection volume	2 µL		
Column temperature	40°C		
Internal Standard	Propyzamide D_3		
Aquired mass transitions			
Propyzamide D_3	259/193		
Avermectin B1a $[M+NH_4]^+$	891/305 T 891/567		
Ememectin B1a	887/82 T 887/158		
3-Hydroxycarbofuran	238/163 T 238/181 238/220		
Ethoxyquin-Dimer	433/216 T 433/188 433/375		

Table 13: Instrumentation details on Method 2[16]

LC	Waters I-Class		
MS/MS	**Sciex 5500QTrap, run in ESI positive mode**		
Column	ChiralArt Cellulose-SB 100x4.6mm, 3µm		
Pre-column	-		
Mobile Phase	A: 5 mmol NH_4-Formiate in Water + 5 % Methanol B: 5 mmol NH_4-Formiate in Methanol		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	Initial	20	0.6
	15	20	0.6
Injection volume	5 µL		
Column temperature	35°C		
Internal Standard	Chlorpyrifos D_{10}		
Aquired mass transitions			
Chlorpyrifos D_{10}	360/199		
Gamma-Cyhalothrin	467/225 T 467/450		

[16] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_Observation_Cyhalothrin_V1.pdf

Table 14: Instrumentation details on Method 3

LC	Agilent Infinity		
MS/MS	Sciex 6500QTrap, run in ESI positive mode		
Column	Zorbax 3,5 µm; Eclipse XDB-C_{18}; 2,1x 50 mm		
Pre-column	C_{18} ODS 4mm x 2mm ID (Phenomenex AJO-4286)		
Mobile Phase	A: 5 mmol NH_4-Formiate in Water + 1 % Formic acid B: 5 mmol NH_4-Formiate in Methanol + 1 % Formic acid		
Gradient	Time (min)	Mobile Phase A (%)	Flow (mL/min)
	0	60	0.4
	2	0	0.4
	7	0	0.4
	7.1	60	0.4
	11	60	0.4
Injection volume	1 µL		
Column temperature	40°C		
Internal Standard	Fentin D_{15}; Propyzamide D_3 when employing A-QuEChERS		
Aquired mass transitions			
Fentin D_{15}	366/120		
Propyzamide D_3	259/193		
Fentin	351/120 T 351/197 349/195		

Table 15: Instrumentation details on Method 4

LC	Agilent Infinity		
MS/MS	Sciex 5500QTrap, run in ESI negative mode		
Column	Acquity UPLC BEH C_{18} 1.7µm 2.1 x 100 mm Column		
Pre-column	Van Guard BEH C_{18} 1.7µm		
Mobile Phase	A: 0.01 % acetic acid in Water + 5 % Acetonitrile B: 0.01 % acetic acid in Acetonitrile		
Gradient	Time (min)	Mobile Phase A (%)	Flow (mL/min)
	0	80	0.4
	4	70	0.4
	7	10	0.4
	8.5	10	0.4
	8.6	80	0.4
	13.5	80	0.4
Injection volume	2 µL		
Column temperature	40°C		
Internal Standard	Propyzamide D_3		
Aquired mass transitions			
Propyzamide D_3	257/231		
Haloxyfop	360/288 T 362/290 360/196		
Diclofop (free acid)	325/253 T 325/255 325/145		

Table 16: *Instrumentation details on Method 5 (corresponds to Method 4.2 (M 4.2): "Quats & Co BEH Amide"; see QuPPe PO[17])*

LC	**Waters I-Class**		
MS/MS	**Sciex 5500QTrap, run in ESI positive mode**		
Column	BEH Amide 2.1 x 100mm 1.7 µm		
Pre-column	BEH Amide 1.7 µm		
Pre-filters	e.g. waters column inline filter 2 µm		
Mobile Phase	A: 50 mmol NH_4-Formiate in Water (adjusted to pH 3 with formic acid) B: Acetonitrile		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	0	3	0.5
	0.5	3	0.5
	4	30	0.5
	5	60	0.5
	6	60	0.5
	6.1	3	0.5
	10	3	0.5
Injection volume	2 µL		
Column temperature	40°C		
Aquired mass transitions			
Amitrole	85/43 T 85/58 85/57		
Amitrole $^{15}N_2$ $^{13}C_2$	89/44		
Nicotine	163/130 T 163/132 163/84		
Nicotine D_4	167/84		
Cotinine	177/80 T 177/98		
Cotinine D_3	180/80		
PTU	117/60 T 117/58 117/72		
PTU D_6	123/64		
Melamine	127/85 T 127/68 127/60		
Melamine $^{15}N_3$	130/87		

[17] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Table 17: Instrumentation details on Method 6 (corresponds to Method 4.1 (M 4.1): "Quats & Co Obelisc R"; see QuPPe PO[18])

LC	Waters I-Class		
MS/MS	**Sciex 5500QTrap, run in ESI positive mode**		
Column	Obelisc R 2.1 x 150 mm 5 µm 100 Å		
Pre-column	Obelisc R 2.1 x 10 mm 5 µm		
Pre-filters	e.g. Supelco column saver 2 µm Filter		
Mobile Phase	A: 50 mmol NH_4-Formiate in Water (adjusted to pH 3 with formic acid) B: Acetonitrile		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	0	20	0.4
	3	80	0.4
	8	80	0.4
	8.1	20	0.4
	14	20	0.4
Injection volume	10 µL		
Column temp.	40°C		
Aquired mass transitions			
Diquat D_8	See Table 19; mind to use MRMs corresponding to those of the native analyte (see below)		
Diquat	See Table 19.		
Paraquat	186/171 93/171 T 93/77		
Paraquat D_8	194/179 97/179 T mind to use MRMs corresponding to those of the native analyte (see below)		

Table 18: Exemplary matrix effects of Diquat in infant formula powder extract, considering mass transitions resulting from different parents (Diquat conc. in the final extract: 0.015 µg/mL)

Analyte	Type of parent ion	Native compound		Corresponding ILIS D_8	
		MRM (m/z)	Matrix effect (%)	MRM (m/z)	Matrix effect (%)
Diquat	**$[M]^{2+}$**	*92/84*	+57	*96/88*	+54
	$[M^{2+} - H^+]^+$	*183/157*	-92	*191/165*	-91
	$[M]^{+\bullet}$	*184/128*	-91	*192/134*	-93

[18] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Table 19: *Individual transitions and MS/MS settings (Sciex API 5500) for Diquat and its respective ILIS on Sciex 5500 QTrap ESI(+). Transitions are grouped by parent type.*

Parent ion Q1 [m/z]	Daughter ion Q1 [m/z]	Suitable ILIS transition	Sensitivity Ranking*	DP (V)	CE (V)	CXP (V)
Diquat $[M]^{2+}$ 92	**84.4**	**ILIS Diquat D_8 $[M]^{2+}$ 96/88**	1	**61**	**21**	**4**
	157		5	**61**	**19**	**12**
	78		8	**61**	**31**	**12**
	130		7	**61**	**25**	**8**
Diquat $[M^{2+} - H^+]^+$ 183	**157**	**ILIS Diquat D_8 $[M^{2+}- H^+]^+$ 191/165**	2	**161**	**31**	**10**
	130		7	**161**	**43**	**8**
	168		6	**161**	**37**	**10**
	78		9	**161**	**51**	**12**
Diquat $[M]^{+\bullet}$ 184	128	ILIS Diquat D_8 $[M]^{+\bullet}$ 192/134	3	60	55	8
	106		5	60	23	8
	78		5	60	65	12
	156		4	60	29	10
	169		5	60	27	12
	155		5	60	43	12
	168		5	60	45	12
ILIS Diquat D_8 $[M]^{2+}$ 96	**88,4**	-		**61**	**21**	**4**
ILIS Diquat D_8 $[M^{2+}- H^+]^+$ 191	**165**	-		**101**	**31**	**10**
ILIS Diquat D_8 $[M]^{+\bullet}$ 192	134	-		156	55	8

* The ranking in this table only refers to the signal to noise ratio. Further experiments are planned to study signal repeatability of various mass transitions also in comparison with the transitions of the respective ILIS.

Table 20: *Instrumentation details on Method 7 (corresponds to Method 1.6 (M 1.6): "Glyphosate & Co. on Torus DEA"; see QuPPe PO[19])*

LC	**Waters I-Class**		
MS/MS	**Sciex 5500QTrap, run in ESI negative mode**		
Column	Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm		
Pre-column	Waters Torus™DEA VanGuard™ 2.1 mm x 5 mm; 1.7 µm		
Pre-filters	Waters ACQUITY UPLC Column In-Line Filter Kit		
Mobile Phase	A: 1.2% formic acid in Water B: 0.5% formic acid in Acetonitrile		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	0	10	0.5
	0.5	10	0.5
	1.5	80	0.5
	4.5	90	0.5
	17.5	90	0.5
	17.6	10	0.5
	23	10	0.5
Injection volume	10 µL		
Column temperature	50°C		
Aquired mass transitions			
Topramezone	362/334 T 362/318 362/194		
MPPA D_3*	154/136		

* IMPORTANT NOTE: Be aware, that the use of ISs other than the isotope labelled analogues of the analytes can introduce significant errors if there are significant differences in the matrix effects between sample and calibration solution. In absence of topramezone ILIS MPPA D_3 was tested as it typically shows little matrix effects. It merely served for correcting for volume deviations, as marix effects were largely compensated by matrix-matched calibrations.

[19] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Table 21: Instrumentation details on Method 8 (corresponds to Method M 11: "Anionic Pesticides with Ion Chromatography", see QuPPe PO[20; 21]

IC	**Thermo Scientific Integrion**		
MS/MS	**Sciex 5500QTrap, run in ESI negative mode**		
Column	Thermo Scientific™ Dionex™ IonPac™ AS19,2x25mm; 32°C		
Pre-column	Thermo Scientific™ Dionex™ IonPac™ AG19,2x5mm		
Mobile Phase	KOH		
Gradient	**Time (min)**	**C (KOH)**	**Flow (mL/min)**
	0	15	0.3
	8	15	0.3
	13	36	0.3
	21	36	0.3
	21.5	70	0.3
	25	70	0.3
	25.5	15	0.3
	30	15	0.3
Injection volume	5 µL of 5-fold diluted extracts		
Column temperature	32°C		
Flow Make-up Solvent before ion source	0.15 mL/min acetonitrile		
Aquired mass transitions			
Trifluoroacetic acid	113/69 T 113/113		
Trifluoroacetic acid $^{13}C_2$	115/70		

Table 22: Instrumentation details on Method 9 (corresponds to M 1.4: "PerChloPhos", see QuPPe PO[22])

LC	**Agilent Infinity**		
MS/MS	**Sciex 6500QTrap+, run in ESI negative mode**		
Column	Hypercarb 2.1 x 100 mm 5 µm		
Pre-column	Hypercarb Guard 2.1 x 10 mm 5 µm		
Mobile Phase	A: 1% acetic acid in water +5% methanol B: 1% acetic acid in methanol		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	0	100	0.4
	10	70	0.4
	10.1	100	0.4
	15	100	0.4
Injection volume	5 µL		
Column temperature	40°C		
Aquired mass transitions			
Chlorate	83/67 T 85/69		
Chlorate $^{18}O_3$	89/71 91/73		
Perchlorate	99/83 T 101/85		
Perchlorate $^{18}O_3$	107/89 109/91		
Phosphonic acid	81/79 T 81/63		
Phosphonic acid $^{18}O_3$	87/85 87/67		
Thiocyanate	58/58		
Thiocyanate ^{13}C ^{15}N	60/60		

[20] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN
[21] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EPRW%202020%20-%20PD87.pdf
[22] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Table 23: Instrumentation details on Method 10 (corresponds to Method 10 (M 10): "Triazole derivative metabolites (TDMs) on Torus DEA", see QuPPe PO[23])

LC	**Waters I-Class**		
MS/MS	**Sciex 5500QTrap, run in ESI positive mode**		
Column	Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm		
Pre-column	Waters Torus™DEA VanGuard™ 2.1 mm x 5 mm; 1.7 µm		
Pre-filters	Waters ACQUITY UPLC Column In-Line Filter Kit		
Mobile Phase	A: 1.2% formic acid in Water B: 0.5% formic acid in Acetonitrile		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	0	10	0.5
	0.5	10	0.5
	1.5	80	0.5
	4.5	90	0.5
	5	90	0.5
	5.5	10	0.5
	10	10	0.5
Injection volume	10 µL		
Column temperature	50°C		
Aquired mass transitions			
1,2,4-Triazole acetic acid	128/70 T 128/43 128/73		
1,2,4-Triazole acetic acid $^{13}C_2$ $^{15}N_3$	133/75		
1,2,4-Triazole acetic acid D_2	130/72		
1,2,4-Triazole lactic acid	158/70 T 158/43 158/112		
1,2,4-Triazole lactic acid $^{13}C_2$ $^{15}N_3$	163/75		
1,2,4-Triazole lactic acid D_2	160/72		
1,2,4-Triazol-1yl-alanine	157/70 T 157/88 157/42		
1,2,4- Triazol-1yl-alanine $^{13}C_2$ $^{15}N_3$	162/75		
1,2,4-Triazol-1yl-alanine D_2	159/42		

[23] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Table 24: Instrumentation details on Method 11 (corresponds to Method (M 1.3): "Glyphosate & Co.Hypercarb", see QuPPe PO[24])

LC	**Agilent Infinity**		
MS/MS	**Sciex 6500QTrap+, run in ESI negative mode**		
Column	Hypercarb 2.1 x 100 mm 5 µm		
Pre-column	Hypercarb Guard 2.1 x 10 mm 5 µm		
Mobile Phase	A: 1% acetic acid in water +5% methanol B: 1% acetic acid in methanol		
Gradient	**Time (min)**	**Mobile Phase A (%)**	**Flow (mL/min)**
	0	100	0.2
	10	70	0.2
	11	70	0.4
	18	70	0.4
	19	10	0.4
	22	10	0.4
	22.1	100	0.2
	30	100	0.2
Injection volume	5 µL		
Column temperature	40°C		
Aquired mass transitions			
Cyanuric acid	128/42 T 128/85		
Cyanuric acid $^{13}C_3$	131/43		

5. General remarks on validation experiments

Initial method validation was conducted the QuEChERS or the QuPPe AO procedure on 'normal' infant formula powder (see **1**; group a) and on homogenized and pasteurized whole cow's milk. Validation were conducted on 2 validation levels with the low level being lower than the MRL required to ensure toxicological safety of the respective compounds in infant formulae.

When analysing cow's milk, a test portion of 10 g is used both in QuEChERS and QuPPe. In the method for infant formulae presented here, 2g of dry infant formula were employed in QuEChERS and QuPPe, which corresponds to 15-16 g reconstituted product (depending on recipe). 10 g of water is added as foreseen for any other dry commodities in QuEChERS and QuPPe.

For the analysis of the 13 toxicologically critical SRM substances in infant formulae, 7 different LC-MS/MS methods were employed. 5 of them involved measurement in the ESI-positive mode and 2 of them in the ESI-negative mode. Matrix-matched calibration solutions were prepared using blank extracts, at the 60% and 120% level of the spiked concentration. In the case of QuPPe analytes and fentin, the results were evaluated using isotopically labelled analogues of the target analytes as internal standards. In the case of QuEChERS analytes propyzamide D_3 was used. Table 26 and Table 33 show validation results for QuEChERS amenable analytes in infant formula and whole cow's milk respectively. Table 27 and Table 34 show validation results of QuPPe amenable analytes in infant

[24] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

formula powder and whole cow's milk respectively. More detailed results for individual MRM-transitions of diquat are shown in Table 28.

Table 29 and Table 35 show validation data of the additionally analysed substances by QuPPe AO in infant formula powder and whole cow's milk respectively. These additionally analysed substances were not included in the initial method validation experiments. They were validated in parallel to the analysis of the samples belonging to category b) to f), see below.

Exemplary chromatograms of spiked infant formula extracts are shown in Table 31 and Table 32. Exemplary chromatograms of spiked milk extracts are shown in Table 36 and Table 37.

In the course of sample analysis additional validation studies were conducted to make sure that the performance criteria are also fulfilled for other types of infant formulae (i.e. of categories b) to f). The respective LOW-Level was spiked and the samples were extracted in quintuplicate (n=5). For quality control purposes, duplicate recovery experiments were run with every batch on samples of category a) at the respective LOW-Level. As regards the ready-to-use infant formulae, duplicate recovery experiments were conducted in parallel to the analysis of the ready-to-use products belonging to category a) and c).

Figure 4 to Figure 9 and Table 30 show average recoveries in category a) to f) according to the measurement methods. Average recoveries on category a) refer to the experiments run in parallel to the sample extractions, not to initial method validation.
In some cases, for the sake of efficiency, it was decided to skip the analysis of certain samples or to run the samples using slightly different, yet equivalent, methods than those applied in the initial validation experiments. All validation experiments that ran in parallel to the analysis of the samples concerned the methods that were actually used for those samples. The differences between the methods used in initial validation and the methods used in the analysis of the samples are summarized below.

- It could be shown A-QuEChERS is suitable for all QuEChERS-amenable compounds (Avermectin B1a, Emamectin B1a, 3-Hydroxy-carbofuran, Ethoxyquin-Dimer, Gamma-Cyhalothrin, Fentin, Haloxyfop, Diclofop (free acid) and topramezone), without whereas the citrate-buffered QuEChERS was not suitable for the analysis of fentin. It was therefore decided to conduct sample analysis and additional method validation only using A-QuEChERS
- Additional validations and sample analysis on Gamma-Cyhalothrin was done by method 1 using a conventional column. This equally sensitive method was used to screen for cyhalothrin isomers in the samples. In case of positive findings, the concerned samples would have been reanalysed by method 2, which applies a chiral column, thus allowing individual quantification of the gamma- isomer.
- After observing that the analysis of milk samples did not show any positive findings of triazole derivative metabolites, it was decided not to continue the analysis with infant formulae. Validations in infant formulae were also skipped. This was backed by information from EFSA reports indicating that TDMs only end up at very low trace levels in milk.
- As Thiocyanate and Ethoxyquin-Dimer were only analysed in samples of infant formula powder and not in milk, additional validations were only performed on infant formula powder

In the case of infant formulae, the lowest spiking levels for validation experiments were chosen to remain below the calculated maximum safe MRL, which depends on the ADI of each compound. Table 25 gives an overview of the LOW and HIGH spiking levels and their relationship to the highest acceptable MRL. This relationship is additionally illustrated in Figure 3.

Some additional comments concerning specific analytes are given below:

Diquat

Diquat produces several parent ions within the ion-source, each one fragmenting into various product ions. The most prominent parent ions observed are the doubly charged parent ($[M]^{2+}$; m/z 92), the singly charged protonated parent ($[M^{2+} - H^+]^+$; m/z 183) and the singly charged radical parent ($[M]^{+\bullet}$; m/z 184). The relative yields of the various parent ions were shown to greatly depend on the co-eluting matrix, which gives an additional dimension to the matrix-effects. Mass transitions from the same parent ion show a much more uniform response towards co-eluting matrix compared to mass transitions from different parents (see Table 18 and Table 38). Same applies to the mass transitions of the ILIS. For a proper equalization of the matrix effects and correct quantifications it is thus paramount that equivalent parent ions (or even better, equivalent mass-transitions) of native analyte and the corresponding ILIS are used. Table 18 shows exemplary matrix effects for various mass-transitions in infant formula powder while Table 19 gives an overview of mass transitions of diquat.

In 'normal' infant formulae extracts the signals derived from the $[M]^{2+}$ parent were not affected much by the presence of co-eluting matrix and even showed signal enhancement. In contrast, the signals derived from the other two parents $[M^{2+} - H^+]^+$ and $[M]^{+}$* were heavily suppressed (ME -90%). The mass transitions of diquat D_8 showed the same pattern. Table 19 gives an overview of mass transitions of diquat. For reducing errors, it is thus of foremost importance using equivalent mass transitions of native analyte and ILIS. Some MRMs of the radical parent ion ($[M]^{+\bullet}$ m/z 184) showed poorer repeatability. This will be studied further.

An important aspect to be considered when working with diquat, is its tendency to interact with surfaces in the injector. This behaviour leads to carry-over into subsequent runs. Injector contamination is more pronounced when injecting solutions in solvent, whereas the carry-over from a contaminated injector to the next run is more pronounced when injecting a matrix extract rather than a solvent. These observations indicate that matrix components compete with diquat reducing its interaction with the surfaces and displacing it from the interaction sites on the surface. Injection of solvent-based standards should be avoided.

Similar observations were made for Paraquat.

Fentin

As described in an EURL-SRM observation document[25], fentin tends to show low recoveries using citrate buffered QuEChERS. Nearly quantitative recoveries are obtained using the acidified FA-QuEChERS. Isotope labelled fentin helps to correct for these recovery losses. When using FA-QuEChERS a generic internal standard (e.g. propyzamide D_3) may be used.

[25] Analysis of Organotin-Pesticides by the QuEChERS Method - Impact of acidifying on the recoveries: **https://www.eurl-pesticides.eu/library/docs/srm/EURL_observations_Organotins.pdf**

This trend was confirmed in validation studies conducted in group b) to f) in infant food formula, see **Figure 5**.

Gamma-Cyhalothrin

Both Lambda and gamma cyhalothrin are approved within the EU. Lambda-cyhalothrin is a 1:1 mixture of two stereoisomers, one of them being gamma cyhalothrin, which is the toxicologically most toxic. Using traditional LC-columns the two constituent isomers of lambda cyhalothrin cannot be separated. For proper risk assessment the separation and separate quantification of the two isomers is, however, necessary. A method for the analysis of lambda- and gamma-cyhalothrin involving QuEChERS extraction and enantioselective LC-Separation of RS and SR-Isomers' was published in the website of the EURL-SRM[26].

Topramezone

Topramezone showed strong signal enhancement (~280 %) when injecting infant formula extract compared to the injection of an equally concentrated standard in pure solvent. Similar effects were also observed in other matrices. Whether these effects are due to losses in the injector in absence of matrix, needs to be further studied. At a later point of the study, extraction of Topramezone using A QuEChERS was also tested and validated successfully in infant formula powder using no internal standard. Sample analysis was therefore performed using A-QuEChERS extraction.

PTU

Validation in infant formula powder of group b) "lactose-free" was not successful. Peak intensities were at that level not sufficient. This is likely caused by stronger suppressions in this group of infant formula.

TFA, Chlorate, Perchlorate and Phosphonic acids

Validation in matrices of group b) to f) was complicated by residues in the blank material used for calibration leading to high intercept. In such cases, the slope of the curve was used for calculations.

[26] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_Observation_Cyhalothrin_V1.pdf

Table 25: Overview on ADI values of toxicologically critical SRM substances, required LOQs, spiking levels on powder and corresponding spiking level in reconstituted product (ready-to-use).

Analyte	Internal Standard	ADI (mg/kg body weight per day)	Required LOQ (mg/kg)	Spiking Level on Powder (mg/kg)	Spiking Level calculated on Reconst. product* (mg/kg)	Percentage of required LOQ
Abamectin	Propyzamide D_3	**0.0025**	**0.0096**	**0.05**	**0.0067**	**69%**
				0.25	**0.0333**	**347%**
Emamectin	Propyzamide D3	**0.005**	**0.0019**	**0.01**	**0.0013**	**7%**
				0.05	**0.0067**	**35%**
3-Hydroxy-carbofuran	Propyzamide D_3	**0.00015**	**0.0006**	**0.004**	**0.00053**	**92%**
				0.02	**0.0027**	**462%**
Gamma-Cyhalothrin	Chlorpyrifos D_{10}	**0.0012**	**0.0048**	**0.032**	**0.0043**	**92%**
Fentin	Fentin D_{15} or Propyzamide D_3	**0.0004**	**0.0015**	**0.01**	**0.0013**	**87%**
				0.05	**0.0067**	**433%**
Haloxyfop	Propyzamide D_3	**0.00065**	**0.0025**	**0.015**	**0.0020**	**80%**
				0.075	**0.0100**	**400%**
Diclofop (free acid)	Propyzamide D_3	**0.001**	**0.0038**	**0.025**	**0.0033**	**87%**
				0.125	**0.0167**	**433%**
Amitrole	Amitrole $^{15}N_2$ $^{13}C_2$	**0.001**	**0.0038**	**0.02**	**0.0027**	**69%**
				0.1	**0.0133**	**347%**
Nicotine	Nicotine D_4	**0.0008**	**0.0031**	**0.02**	**0.0027**	**87%**
				0.1	**0.0133**	**433%**
Cotinine	Cotinine D_3	**0.0008**	**0.0031**	**0.005**	**0.00067**	**22%**
				0.025	**0.0033**	**108%**
PTU	PTU D_6	**0.0003**	**0.0012**	**0.005**	**0.00067**	**58%**
				0.025	**0.0033**	**289%**
Diquat	Diquat D_8	**0.002**	**0.0076**	**0.05**	**0.0067**	**87%**
				0.25	**0.0333**	**433%**
Topramezone	*MPPA D_3***	**0.001**	**0.0038**	**0.005**	**0.00067**	**17%**
				0.025	**0.0033**	**87%**

**Calculated based on a conversion factor 7.5*

*** Please read important note on MPPA D3 under Table 20*

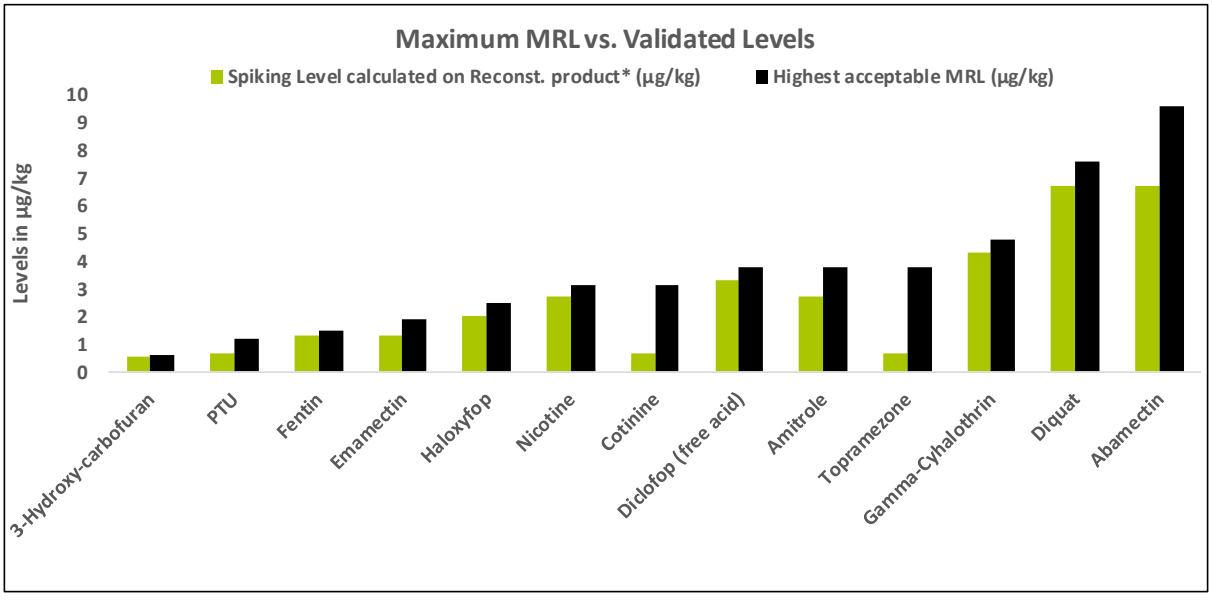


Figure 3: Levels of highest acceptable MRL in µg/kg in comparison to actually validated spiked levels calculated on reconstituted products in µg/kg.

5.1 Analytical Performance data for Infant formulae

*Table 26: QuEChERS AO and A-QuEChERS recovery data of toxicologically critical SRM substances **in 'normal' infant formula powder**. Matrix-matched calibration using internal standard **(n = 5)**.*

Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)[A]	Mean Rec. (%)	RSD (%)
Avermectin B1a (NH_4-Adduct was used as parent)	891/305 T	Propyzamide D_3	QuEChERS	2 g	**0.05 (0.0067)**	107	4.3
	891/567					110	7.1
	891/305 T				**0.25 (0.0333)**	105	6.4
	891/567					107	5.9
	891/305 T		A-QuEChERS	2 g	**0.05 (0.0067)**	96	13.6
	891/567					104	12.6
	891/305 T				**0.25 (0.0333)**	104	8.2
	891/567					109	11.8
Emamectin B1a	887/82 T	Propyzamide D_3	QuEChERS	2 g	**0.01 (0.0013)**	102	6.1
	887/158					104	5.5
	887/82 T				**0.05 (0.0067)**	103	4.1
	887/158					103	4.2
	887/82 T		A-QuEChERS	2 g	**0.01 (0.0013)**	104	5.6
	887/158					96	6.9
	887/82 T				**0.05 (0.0067)**	99	1.8
	887/158					105	3.7
3-Hydroxycarbofuran	238/163 T	Propyzamide D_3	QuEChERS	2 g	**0.004 (0.00053)**	100	4.2
	238/181					99	9.0
	238/220					93	14.1
	238/163 T				**0.02 (0.0027)**	98	1.9
	238/181					98	4.9
	238/220					104	6.1
	238/163 T		A-QuEChERS	2 g	**0.004 (0.00053)**	105	6.2
	238/181					105	10.6
	238/220					107	16.9
	238/163 T				**0.02 (0.0027)**	105	6.2
	238/181					100	4.6
	238/220					95	6.6
Gamma-Cyhalothrin	467/225 T	Chlorpyrifos D_{10}	QuEChERS	2 g	**0.032 (0.0043)**	99	1.7
	467/450					103	7.7
	467/225 T			2 g	**0.064 (0.0086)**	95	6.7
	467/450					93	7.6
Haloxyfop	360/288 T	Propyzamide D_3	QuEChERS	2 g	**0.015 (0.0020)**	103	3.1
	362/290					100	4.3
	360/196					109	3.3
	360/288 T				**0.075 (0.0100)**	98	2.5
	362/290					99	2.9
	360/196					103	4.0
	360/288 T		A-QuEChERS	2 g	**0.015 (0.0020)**	105	2.1
	362/290					107	2.8
	360/196					101	3.8
	360/288 T				**0.075 (0.0100)**	105	2.1
	362/290					106	4.3
	360/196					105	2.0

Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)[A]	Mean Rec. (%)	RSD (%)
Fentin	351/120 T	Fentin D_{15}	QuEChERS	2 g	**0.01 (0.0013)**	99	8.0
	351/197					95	3.1
	349/195					96	1.7
	351/120 T				**0.05 (0.0067)**	99	2.5
	351/197					98	1.6
	349/195					97	1.5
	351/120 T	Propyzamide D_3		2 g	**0.01 (0.0013)**	37	11.4
	351/197					36	5.0
	349/195					35	7.1
	351/120 T				**0.05 (0.0067)**	43	12.2
	351/197					41	15.0
	349/195					42	13.4
	351/120 T	Fentin D_{15}	A-QuEChERS	2 g	**0.01 (0.0013)**	102	3.3
	351/197					100	1.3
	349/195					104	3.0
	351/120 T				**0.05 (0.0067)**	103	1.0
	351/197					102	0.9
	349/195					103	1.9
	351/120 T	Propyzamide D_3		2 g	**0.01 (0.0013)**	102	3.5
	351/197					100	3.1
	349/195					103	4.4
	351/120 T				**0.05 (0.0067)**	100	2.2
	351/197					99	2.2
	349/195					100	1.6
Diclofop (free acid)	325/253 T	Propyzamide D_3	QuEChERS	2 g	**0.025 (0.0033)**	106	7.5
	325/255					99	5.2
	325/145					100	4.3
	325/253 T				**0.125 (0.0167)**	99	7.2
	325/255					101	2.4
	325/145					104	5.6
	325/253 T		A-QuEChERS	2 g	**0.025 (0.0033)**	106	4.3
	325/255					104	2.7
	325/145					103	6.7
	325/253 T				**0.125 (0.0167)**	108	3.3
	325/255					105	3.8
	325/145					107	1.5
Topramezone	362/334 T	-	A-QuEChERS	2g	**0.005* (0.00067)**	101	6.2
	362/318					76	10.5
	362/194					88	5.1

[A] Spiking level calculated on reconstituted product based on a conversion factor 7.5 in mg/kg in parentheses.
* validated in category b) lactose-free

*Table 27: QuPPe-AO recovery data of toxicologically critical SRM substances in **'normal' infant formula powder**. Matrix-matched calibration using internal standard **(n = 5)**.*

Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)[A]	Mean Rec. (%)	RSD (%)
Amitrole	85/43 T	Amitrole $^{15}N_2$ $^{13}C_2$	QuPPe AO	2 g	**0.02 (0.0027)**	99	3.7
	85/58					105	5.7
	85/57					106	5.2
	85/43 T				**0.1 (0.0133)**	104	2.1
	85/58					101	2.3
	85/57					96	5.7
Nicotine	163/130 T	Nicotine D_4	QuPPe AO	2 g	**0.02 (0.0027)**	99	13.8
	163/132					97	15.6
	163/84					102	13.4
	163/130 T				**0.1 (0.0133)**	119	15.9
	163/132					117	17.6
	163/84					118	15.2
Cotinine	177/80	Cotinine D_3	QuPPe AO	2 g	**0.005 (0.00067)**	98	5.7
	177/98					96	4.0
	177/80				**0.025 (0.0033)**	102	4.7
	177/98					101	4.6
PTU	117/60 T	PTU D_6	QuPPe AO	2 g	**0.005 (0.00067)**	101	3.8
	117/58					117	10.8
	117/72					126	25.1
	117/60 T				**0.025 (0.0033)**	103	4.6
	117/58					116	6.7
	117/72					116	16.7
Diquat	92/84	Diquat D_8 *	QuPPe AO	2 g	**0.05 (0.0067)**	109	16.4
	183/157					91	10.7
	92/157					90	13.5
	92/84				**0.25 (0.033)**	98	3.9
	183/157					96	5.2
	92/157					100	4.1
Topramezone	362/334 T	MPPA D_3**	QuPPe AO	2 g	**0.005 (0.00067)**	89	9.5
	362/318					95	4.5
	362/194					92	3.0
	362/334 T				**0.025 (0.0033)**	86	8.5
	362/318					90	6.8
	362/194					85	5.6

[A] Spiking level calculated on reconstituted product based on a conversion factor 7.5 in mg/kg in parentheses.

* It is important to use an MRM of the ILIS that corresponds to that of the native substance (equivalent parent ion)

** Please read important note on MPPA D3 under **Table 20**

Table 28: Detailed validation results in infant food formula for individual transitions of Diquat using the corresponding parent masses of ILIS.

Transition of Diquat native substance	MRM	Transition of Diquat D_8 ILIS	Extraction method	Sample Weight	Spiking Level (mg/kg)[A]	Mean Rec. (%)	RSD (%)
Diquat $[M]^{2+}$	92/84	Diquat D_8 $[M]^{2+}$ 96/88	QuPPe AO	2 g	**0.05 (0.0067)**	109	16.4
	92/157					90	13.5
	92/78					- [1]	- [1]
	92/130					- [1]	- [1]
Diquat $[M^{2+} - H^+]^+$	183/157	Diquat D_8 $[M^{2+} - H^+]^+$ 191/165				91	10.7
	183/130					- [1]	- [1]
	183/168					- [1]	- [1]
	183/78					- [1]	- [1]
Diquat $[M]^{+\bullet}$	184/128	Diquat D_8 $[M]^{+\bullet}$ 192/134				87	13.6
	184/106					92	8.9
	184/78					- [1]	- [1]
	184/156					- [1]	- [1]
	184/169					- [1]	- [1]
	184/155					109	20.0
	184/168					109	7.1
Diquat $[M]^{2+}$	92/84	Diquat D_8 $[M]^{2+}$ 96/88	QuPPe AO	2 g	**0.25 (0.0333)**	98	3.9
	92/157					100	4.1
	92/78					109	14.3
	92/130					91	3.9
Diquat $[M^{2+} - H^+]^+$	183/157	Diquat D_8 $[M^{2+} - H^+]^+$ 191/165				96	5.2
	183/130					101	6.6
	183/168					*103* [2]	*8.9* [2]
	183/78					92	12.1
Diquat $[M]^{+\bullet}$	184/128	Diquat D_8 $[M]^{+\bullet}$ 192/134				92	9.8
	184/106					109	6.7
	184/78					98	4.6
	184/156					102	9.5
	184/169					94	5.7
	184/155					98	12.9
	184/168					88	11.1

[A] Spiking level calculated on reconstituted product based on a conversion factor 7.5 in mg/kg in parentheses.
1) Poor peak intensity affecting repeatability and accuracy
2) Poor Linearity of Calibration

*Table 29: QuPPe-AO recovery data of additionally analysed substances in **'normal' infant formula powder**. Matrix-matched calibration using internal standard **(n = 5)**.*

Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)[A]	Mean Rec. (%)	RSD (%)
Paraquat	93/171	Paraquat D_8	QuPPe AO	2 g	**0.05 (0.0067)**	103	15.5
	186/171					101	12.1
	93/77					97	**24.5**
	93/171				**0.25 (0.0333)**	103	4.9
	186/171					95	4.1
	93/77					108	6.6
Trifluoracetic acid	113/69	Trifluoro acetic acid $^{13}C_2$	QuPPe AO	2 g	**0.05* (0.0067)**	98	6.3
	113/113					118	9.8
Triazole acetic acid	128/70	1,2,4-Triazole acetic acid $^{13}C_2$ ^{15}N	QuPPe AO	2 g	**0.05 (0.0067)**	100	8.6
	128/73					76	7.0
	128/43					98	12.2
	128/70				**0.1 (0.0133)**	100	2.0
	128/73					83	8.4
	128/43					102	7.4
Triazole lactic acid	158/70	1,2,4-Triazole lactic acid $^{13}C_2$ ^{15}N	QuPPe AO	2 g	**0.02 (0.0027)**	110	4.3
	158/43					94	14.4
	158/112					81	**38.7**
	158/70				**0.04 (0.0054)**	105	4.0
	158/43					106	15.5
	158/112					99	18.1
Triazole alanine	157/70	1,2,4-Triazole-1yl-alanine $^{13}C_2$ ^{15}N	QuPPe AO	2 g	**0.05 (0.0067)**	94	14.0
	157/88					91	**20.9**
	157/42					103	16.3
Melamine	127/85	Melamine $^{15}N_3$	QuPPe AO	2 g	**0.02* (0.0027)**	**124**	4.0
	127/68					**123**	6.4
Chlorate	83/67	Chlorate $^{18}O_3$	QuPPe AO	2 g	**0.02* (0.0027)**	107	8.7
	85/69					119	13.5
Perchlorate	99/83	Perchlorate $^{18}O_4$	QuPPe AO	2 g	**0.02* (0.0027)**	110	5.5
	101/85					100	14.1
Phosphonic acid	81/79	Phosphonic acid $^{18}O_3{}^{18}O_3$	QuPPe AO	2 g	**0.05* (0.0067)**	102	15.7
	81/63					103	18.2
Thiocyanate	58/58	Thiocyanate ^{13}C ^{15}N	QuPPe AO	2 g	**0.5** (0.067)**	98	12.2
Ethoxyquin-Dimer	433/216	Propyzamide D_3	A-QuEChERS	2g	**0.005** (0.00067)**	96	5.8
	433/188					90	11.7
	433/375					91	6.0

[A] Spiking level calculated on reconstituted product based on a conversion factor 7.5 in mg/kg in parentheses.

**validation on category b) lactose-free*

*** validation on category c) hypoallergenic*

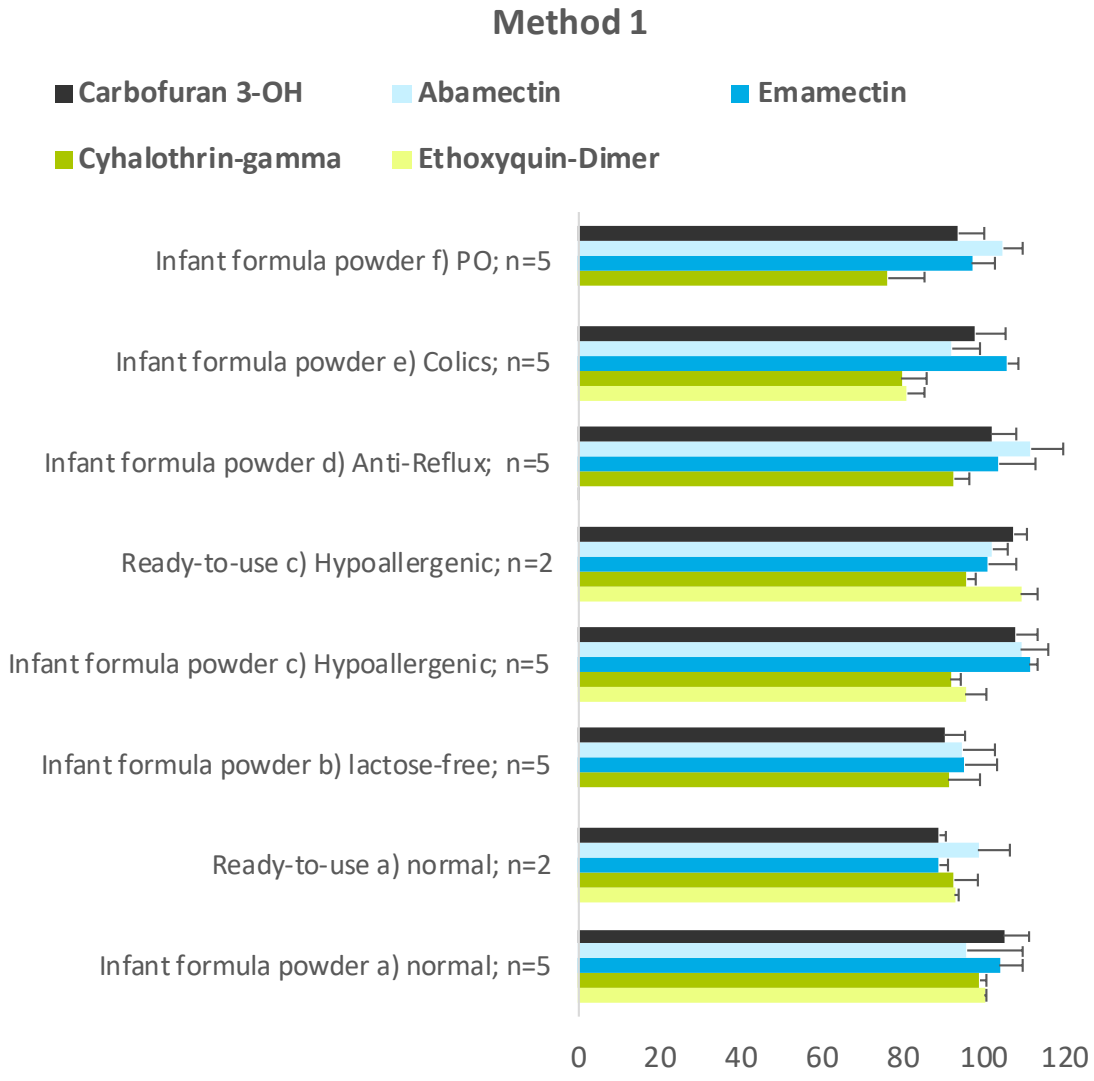


Figure 4: *Average Recovery rates of target transitions in % in category a) to f) in infant formula powder (IFP) respectively ready-to-use products (IFRTU) of analytes covered by method 1 (n=2 or n=5, see labelling on the left of the diagram). Cyhalothrin-gamma was analysed on the C_{18} column that does not result in a chiral separation. Spiking Levels: Carbofuran-3-OH: 0.004 mg/kg in IFP and 0.0008 mg/kg in IFRTU; Abamectin: 0.05 mg/kg in IFP and 0.01 mg/kg in IFRTU; Emamectin: 0.01mg/kg in IFP and 0.002 mg/kg in IFRTU; Cyhalothrin-gamma: 0.032 mg/kg in IFP and 0.0064 mg/kg in IFRTU; Ethoxyquin-Dimer: 0.005mg/kg.*

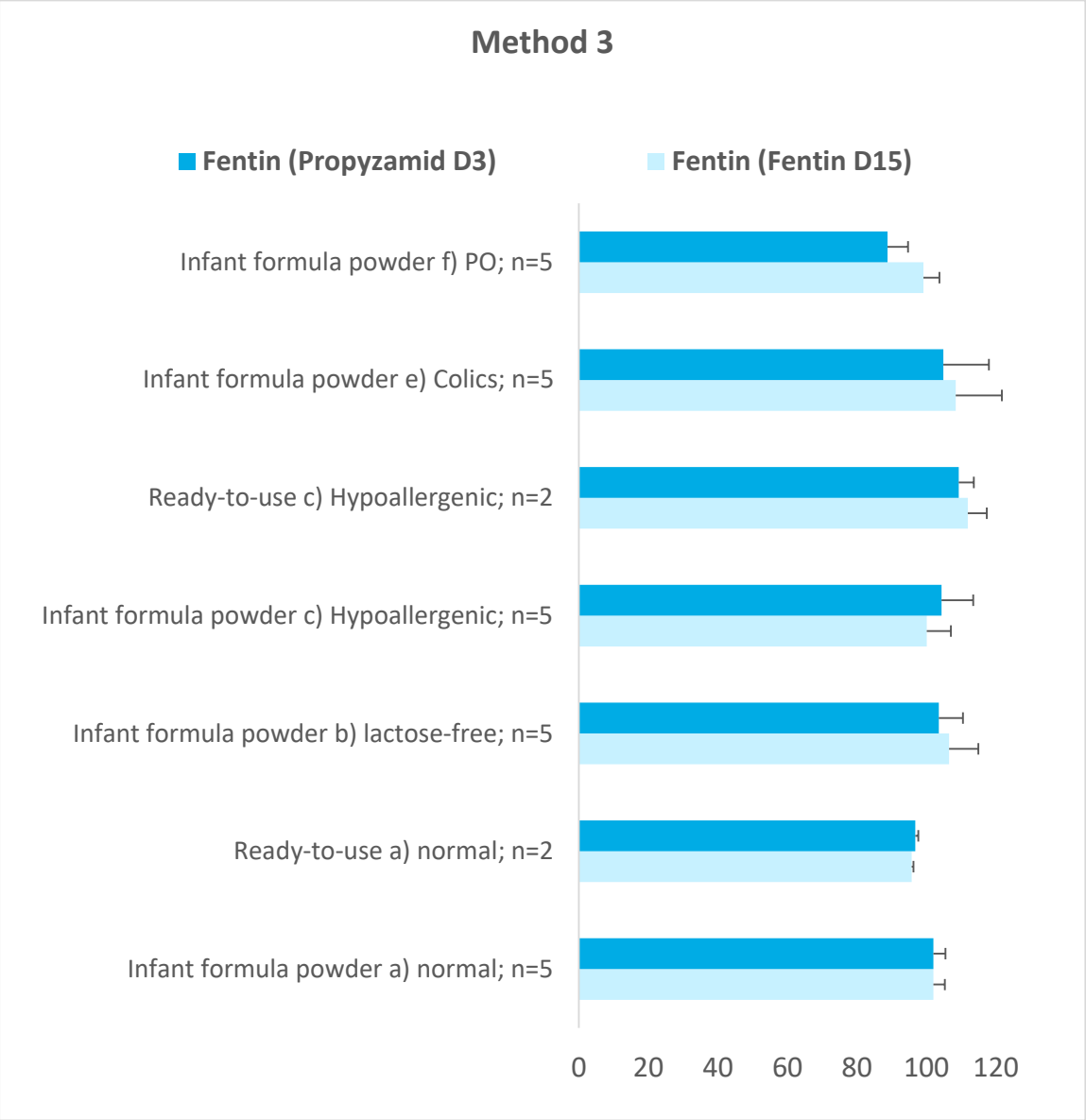


Figure 5: *Average Recovery rates of target transitions in % in category a) to f) in infant formula powder (IFP) respectively ready-to-use products (IFRTU) of analytes covered by method 3 (n=2 or n=5, see labelling on the left of the diagram) and comparison of results using Fentin D_{15} or Propyzamide D_3 ILIS for the determination of Fentin. Spiking Levels: Fentin: 0.01 mg/kg in IFP and 0.002 mg/kg in IFRTU.*

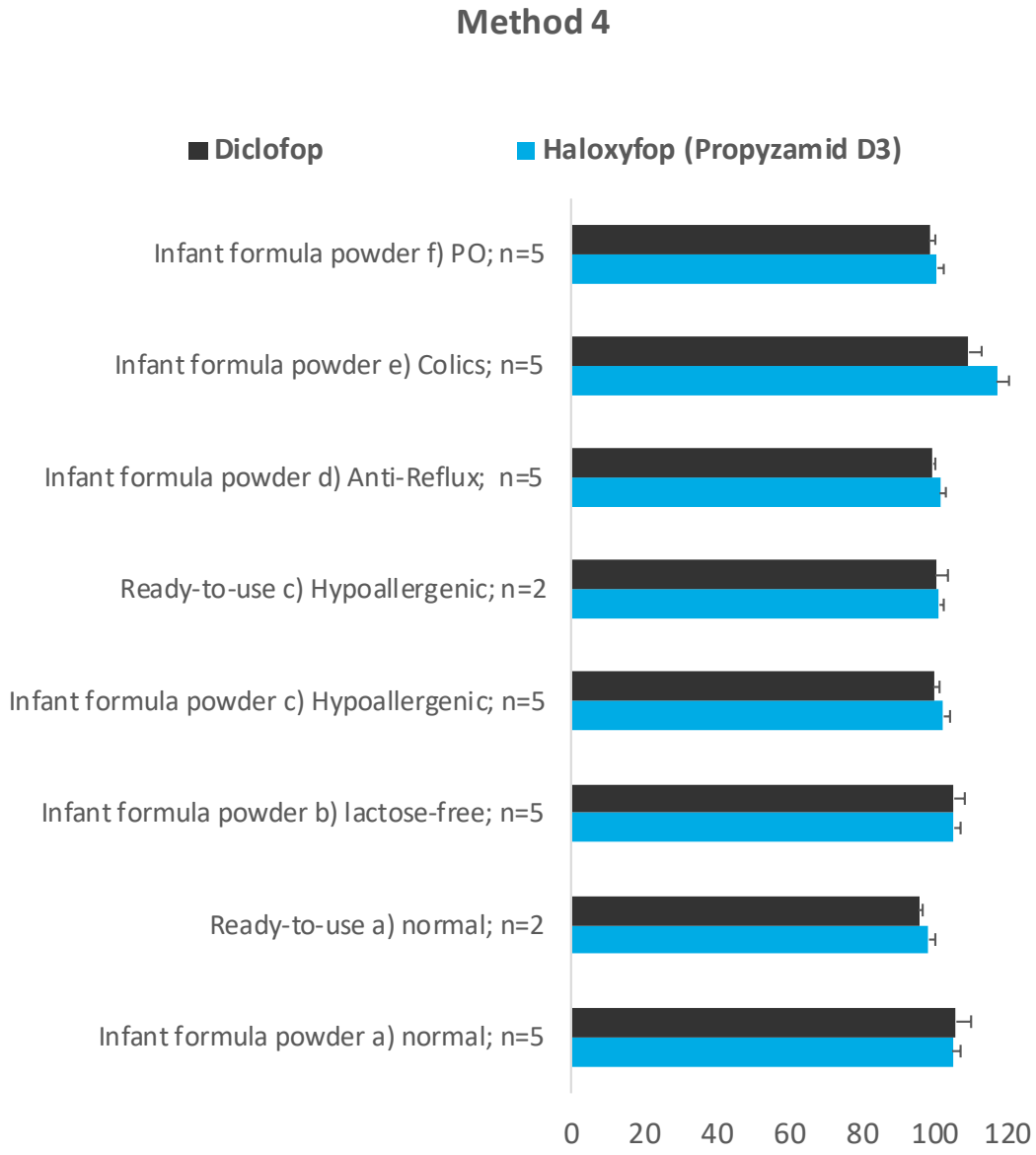


Figure 6: *Average Recovery rates of target transitions in % in category a) to f) in infant formula powder (IFP) respectively ready-to-use products (IFRTU) of analytes covered by method 4 (n=2 or n=5, see labelling on the left of the diagram). Spiking Levels: Haloxyfop: 0.015 mg/kg in IFP and 0.003 mg/kg in IFRTU; Diclofop: 0.025 mg/kg in IFP and 0.005 mg/kg in IFRTU.*

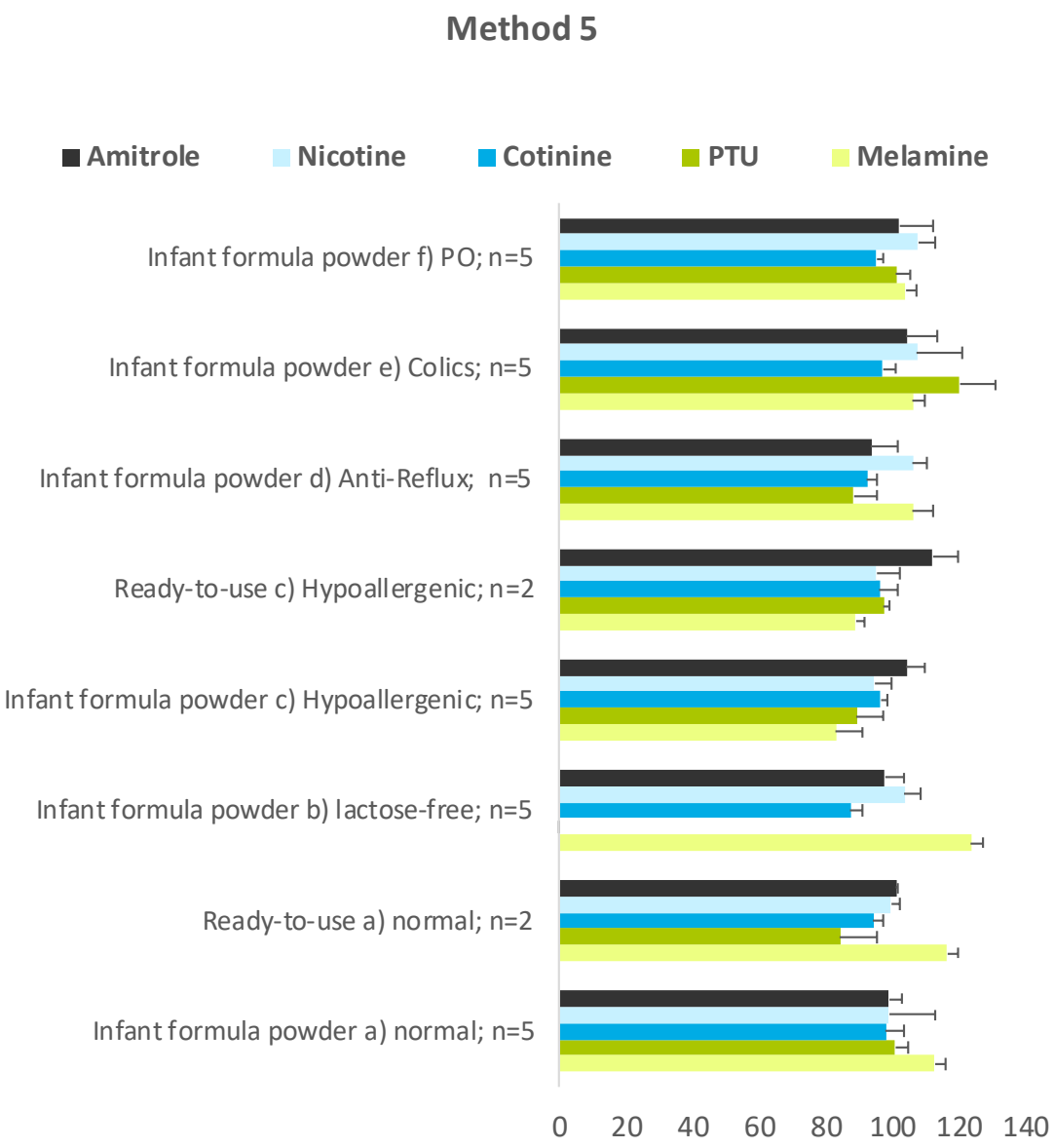


Figure 7: *Average Recovery rates of target transitions in % in category a) to f) in infant formula powder (IFP) respectively ready-to-use products (IFRTU) of analytes covered by method 5 (n=2 or n=5, see labelling on the left of the diagram). Spiking Levels: Amitrole: 0.02 mg/kg in IFP and 0.004 mg/kg in IFRTU; Nicotine: 0.02 mg/kg in IFP and 0.004 mg/kg in IFRTU; Cotinine: 0.005 mg/kg in IFP and 0.001 mg/kg in IFRTU; PTU: 0.005 mg/kg in IFP and 0.001 mg/kg in IFRTU; Melamine: 0.02 mg/kg in IFP and 0.004 mg/kg in IFRTU.*

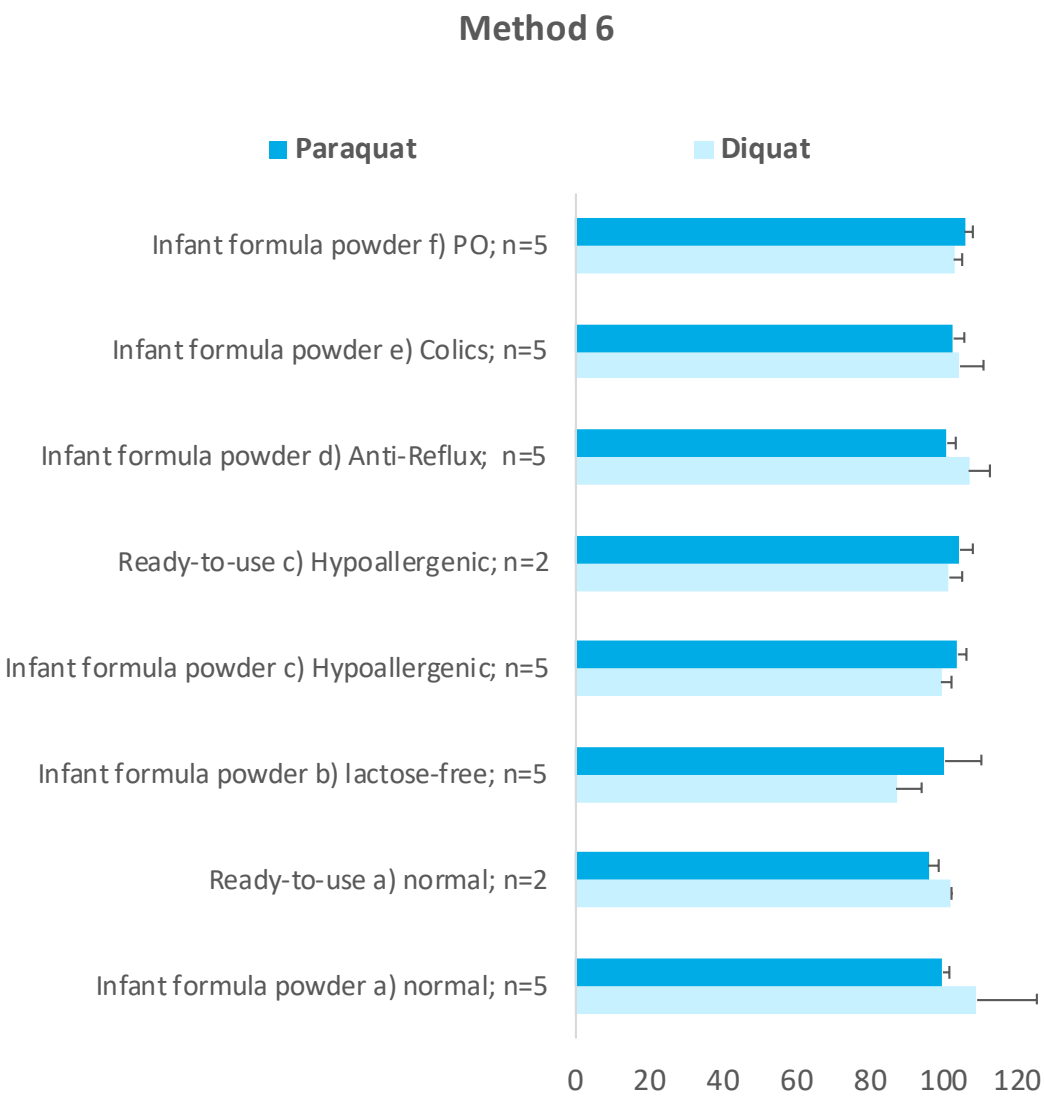


***Figure 8**: Average Recovery rates of target transitions in % in category a) to f) in infant formula powder (IFP) respectively ready-to-use products (IFRTU) of analytes covered by method 6 (n=2 or n=5, see labelling on the left of the diagram). Spiking Levels: Diquat: 0.05 mg/kg in IFP and 0.01 mg/kg in IFRTU; Paraquat: 0.05 mg/kg in IFP and 0.01 mg/kg in IFRTU.*

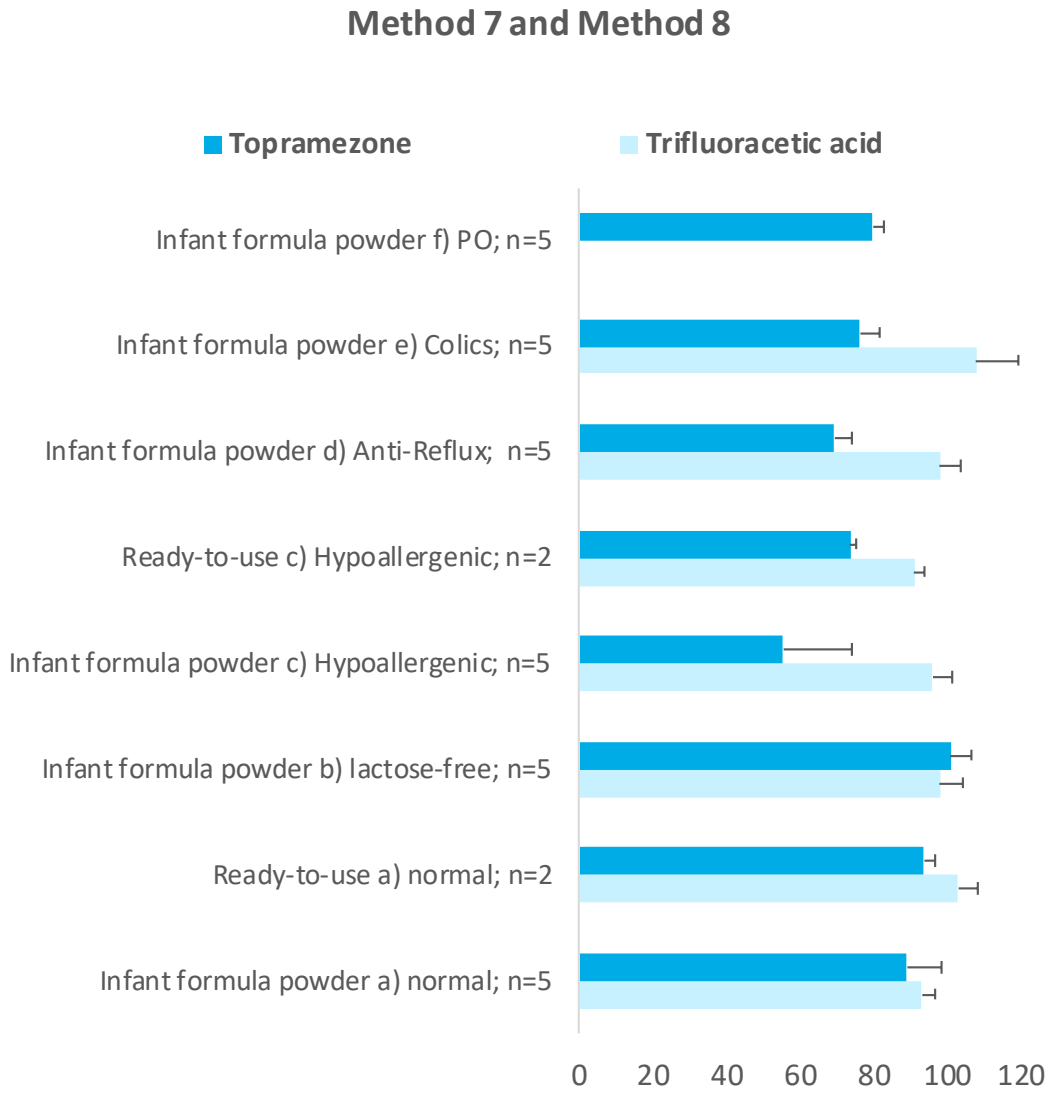


***Figure 9**: Average Recovery rates of target transitions in % in category a) to f) in infant formula powder (IFP) respectively ready-to-use products (IFRTU) of analytes covered by method 7 and 8 (n=2 or n=5, see labelling on the left of the diagram). Spiking Levels: Topramezone: 0.005 mg/kg in IFP and 0.001 mg/kg in IFRTU; Trifluoracetic acid: 0.05 mg/kg in IFP and 0.01 mg/kg in IFRTU.*

Table 30: Average Recovery rates of target transitions in % in category a) to f) in infant formula powder (IFP) respectively ready-to-use products (IFRTU) of analytes covered by method 9 (n=2 or n=5).

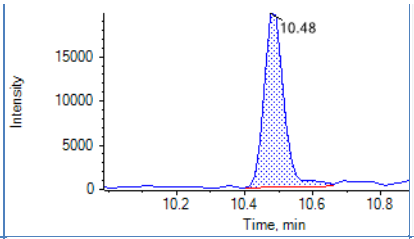
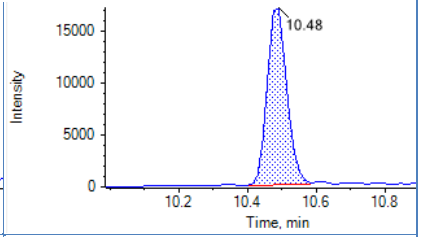
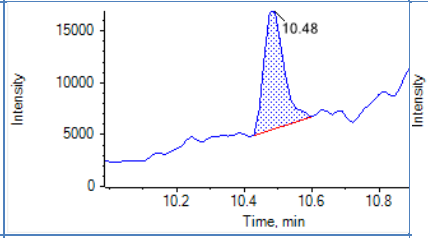
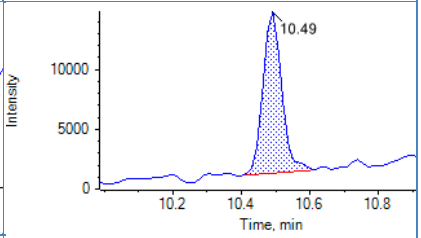
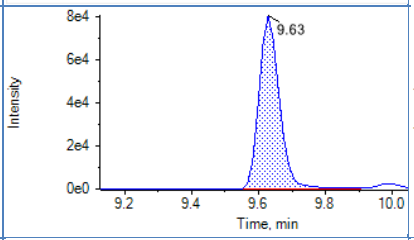
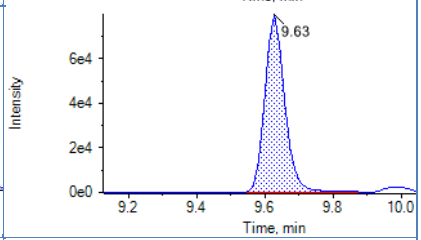
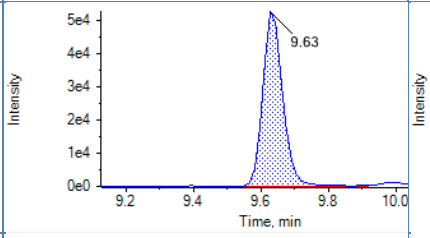
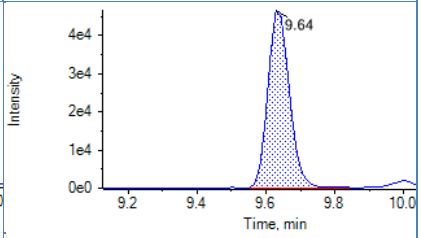
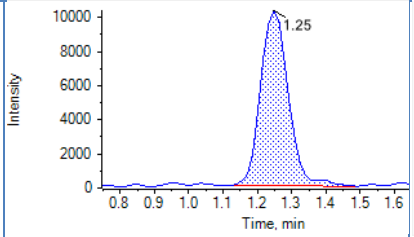
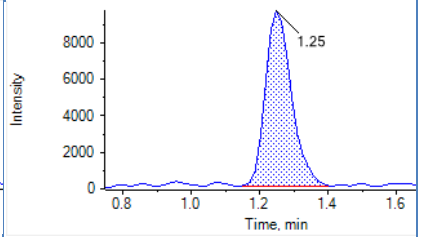
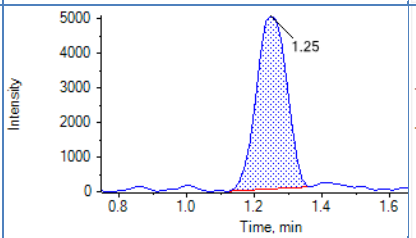
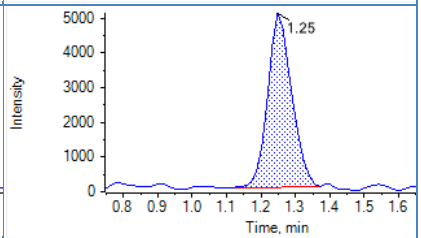
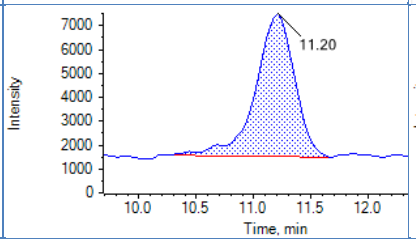
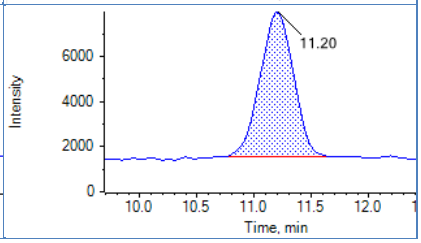
Category	Details**	n	Chlorate	Perchlorate	Phosphonic acid
a) Normal IFP	Spiking Level in mg/kg	n=5	0.02	0.02	0.05
	Recovery in %		132	109	*
	RSD in %		3.6	4.3	
a) Normal IFRTU	Spiking Level in mg/kg	n=2	0.004	0,004	0.01
	Recovery in %		124	*	114
	RSD in %		3.9		19.2
b) Lactose-free IFP	Spiking Level in mg/kg	n=5	0.02	0.02	0.05
	Recovery in %		107	110	102
	RSD in %		8.7	5.5	15.7
c) Hypoallergenic IFP	Spiking Level in mg/kg	n=5	0.02	0.02	0.05
	Recovery in %		84	110	*
	RSD in %		12.8	12.5	
c) Hypoallergenic IFRTU	Spiking Level in mg/kg	n=2	0.004	0.004	0.01
	Recovery in %		88	115	*
	RSD in %		8.9	3.1	
d) Anti-reflux IFP	Spiking Level in mg/kg	n=5	0.02	0.02	0.05
	Recovery in %		111	126	126
	RSD in %		21.4	9.2	12.8
e) Anti-colic IFP	Spiking Level in mg/kg	n=5	0,02	0.02	0.05
	Recovery in %		*	93	97
	RSD in %			9.1	10.2
f) Plant-based IFP	Spiking Level in mg/kg	n=5	0,02	0.02	0.05
	Recovery in %		*	99	*
	RSD in %			9.0	

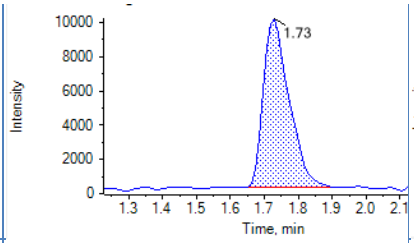
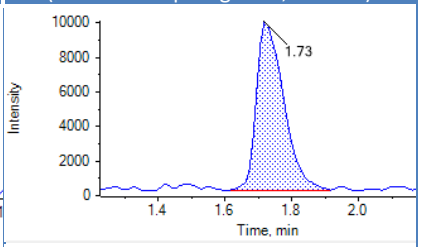
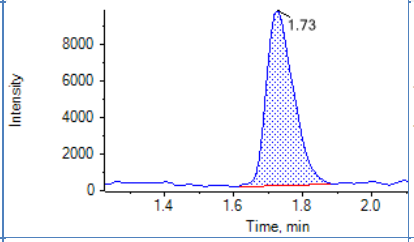
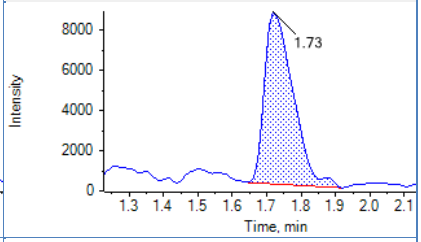
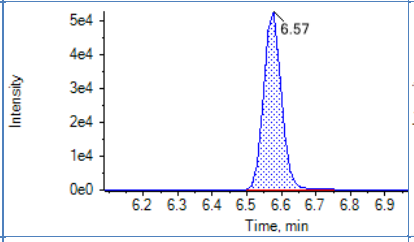
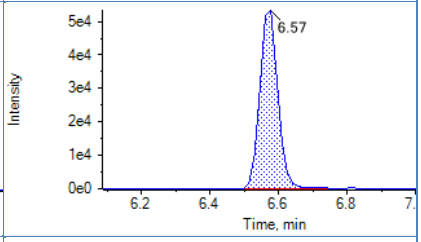
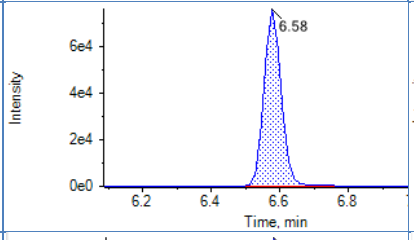
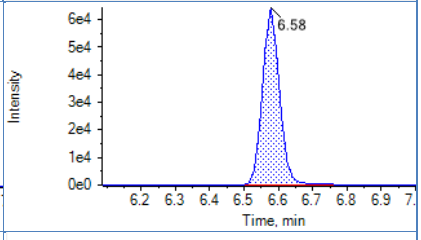
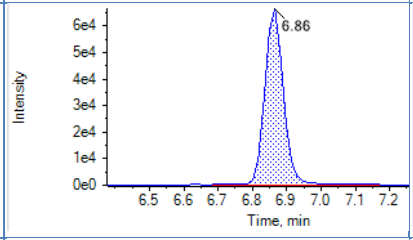
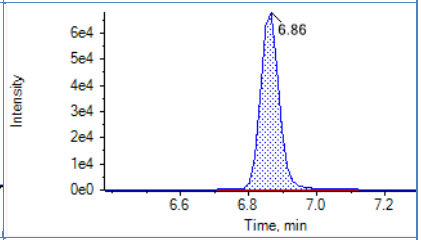
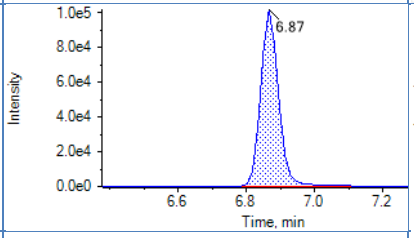
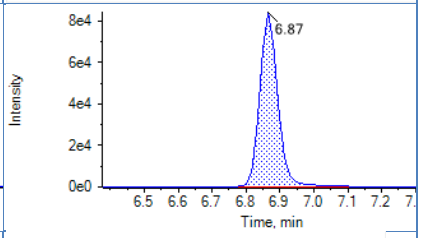
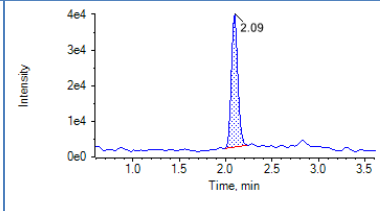
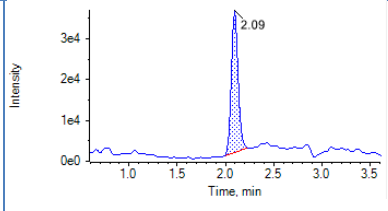
* not validated because of too high residue levels in blank matrix

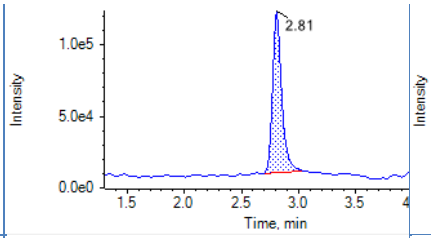
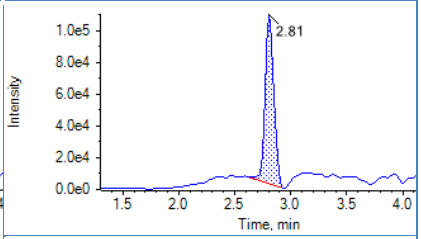
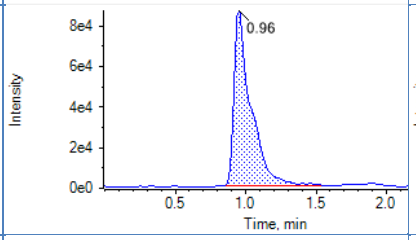
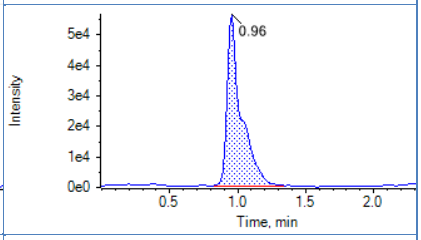
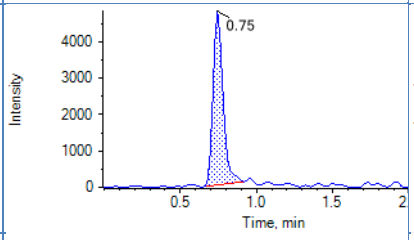
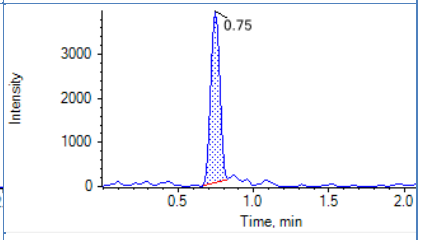
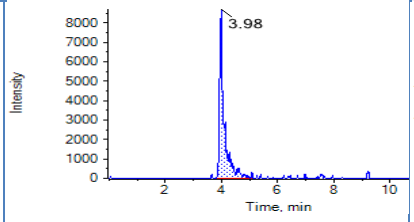
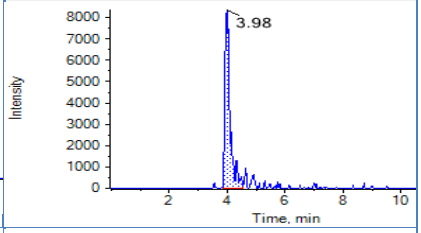
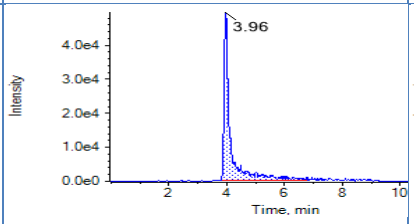
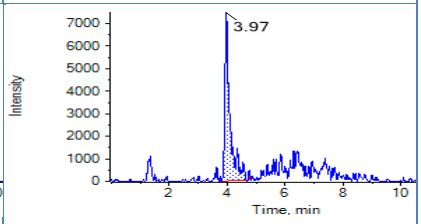
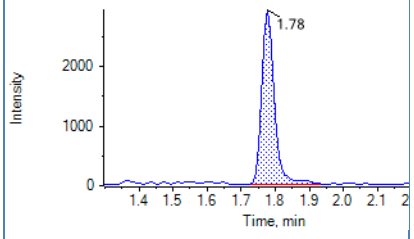
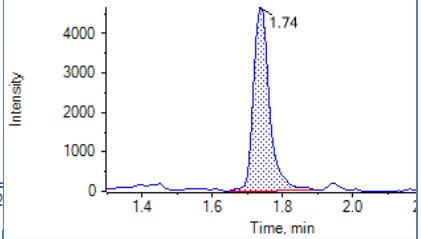
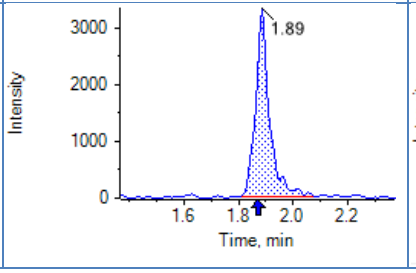
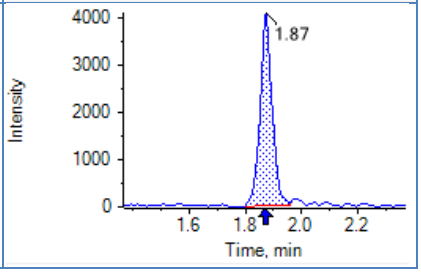
** spiking levels refer to the product listed under “Category”

IFP: Infant formula powder; IFIFRTU: Infant formula ready to use

Table 31: *Exemplary chromatograms of target analytes in infant food formula derived from injecting solvent-based and matrix-matched calibration standards at 120% of the respective lowest spiking level in validation experiments using QuEChERS, A-QuEChERS and QuPPe AO.*

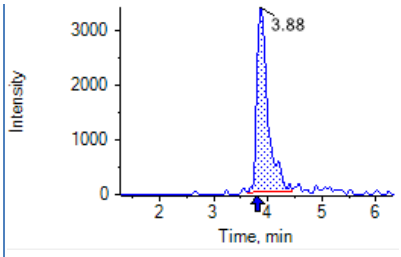
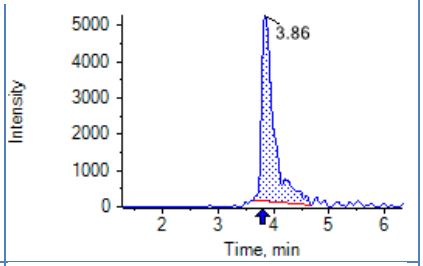
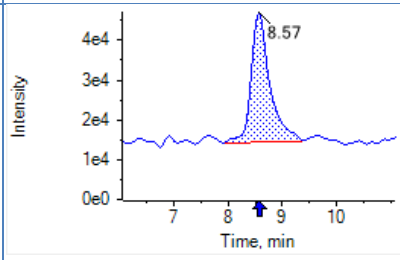
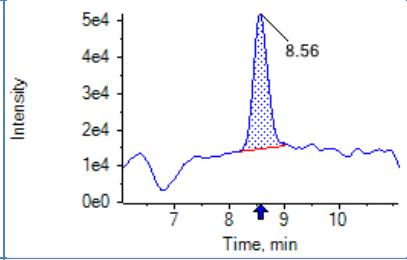
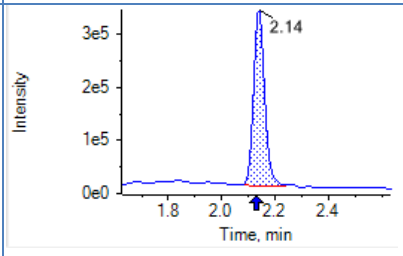
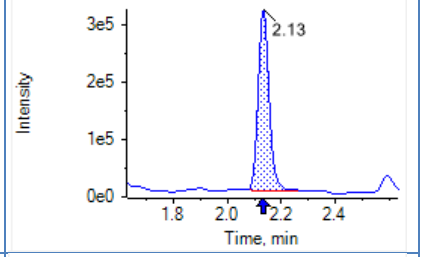
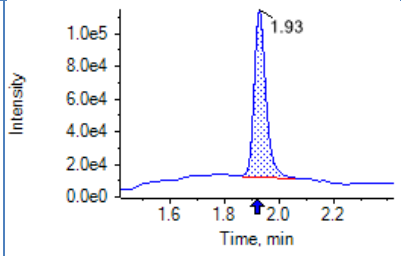
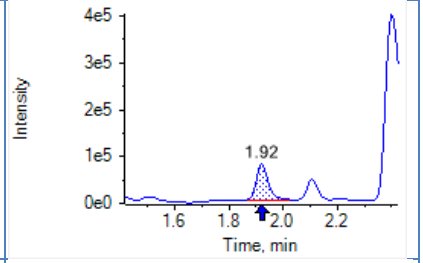
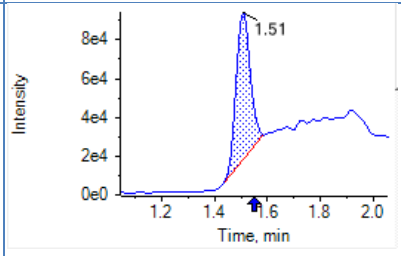
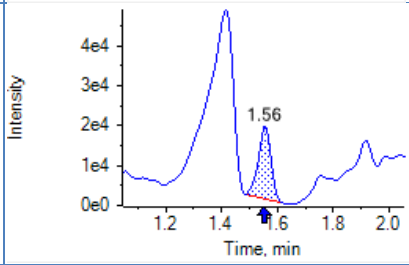
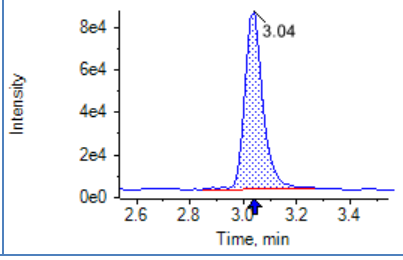
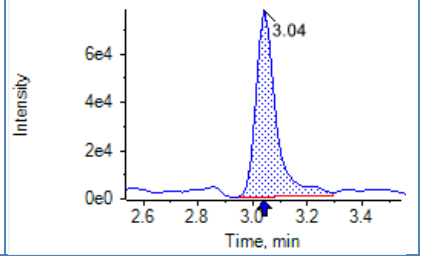
Analyte Transition	Spiking Level on powder (mg/kg)[A]	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in infant formula powder of category a) (at 120% of spiking level, see left)
Avermectin B1a 891/305 [M+NH$_4$]$^+$	0.05 (0.0067) - **QuEChERS**		
	0.05 (0.0067) - **A-QuEChERS**		
Emamectin B1a 887/82	0.01 (0.0013) - **QuEChERS**		
	0.01 (0.0013) - **A-QuEChERS**		
3-Hydroxy-carbofuran 238/163	0.004 (0.00053) - **QuEChERS**		
	0.004 (0.00053) - **A-QuEChERS**		
Gamma-cyhalothrin 467/225	0.032 (0.0043) - **QuEChERS**		

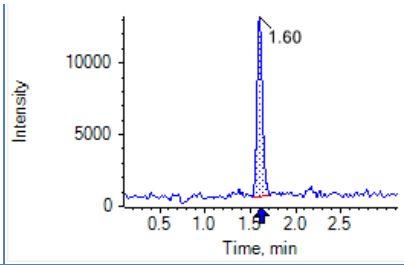
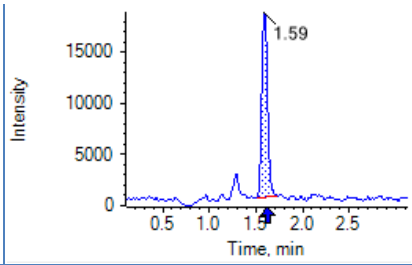
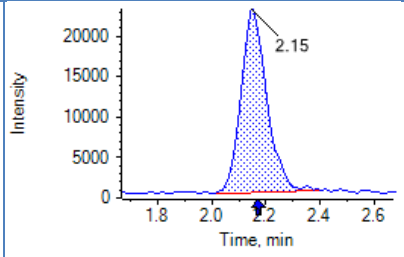
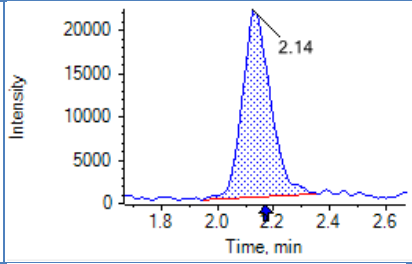
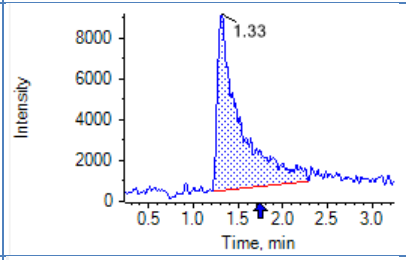
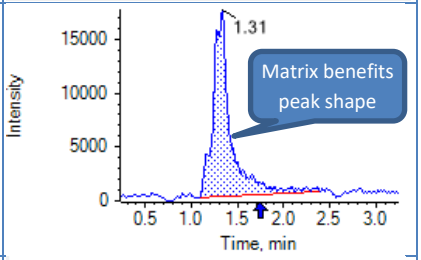
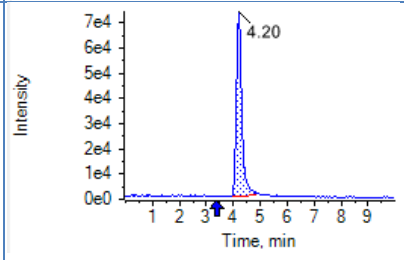
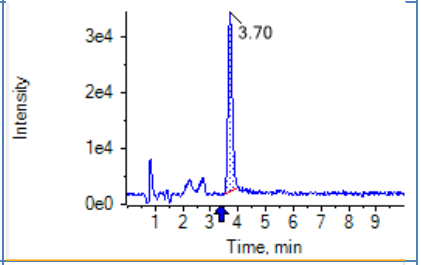
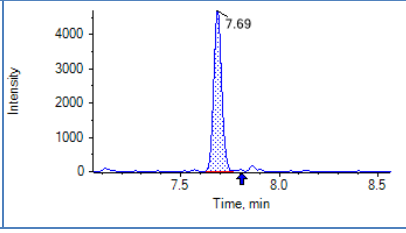
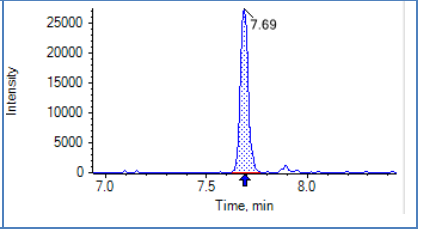
Analyte Transition	Spiking Level on powder (mg/kg)[A]	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in infant formula powder of category a) (at 120% of spiking level, see left)
Fentin 351/120	0.01 (0.0013) - **QuEChERS**		
	0.01 (0.0013) - **A-QuEChERS**		
Haloxyfop 360/288	0.015 (0.0020) - **QuEChERS**		
	0.015 (0.0020) - **A-QuEChERS**		
Diclofop 325/253	0.025 (0.0033) - **QuEChERS**		
	0.025 (0.0033) - **A-QuEChERS**		
Amitrole 85/43	0.02 (0.0027) - **QuPPe AO**		

Analyte Transition	Spiking Level on powder (mg/kg)[A]	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in infant formula powder of category a) (at 120% of spiking level, see left)
Nicotine 163/130	0.02 (0.0027) - **QuPPe AO**		
Cotinine 177/80	0.005 (0.00067) - **QuPPe AO**		
PTU 117/60	0.005 (0.00067) - **QuPPe AO**		
Diquat 92/84 $[M]^{2+}$	0.05 (0.0067) - **QuPPe AO**		
Diquat 183/157 $[M^{2+} - H^{+}]^{+}$	0.05 (0.0067) - **QuPPe AO**		
Topramezone 362/334	0.005 (0.00067) - **QuPPe AO**		
Topramezone 362/334*	0.005 (0.00067) - **A-QuChERS**		

[A] Spiking level calculated on reconstituted product based on a conversion factor 7.5 in mg/kg in parentheses.
*in category b) lactose-free

Table 32: Exemplary chromatograms of target analytes of additionally analyzed compounds in infant food formula derived from injecting solvent-based and matrix-matched calibration standards at 120% of the respective lowest spiking level in validation experiments using QuPPe AO.

Analyte Transition	Spiking Level on powder (mg/kg)[A]	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in infant formula powder of category a) (at 120% of spiking level, see left)
Paraquat 93/171 [M^{2+}]	0.05 (0.0067) - **QuPPe AO**		
Trifluoracetic acid 113/69	0.05 (0.0067) - **QuPPe AO**		
Triazole acetic acid 128/70	0.05 (0.0067) - **QuPPe AO**		
Triazole lactic acid 158/70	0.02 (0.0027) - **QuPPe AO**		
Triazole alanine 157/70	0.05 (0.0067) - **QuPPe AO**		
Melamine 127/85*	0.02 (0.0027) - **QuPPe AO**		

Analyte Transition	Spiking Level on powder (mg/kg)[A]	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in infant formula powder of category a) (at 120% of spiking level, see left)
Chlorate 83/67*	0.02 (0.0027) - **QuPPe AO**		
Perchlorate 99/83*	0.02 (0.0027) - **QuPPe AO**		
Phosphonic acid 81/79*	0.05 (0.0067) - **QuPPe AO**		
Thiocyanate 58/58**	0.5 (0.067) - **QuPPe AO**		
Ethoxyquin-Dimer 433/216**	0.005 (0.00067) - **A-QuEChERS**		

[A] Spiking level calculated on reconstituted product based on a conversion factor 7.5 in mg/kg in parentheses.

* In category b) lactose-free

** In category c) hypoallergenic; peak in solvent at 0.1 µg/mL

5.2 Milk

Table 33: *QuEChERS AO and A-QuEChERS recovery data of toxicologically critical SRM substances in* ***whole cow's milk****. Matrix-matched calibration using internal standard* ***(n = 5)****.*

Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)	Mean Rec. (%)	RSD (%)
Avermectin B1a (NH_4-Adduct was used as parent)	891/305 T	Propyzamide D_3	QuEChERS	10g	**0.002**	101	8.8
	891/567					92	17.2
	891/305 T				**0.005**	108	9.4
	891/567					100	14.8
	891/305 T		A-QuEChERS	10g	**0.002**	110	6.5
	891/567					94	8.0
	891/305 T				**0.005**	98	3.2
	891/567					105	4.6
Emamectin B1a	887/82 T	Propyzamide D_3	QuEChERS	10g	**0.002**	103	3.0
	887/158					103	4.6
	887/82 T				**0.005**	109	4.4
	887/158					112	4.5
	887/82 T		A-QuEChERS	10g	**0.002**	109	2.6
	887/158					101	3.5
	887/82 T				**0.005**	106	2.8
	887/158					107	3.3
3-Hydroxycarbofuran	238/163 T	Propyzamide D_3	QuEChERS	10g	**0.002**	96	3.8
	238/181					99	2.5
	238/220					102	5.3
	238/163 T				**0.005**	103	6.9
	238/181					104	5.2
	238/220					104	4.8
	238/163 T		A-QuEChERS	10g	**0.002**	104	3.9
	238/181					109	4.7
	238/220					104	4.8
	238/163 T				**0.005**	100	3.2
	238/181					103	4.6
	238/220					106	3.1
Haloxyfop	360/288 T	Propyzamide D_3	QuEChERS	10g	**0.002**	106	1.6
	362/290					104	3.0
	360/196					111	4.4
	360/288 T				**0.005**	107	0.9
	362/290					107	3.7
	360/196					108	5.9
	360/288 T				**0.002**	113	8.1
	362/290					114	5.4
	360/196					111	4.9
	360/288 T				**0.005**	105	1.9
	362/290					107	2.6
	360/196					109	5.6

Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)	Mean Rec. (%)	RSD (%)
Fentin	351/120 T	Propyzamide D_3	A-QuEChERS	10g	**0.002**	92	5.9
	349/195					92	2.8
	351/197					89	4.8
	351/120 T				**0.005**	95	3.4
	349/195					91	2.4
	351/197					89	1.6
	351/120 T				**0.002**	110	2.4
	349/195					103	1.4
	351/197					104	6.3
	351/120 T				**0.005**	103	3.9
	349/195					102	1.8
	351/197					99	2.6
Diclofop (free acid)	325/253 T	Propyzamide D_3	QuEChERS	10g	**0.002**	106	2.7
	325/255					105	7.3
	325/145					101	8.9
	325/253 T				**0.005**	102	6.1
	325/255					101	1.8
	325/145					101	4.0
	325/253 T		A-QuEChERS	10g	**0.002**	104	4.6
	325/255					106	6.0
	325/145					105	4.0
	325/253 T				**0.005**	107	3.6
	325/255					106	2.6
	325/145					111	7.3
Topramezone	362/334 T	-	QuEChERS	10g	**0.002**	46	22.5
	362/318					42	33.3
	362/194					47	21.8
	362/334 T				**0.005**	40	33.7
	362/318					40	30.1
	362/194					40	42.4
	362/334 T		A-QuEChERS	10g	**0.002**	85	2.9
	362/318					83	2.9
	362/194					86	3.9
	362/334 T				**0.005**	86	2.0
	362/318					83	4.5
	362/194					99	9.5

*Table 34: QuPPe-AO recovery data of toxicologically critical SRM substances in **whole cow's milk**. Matrix-matched calibration using internal standard (n = 5).*

Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)	Mean Rec. (%)	RSD (%)
Amitrole	85/43 T	Amitrole $^{15}N_2$ $^{13}C_2$	QuPPe AO	10 g	**0.01**	98	2.7
	85/58					94	2.7
	85/57					99	2.0
	85/43 T				**0.05**	101	1.9
	85/58					102	1.7
	85/57					103	2.1
Nicotine	163/130 T	Nicotine D_4	QuPPe AO	10 g	**0.01**	103	4.6
	163/132					105	7.6
	163/84					103	3.7
	163/130 T				**0.05**	103	2.2
	163/132					103	1.1
	163/84					105	2.8
Cotinine	177/80	Cotinine D_3	QuPPe AO	10 g	**0.01**	103	1.3
	177/98					102	1.9
	177/80				**0.05**	102	1.6
	177/98					102	1.5
PTU	117/60 T	PTU D_6	QuPPe AO	10 g	**0.01**	104	3.6
	117/58					102	2.1
	117/72					107	9.0
	117/60 T				**0.05**	102	3.0
	117/58					99	2.1
	117/72					102	2.8
Diquat	92/84	Diquat D_8 *	QuPPe AO	10 g	**0.01**	106	16.4
	183/157					95	5.7
	92/157					88	8.3
	92/84				**0.05**	108	7.4
	183/157					102	1.0
	92/157					105	9.7
Topramezone	362/334 T	MPPA D_3**	QuPPe AO	10 g	**0.01**	80	10.6
	362/318					81	13.5
	362/194					79	9.6
	362/334 T				**0.05**	86	9.4
	362/318					88	12.2
	362/194					89	11.3

* It is important to use an MRM of the ILIS that corresponds to that of the native substance (equivalent parent ion)

** Please read important note on MPPA D3 under **Table 20**

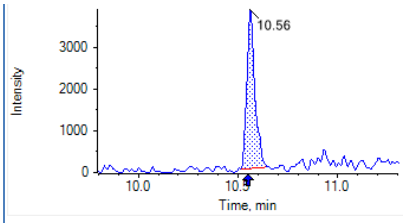
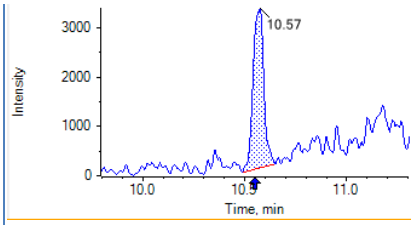
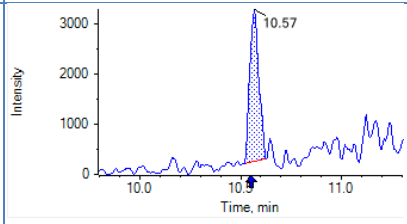
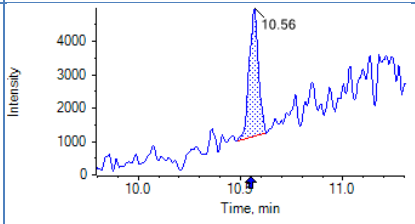
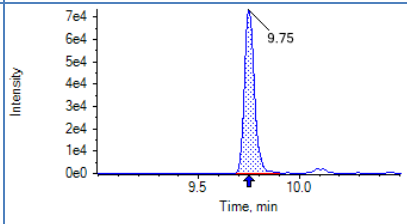
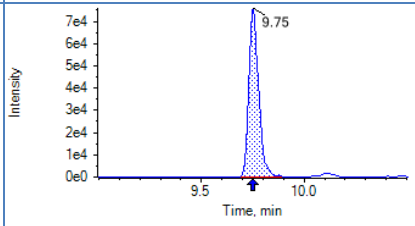
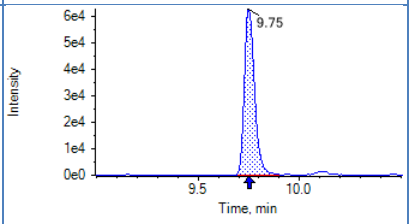
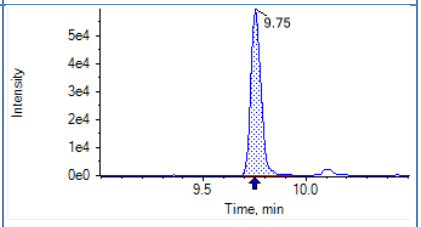
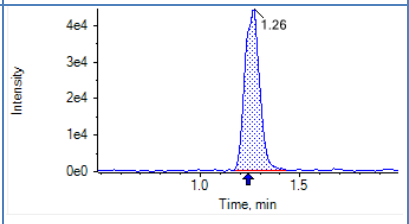
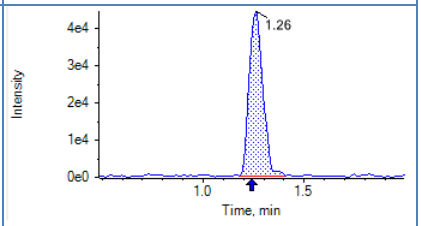
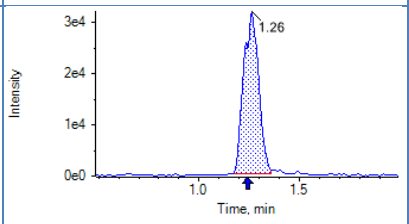
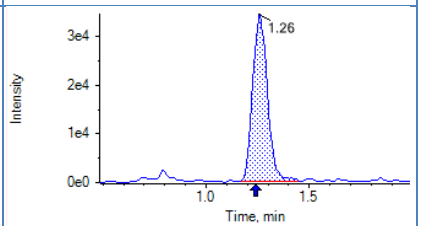
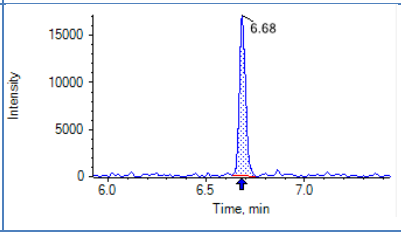
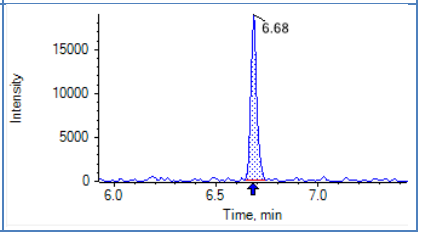
*Table 35: QuPPe-AO recovery data of additionally analysed substances in **whole cow's milk**. Matrix-matched calibration using internal standard **(n = 5)**.*

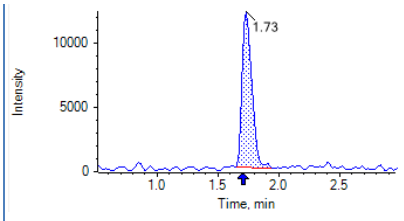
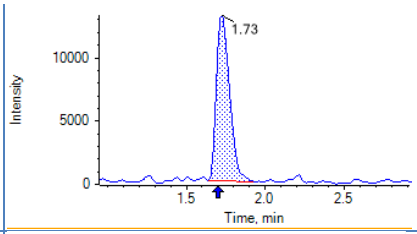
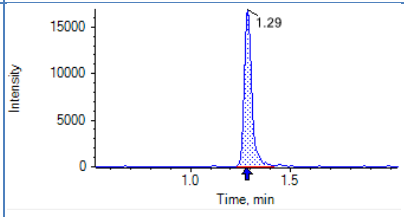
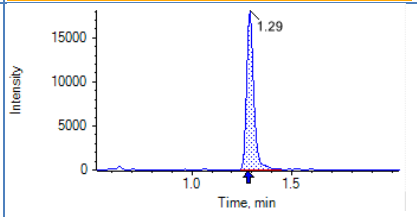
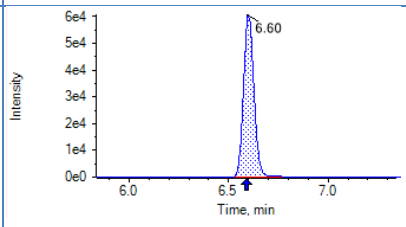
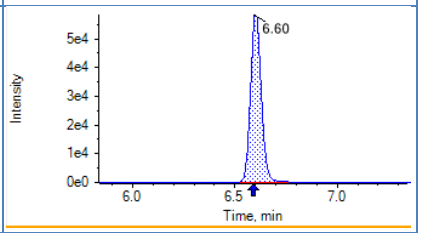
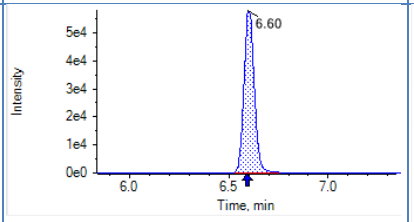
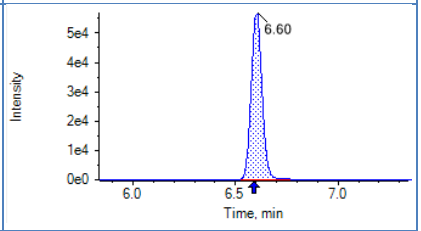
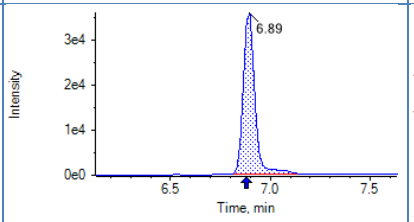
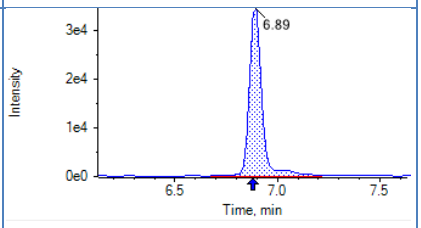
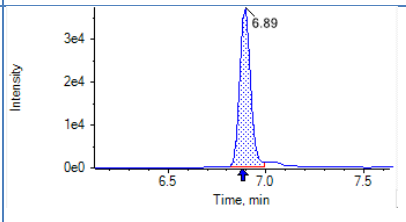
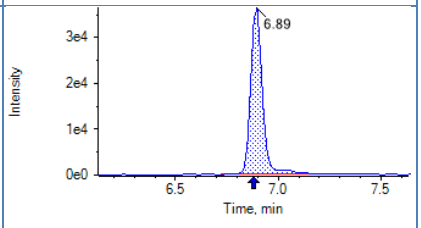
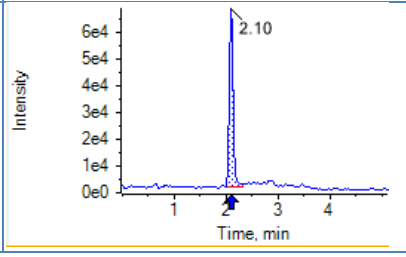
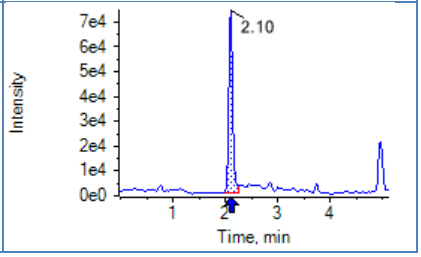
Analyte	Transition	Internal Standard	Extraction method	Sample Weight	Spiking Level (mg/kg)	Mean Rec. (%)	RSD (%)
Paraquat	93/171	Paraquat D_8	QuPPe AO	10 g	**0.01**	103	9.9
	186/171					96	3.5
	93/77					98	19.2
	93/171				**0.05**	121	11.6
	186/171					103	4.3
	93/77					121	21.1
Trifluoracetic acid	113/69	Trifluoro acetic acid $^{13}C_2$	QuPPe AO	10 g	**0.01**	110	4.5
	113/113					*	*
	113/69				**0.02**	110	5.6
	113/113					129	7.7
Triazole acetic acid	128/70	1,2,4-Triazole acetic acid $^{13}C_2$ ^{15}N	QuPPe AO	10 g	**0.05**	99	5.7
	128/73					89	8.2
	128/43					101	5.2
	128/70				**0.1**	76	5.9
	128/73					68	11.2
	128/43					73	6.3
Triazole lactic acid	158/70	1,2,4-Triazole lactic acid $^{13}C_2$ ^{15}N	QuPPe AO	10 g	**0.05**	102	1.9
	158/43					105	3.9
	158/112					110	10.5
	158/70				**0.1**	98	1.4
	158/43					96	1.9
	158/112					96	3.2
Triazole alanine	157/70	1,2,4-Triazole-1yl-alanine $^{13}C_2$ ^{15}N	QuPPe AO	10 g	**0.05**	101	11.3
	157/88					109	10.3
	157/42					101	10.9
	157/70				**0.1**	100	10.4
	157/88					110	11.8
	157/42					106	9.2
Melamine	127/85	Melamine $^{15}N_3$	QuPPe AO	10 g	**0.01**	89	5.2
	127/68					82	7.4
	127/85				**0.05**	94	5.7
	127/68					87	5.5
Chlorate	83/67	Chlorate $^{18}O_3$	QuPPe AO	10 g	**0.01**	96	5.6
	85/69					102	5.1
	83/67				**0.02**	98	15.5
	85/69					100	9.0
Perchlorate	99/83	Perchlorate $^{18}O_4$	QuPPe AO	10 g	**0.01**	96	2.7
	101/85					88	13.3
Phosphonic acid	81/79	Phosphonic acid $^{18}O_3$	QuPPe AO	10 g	**0.05**	87	7.4
	81/63					**	**
	81/79				**0.1**	101	3.6
	81/63					**	**
Cyanuric acid	128/42	Cyanuric acid $^{13}C_3$	QuPPe AO	10 g	**0.05**	111	8.5
	128/85					104	15.6
	128/42				**0.1**	100	4.3
	128/85					108	6.5

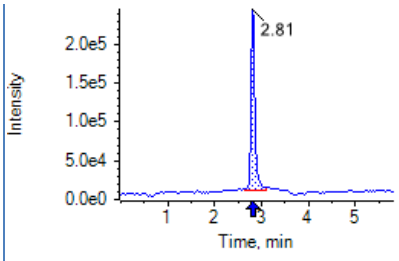
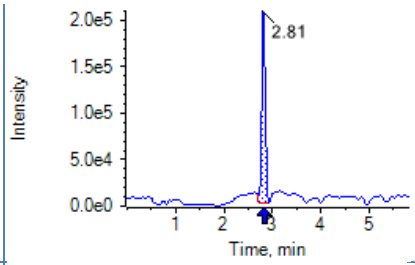
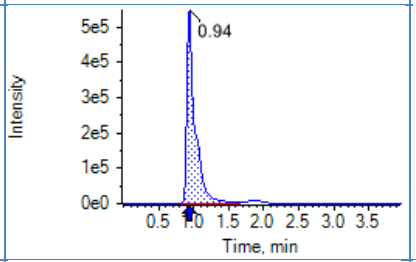
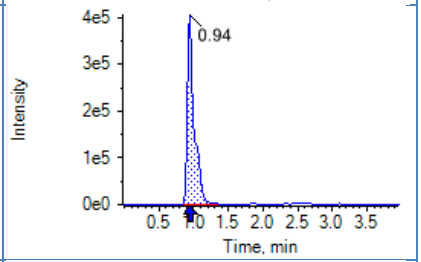
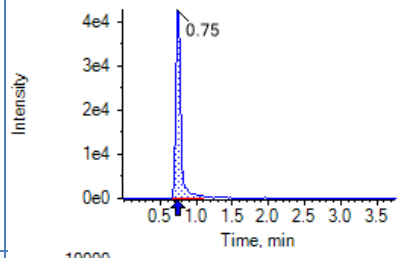
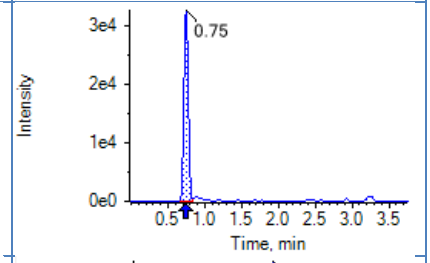
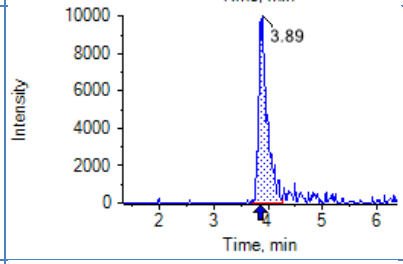
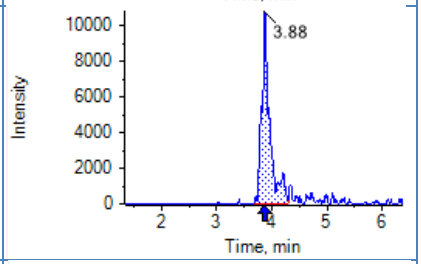
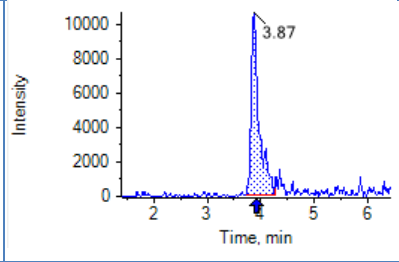
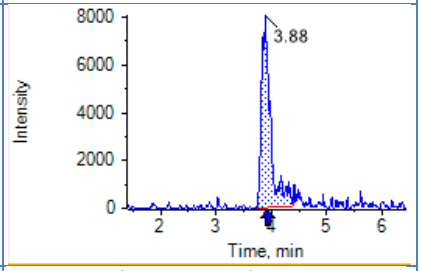
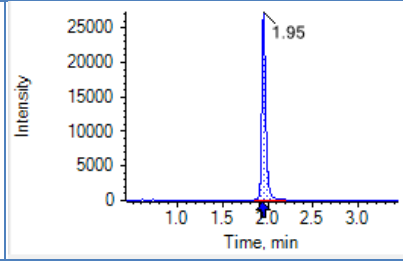
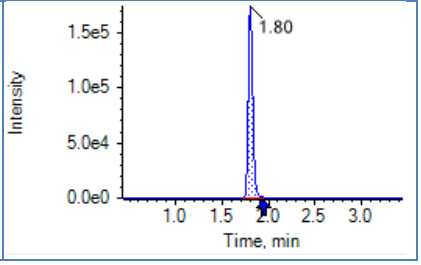
* The "transition" 113/113 is much more interfered compared to the target transition. Estimated LOQ of target transition is lower than 0.01 mg/kg.

** Unable to quantify using this method (Method 9) due to a strong interference of phosphate on this transition.

__Table 36__: Exemplary chromatograms of target analytes in whole cow's milk derived from injecting solvent-based and matrix-matched calibration standards at 120% of the respective lowest spiking level in validation experiments using QuEChERS, A-QuEChERS and QuPPe AO.

Analyte Transition	Spiking Level on milk (mg/kg)	Peak resulting from injecting <u>solvent–based</u> standard (at 120% of spiking level, see left)	Peak resulting from injecting <u>matrix–based</u> standard in whole cow's milk (at 120% of spiking level, see left)
Avermectin B1a 891/305 [M+NH_4]$^+$	0.002 - **QuEChERS**		
	0.002 - **A-QuEChERS**		
Emamectin B1a 887/82	0.002 - **QuEChERS**		
	0.002 - **A-QuEChERS**		
3-Hydroxy-carbofuran 238/163	0.002 - **QuEChERS**		
	0.002 - **A-QuEChERS**		
Gamma-cyhalothrin 467/225 T (as lambda-cyhalothrin on C_{18} column)	0.032 - **QuEChERS**		

Analyte Transition	Spiking Level on milk (mg/kg)	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in whole cow's milk (at 120% of spiking level, see left)
Fentin 351/120	0.002 - **QuEChERS**		
	0.002 - **A-QuEChERS**		
Haloxyfop 360/288	0.002 - **QuEChERS**		
	0.002 - **A-QuEChERS**		
Diclofop 325/253	0.002 - **QuEChERS**		
	0.002 - **A-QuEChERS**		
Amitrole 85/43	0.01 - **QuPPe AO**		

Analyte Transition	Spiking Level on milk (mg/kg)	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in whole cow's milk (at 120% of spiking level, see left)
Nicotine 163/130	0.01 - **QuPPe AO**		
Cotinine 177/80	0.01 - **QuPPe AO**		
PTU 117/60	0.01 - **QuPPe AO**		
Diquat 92/84 $[M]^{2+}$	0.01 - **QuPPe AO**		
Diquat 183/157 $[M^{2+} - H^{+}]^{+}$	0.01 - **QuPPe AO**		
Topramezone 362/334	0.01 - **QuPPe AO**		

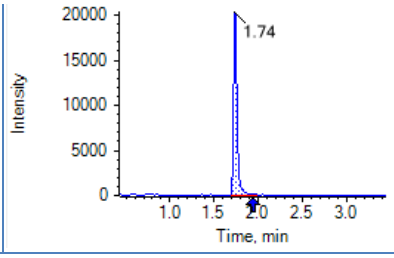
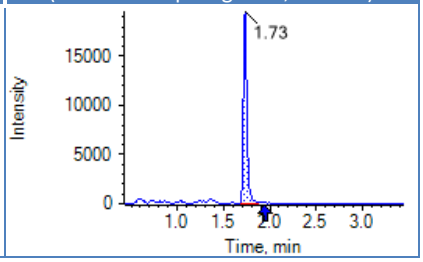
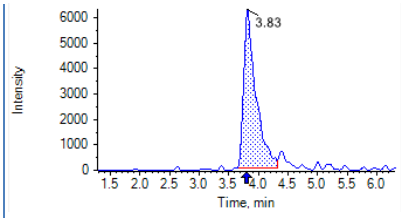
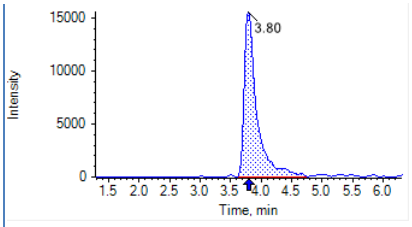
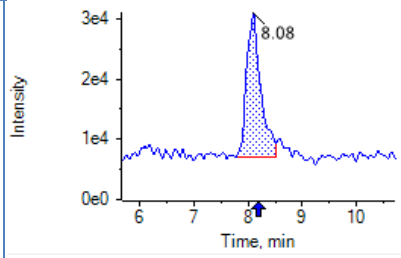
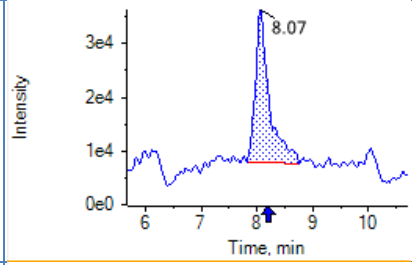
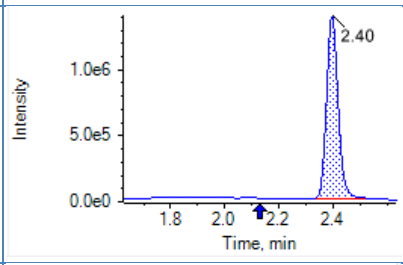
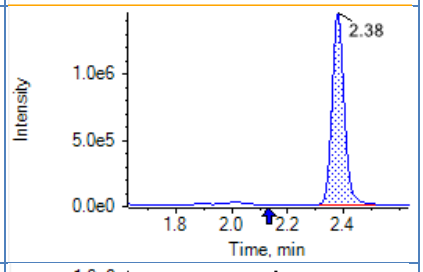
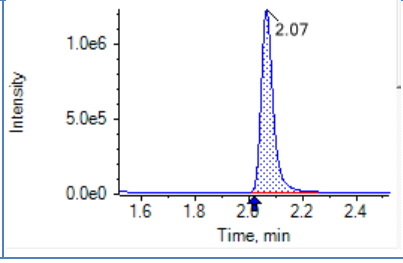
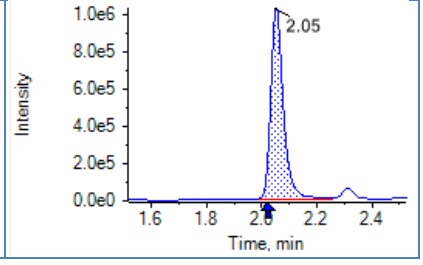
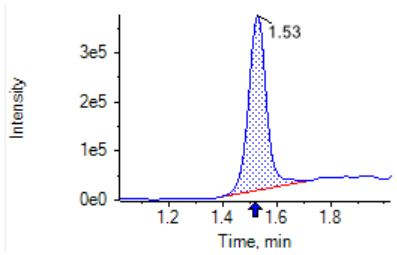
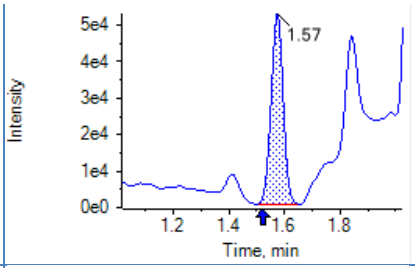
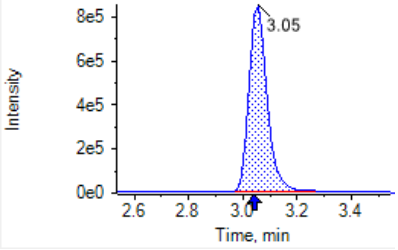
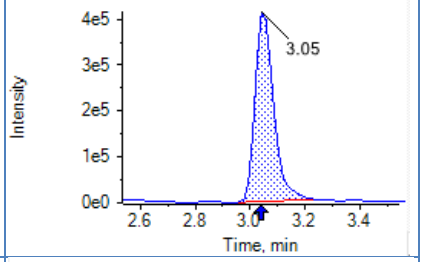
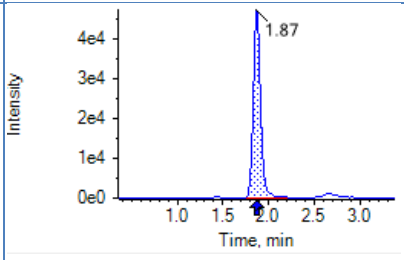
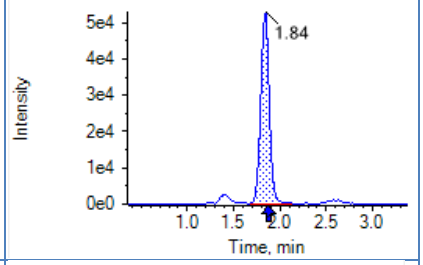
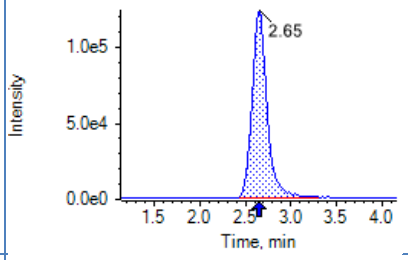
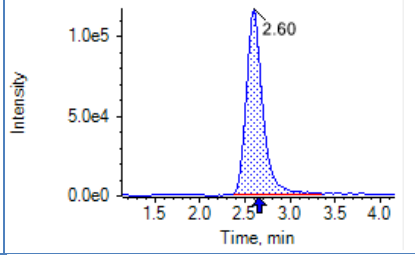
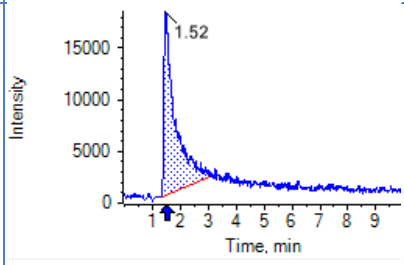
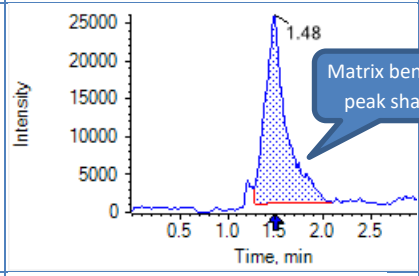
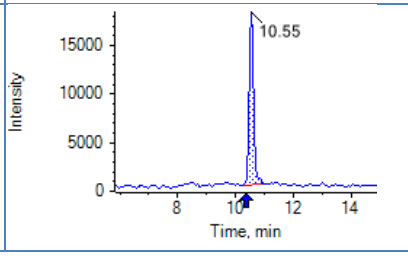
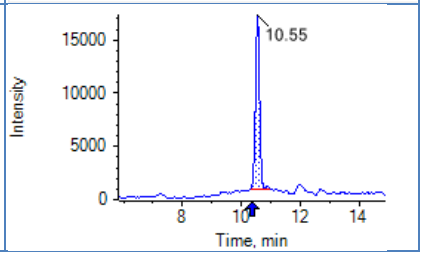
Analyte Transition	Spiking Level on milk (mg/kg)	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in whole cow's milk (at 120% of spiking level, see left)
Topramezone 362/334	0.01 - **A-QuChERS**		

__Table 37__: Exemplary chromatograms of target analytes of additionally analysed compounds in whole cow's milk derived from injecting solvent-based and matrix-matched calibration standards at 120% of the respective lowest spiking level in validation experiments using QuPPe AO.

Analyte Transition	Spiking Level on milk (mg/kg)	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in whole cow's milk (at 120% of spiking level, see left)
Paraquat 93/171 [M^{2+}]	0.01 - **QuPPe AO**		
Trifluoracetic acid 113/69	0.01 - **QuPPe AO**		
Triazole acetic acid 128/70	0.05 - **QuPPe AO**		
Triazole lactic acid 158/70	0.05 - **QuPPe AO**		

Analyte Transition	Spiking Level on milk (mg/kg)	Peak resulting from injecting solvent–based standard (at 120% of spiking level, see left)	Peak resulting from injecting matrix–based standard in whole cow's milk (at 120% of spiking level, see left)
Triazole alanine 157/70	0.05 - **QuPPe AO**		
Melamine 127/85	0.02 - **QuPPe AO**		
Chlorate 83/67	0.01 - **QuPPe AO**		
Perchlorate 99/83	0.01 - **QuPPe AO**		
Phosphonic acid 81/79	0.05 - **QuPPe AO**		
Cyanuric acid 128/42*	0.05 - **QuPPe AO**		

* at 0.024 mg/kg

6. Matrix effects

Matrix effects were determined by comparing peak areas obtained from injecting solvent-based standards with the areas obtained when injecting equally concentrated standards prepared in blank extract. Calculation was accomplished using the following equation:

$$ME\ (\%) = \frac{Area_B}{Area_A} \cdot 100 - 100$$

Where:
ME (%) = Matrix effect in %
$Area_A$ = Peak area in counts in solvent standard
$Area_B$ = Peak area in counts in matrix standard

Calibration standards in solvent and matrix extract corresponding to 120% of the respective HIGH Level (spiking on powder) were chosen to determine the matrix effects. **Table 38** shows matrix effects for target transitions in the respective matrix extracts.

Table 38: *Matrix effects in % of target transitions of the analytes within the scope in extracts of QuEChERS AO, A-QuEChERS and QuPPe AO*

Analyte	MRM [m/z]	Extraction method	ME (%)
QuEChERS-Compounds			
Avermectin B1a	891/305	QuEChERS	-7
		A-QuEChERS	-15
Emamectin B1a	887/82	QuEChERS	8
		A-QuEChERS	1
3-Hydroxycarbofuran	238/163	QuEChERS	-12
		A-QuEChERS	-13
Gamma-Cyhalothrin	467/225	QuEChERS	-8
Fentin	351/120	QuEChERS	7
		A-QuEChERS	-6
Haloxyfop	360/288	QuEChERS	3
		A-QuEChERS	-12
Diclofop	325/253	QuEChERS	6
		A-QuEChERS	-15
QuPPe-Compounds			
Amitrole	85/43	QuPPe AO	-18
Nicotine	163/130	QuPPe AO	-32
Cotinine	177/80	QuPPe AO	-46
PTU	117/60	QuPPe AO	-31
Diquat $[M]^{2+}$	92/84	QuPPe AO	6
Diquat $[M^{2+} - H^+]^+$	183/157	QuPPe AO	-87.6
Diquat $[M]^{+\bullet}$	184/128	QuPPe AO	-93.9
Topramezone	362/334	QuPPe AO	282

7. Short summary

With the aim of checking whether highly toxic compounds can be effectively monitored in infant formulae for children up to 16 weeks of age and of running a pilot monitoring program of infant formulae from the market, a method was developed. The focus was on the analysis of compounds not amenable to multiresidue methods that show a high toxicity (low ADI-values), and for which the default MRL for infant formulae of 0.01 mg/kg is considered unsafe for children of the abovementioned age. Several additional compounds deemed as being of relevance to milk products were also included in the scope, irrespective of their toxicological profile. The following compounds were included in the study:

Compounds covered by QuEChERS and LC-MS/MS: Abamectin, Emamectin, Diclofop, Haloxyfop, Gamma-Cyhalothrin, 3-Hydroxycarbofuran and Fentin. Additional: Ethoxyquin-Dimer.

Compounds covered by QuPPe and LC-MS/MS: Amitrole, Nicotine, PTU, Diquat and Topramezone. Additional: Trifluoracetic acid, Triazole acetic acid, Triazole lactic acid, Triazole alanine, Chlorate, Perchlorate, Phosphonic acid, Thiocyanate, Paraquat and Melamine.

Recoveries of fentin and Toprametazone were low using CEN QuEChERS. The use of FA-QuEChERS, where acetonitrile with 1% FA and no citrate buffer salts is used, resulted in much higher recovery rates.

Document History

Action	When	Document Version
Initial Experiments	May – December 2019	
Further Validation Experiments	November 2019 – January 2020	
Observation document placed on-line	March 2020	V1
Updated version	March 2021	V2