EURL for Cereals and Feeding stuff
National Food Institute
Technical University of Denmark

Validation Report 31B

Determination of pesticide residues in rice based babyfood by LC-MS/MS and GC-MS/MS

(QuEChERS method)

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CONTENT:

1. Introduction

EFSA drafted a Scientific Opinion on pesticides in foods for infants and young children (published 28 June 2018)[1], which concluded that the default MRL 0.01 mg/kg might now not be sufficiently protective for infants below the age of 16 weeks. This default MRL corresponds to an ADI of 0.0026 mg/kg bw (based on a 260 g/kg bw intake of infant formula. However, a row of pesticides have ADI below this value. The Commission then requested from the EURLs information on the lowest achievable LOQs for the pesticides on the EFSA list. Recently the EURL-CF performed a validation on 231 pesticides or metabolites using LC-MSMS and GC-MSMS, 192 at 0.001 mg/kg, 20 at 0.002 mg/kg, 10 at 0.005 mg/kg and 9 at 0.01 mg/kg. Among these pesticides were 36 of which 23 were validated at an acceptable levels according to the LOQs needed. These pesticides were: cadusafos, chlordane, chlorpyrifos, DDTs, demeton-S-methyl, dicofol, dimethoate, ethion, ethoprophos, fenamiphos, fensulfothion, fluquinconazole, flusilazole, mecarbam, methamidophos, methidathion, methomyl, nitrofen, omethoate, oxamyl, oxydemeton-methyl, phosphamidon and triazophos. However, 13 did not obtain LOQs low enough to fulfil the MRLs need (Aldrin, carbofuran, Chlordane, diazinon, dichlorvos, dieldrin, endrin, fipronil, cyhalothrin-lambda, monocrotophos, parathion, disulfoton and nitrofen). For some of the pesticides the lowest, spike level were too high and for other pesticide the instruments were not sensitive enough. Twelve pesticides were not included in the study (carbophenothion, dioxathion, fluometuron, gamma-cyhalothrin, heptachlor, isofenphos, methomyl, phorate, quinoclamine, tembotrione, topramezone and triazoxide).

It was then decided to make a new validation study at two low levels, 0.0005 and 0.001 mg/kg. To obtain better results the instrument methods would be optimized by injecting higher volume of the extractions into the instrument than the 1µl used in this study; 5µl for the GCMSMS and 2 µl for the LCMSMS.

2. Principle of analysis

Sample preparation: The baby food was analysed as ready to consume, so 25 g powder was mixed with 25 ml 50 ºC MiliQ water, that was boiled and then cooled to 50 ºC.

The extraction procedure is outlines in Appendix 4 and described briefly in the following.

Extraction: 5 g porridge was added 5 ml water and mixed well using ceramic homogenizers. The samples was then extracted with 10 ml acetonitrile using a Geno grinder and a salt and buffer mixture is added and the sample is shaken again.

Clean-up: After centrifugation the supernatant is transferred to a clean tube and put in -80 degree freezer for minimum 15 minutes. The extracts are then allowed to thaw until almost liquid state and

then centrifuged. At this point an aliquot is withdrawn and filtered, diluted 1:1 with acetonitrile and analysed by LC-MS/MS. The rest of the supernatant is transferred to a tube containing PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract is diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the matrix matched calibration standards.

Quantification and qualification: The final extracts are analysed by GC-MS/MS. Crude extract withdrawn before PSA clean-up was analysed by LC-MS/MS.

GC-MS/MS: The GC used was Trace 1300 series with an TriPlus RSH Autosampler-GC Liquids. The column was TG-5SILMS 30mx0.25mmx0.25µm. Injection volume was 5 µL on a PTV. Mass spectrometer was Thermo TSQ 8000 Evo, upgrade of the MS to TSQ 9000 Evo with a new ion source, the Advanced Electron Ionization source, AEI. The quadrupole was operating in the multiple reaction monitoring mode (MRM) with electron energy at 50 eV, source temperature at 250°C and transfer line at 250°C. For each pesticide minimum two sets of precursor and product ions were determined. One for quantification and one-three for qualification. The MRM transitions for the pesticides and degradation products are given in Appendix 1a.

LC-MS/MS: The pesticide residues are separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI), Bruker EVOQ. The injection volume was 2 µl. The validation includes pesticides determined in positive and negative mode. For each pesticide or metabolite a precursor ion and 2 product ions were determined. One product ion for quantification and one-two for qualification. The MRM transitions for the pesticides and degradation products sought validated are given in Appendix 1b.

3. Validation design

The method was sought validated for 48 pesticides or metabolites in rice based babyfood, see **Appendix 1**. The validation was performed on 3 times 6 replicates of the two spiking levels; 0.0005 and 0.001 mg/kg. A blank sample was included.

4. Calibration curves and linearity

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels within the range of 0.1 to 33 ng/ml. The quantification was performed from the mean of two bracketing calibration curves. The calibration curves were fitted to a linear curve. The majority of the correlation coefficients (R) were higher or equal to 0.99 but none were lower than 0.97. Thus, good linearity was observed within the relevant concentration range.

5. Specificity

The ion ratios for sample extracts were within ±30% (relative) of average of relevant calibration standards from same sequence. The ion ratios may vary slightly depending on concentration level and in some cases the average of calibration standard are based on the lower calibration levels for the low spike samples.

6. Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides and degradation products on all three spiking levels (0.0005and 0.001 mg/kg). Repeatability is given as the relative standard deviation on the result from two or more analysis at the same sample, done by the same technician, on the same instrument and within a short period of time.

Repeatability (RSD_r) in this validation was calculated from the 5-6 replicate determinations. Repeatability were calculated as given in ISO 5725-2[2].

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.0005and 0.001 mg/kg) with the relevant pesticides, isomers and degradation products.

Robustness

The QuEChERS method has, in connection with the development of the method, been shown to be robust by Anastassiades et al. 2003[3].

Limit of quantification, LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria (se Section 6) were meet.

7. Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:

1. The relative standard deviation of the repeatability and internal reproducibility should be ≤20%[4].
2. The average relative recovery must be between 70 and 120%[3].

If the above mentioned criteria have been meet, the quantification limits, LOQs is stated.

The expanded uncertainty is calculated to demonstrate that it is less than 50%. The expanded uncertainty is given by:

$$U = \sqrt{RSD^2 + Bias^2 + (RSD^2/n)} * 2$$

Where RSD is the intra-laboratory uncertainty (RSD_R),
Bias is 100 minus the recovery,
RSD^2/n is the uncertainty of the bias,
n is the number of recoveries included in the bias and
2 is the coverage factor corresponding to 95% confidence level.

If the expanded uncertainty is higher than 50%, the analytical results must be corrected for recovery and the combined uncertainty is then given by:

$$U_c = \sqrt{RSD^2 + (RSD^2/n)}$$

Where RSD, in this validation, is the repeatability uncertainty (RSD_r),
RSD^2/n is the uncertainty of the bias,
n is the number of recoveries included in the bias and
2 is the coverage factor corresponding to 95% confidence level.

The bias/recovery used for correction will be the bias/recoveries determined for the individual analytes during the initial validation and/or ongoing method validation. However, if it is evaluated that the type of sample being analysed is significantly different from the matrices employed for the method validation it is possible to correct for bias/recoveries based on recovery from spiked samples included in the analytical batch in question. However, minimum of 5 recovery samples must be included then.

The obtained results including recovery, RSD_r, RSD_R, expanded uncertainty (U, Uc and limit of quantification (LOQ) are presented in Appendix 2.

8. Results and conclusion

The validation results obtained for the 55 pesticides, isomers or metabolites using LC-MSMS and GC-MSMS are presented in Appendix 2. In total, 55 compounds were validated, 44 at 0.0005 mg/kg and 9 at 0.001 mg/kg. However, 2 compounds were not validated in this study, topramezone and triazoxide. Other validation studies on cereals have resulted in LOQ of 0.01 mg/kg but it was not possible to have lower LOQ.

Generally the combined uncertainties were lower than 50%, indicating that recovery for correction is not needed.

The EFSA list of pesticides that have MRLs lower than 0.01 mg/kg, see Appendix 3, includes 66 pesticides. Among them, 18 has to be analysed by Single Residues Methods (SRM). The remaining 48 pesticides (residue definition pesticides) can be analysed by Multi Residue Methods (MRM) like the QuEChERS method and are included in this validation study. Of the 48 MRM compounds (residue definition pesticides), lower LOQ was achieved than the required LOQ for 40 compounds, see appendix 2 and 3.

The following 8 pesticides were not validated successfully at low spiking level:
Aldrin, dieldrin and heptachlor require an LOQ of 0.0004 mg/kg, but the lowest spike level was 0.0005 mg/kg. However, it is expected that the compounds could be validated at the 0.0004 mg/kg level.
Dichlorvos requires LOQ at 0.0003 mg/kg but the achieved LOQ was 0.001mg/kg and it is not expected that a lower LOQ can be achieved unless the extract is up-concentrated.
Endrin requires LOQ at 0.0008 mg/kg but the achieved LOQ was 0.001 mg/kg. A recalculation of the data with a more sensitive transition might result in better results.
Fipronil requires LOQ at 0.0008 mg/kg but the achieved LOQ was 0.001 mg/kg. However, the LOQs achieved for the metabolites fipronil-desulfinyl and fipronil-sulfide were 0.0005 mg/kg.
Topramezone and triazoxide require LOQ at 0.0038 mg/kg and 0.0008 mg/kg, respectively. But none of the compounds was validated in this study. In a validation study on cereals an LOQ of 0.01 mg/kg has been achieved and it is not expected to be possible to lower the LOQs.

9. References

1 Scientific opinion on pesticides in foods for infants and young children. EFSA Panel on Plant Protection Products and their Residues (PPR). EFSA Journal 2018;16(6):5286.
DOI: 10.2903/j.efsa.2018.5286.
2 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

3 EN 15662:2008. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method

4 Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed, Document SANTE/12682/2019, European Commission, Brussels, 2019.

Appendix 1a. MRM transitions for GC-MS/MS

Name	RT	Parent Mass	Product Mass	Collision Energy
Aldrin	15.73	262.7	192.9	32
Aldrin	15.73	292.9	257.9	10
Cadusafos	11.99	159	96.9	16
Cadusafos	11.99	159	130.9	8
Cadusafos	11.99	213	89.1	12
Carbofuran	12.47	164	149.1	8
Carbofuran	12.47	221	164	10
Carbophenothion	20.75	157	45	12
Carbophenothion	20.75	199	142.9	10
Carbophenothion	20.75	342	157	10
Chlordane-cis	17.87	271.7	236.9	12
Chlordane-cis	17.87	372.8	265.8	20
Chlordane-cis	17.87	376.6	268	20
Chlorfenvinphos	16.75	266.9	159	16
Chlorfenvinphos	16.75	269	161	15
Chlorfenvinphos	16.75	323	266.9	14
Chlorpyrifos	15.58	196.7	107	36
Chlorpyrifos	15.58	196.7	168.9	12
Chlorpyrifos	15.58	313.9	257.9	12
Chlorpyrifos	15.58	316.1	260	15
Cyhalothrin-gamma (I)	24.75	180.9	151.9	22
Cyhalothrin-gamma (I)	24.75	197	141.1	10
Cyhalothrin-gamma (II)	25.04	181	151.9	22
Cyhalothrin-gamma (II)	25.04	208.1	151.8	10
Cyhalothrin-lambda (I)	24.75	180.9	151.9	22
Cyhalothrin-lambda (I)	24.75	197	141.1	10
Cyhalothrin-lambda (I)	24.75	208.1	180.9	8
Cyhalothrin-lambda (II)	25.04	181	151.9	22
Cyhalothrin-lambda (II)	25.04	208.1	151.8	10
Cyhalothrin-lambda (II)	25.04	208.1	180.9	8
DDD-op	18.76	234.97	164.98	20
DDD-op	18.76	235	199	14
DDD-op	18.76	236.8	165	20
DDD-op	18.76	236.97	164.98	20
DDD-pp	19.84	235	199	14
DDD-pp	19.84	236.8	165	20
DDE-op	17.54	246	176.1	26
DDE-op	17.54	246.5	210.2	10
DDE-op	17.54	317.8	246	20
DDE-pp	18.55	246	176.1	26
DDE-pp	18.55	317.8	246	20
DDE-pp	18.55	317.8	248	18
DDD-pp	19.84	235	199	14

Name	RT	Parent Mass	Product Mass	Collision Energy
DDD-pp	19.84	236.8	165	20
DDT-op	20.01	235	165	22
DDT-op	20.01	235	199.5	14
DDT-op	20.01	236.8	165.1	20
DDT-pp	21.15	235	165.1	22
DDT-pp	21.15	235	199.5	14
DDT-pp	21.15	236.8	165	20
Demeton-S-methyl	11.27	88	59.8	6
Demeton-S-methyl	11.27	109	79	6
Demeton-S-methyl	11.27	141.9	79	12
Diazinon	13.01	137.1	84.1	12
Diazinon	13.01	199	93	15
Diazinon	13.01	304.1	179.1	10
Dicofol, p,p'-	19.6	111	74.9	14
Dicofol, p,p'-	19.6	139	111	12
Dicofol, p,p'-	19.6	250.9	139	12
Dieldrin	19.37	262.8	192.9	30
Dieldrin	19.37	276.9	206.9	20
Dieldrin	19.37	276.9	240.9	10
Dioxathion	12.84	96.9	65	16
Dioxathion	12.84	125	97	6
Disulfoton	13.3	185.9	96.9	16
Disulfoton	13.3	186	153	5
Disulfoton	13.3	274.1	88.2	10
Endrin	19.73	245	173	22
Endrin	19.73	262.8	192.9	30
Ethion	19.88	153	97	10
Ethion	19.88	230.9	128.9	22
Ethion	19.88	230.9	174.9	12
Ethoprophos	11.4	157.9	96.9	16
Ethoprophos	11.4	157.9	113.9	6
Ethoprophos	11.4	200	158	6
Fluometuron	11.34	232	72.1	12
Fluometuron	11.34	232	174.1	14
Fluometuron	11.34	232	186.1	12
Fensulfothion	19.62	140	125	10
Fensulfothion	19.62	291.8	156	15
Fensulfothion	19.62	307.9	153.1	12
Fluquinconazole	26.3	340	108.1	36
Fluquinconazole	26.3	340	298	16
Fluquinconazole	26.3	340	313	14
Heptachlor	14.71	99.8	39	26
Heptachlor	14.71	99.8	65	12
Heptachlor	14.71	271.8	236.9	12
Hexachlorobenzene	12.34	283.8	213.8	28

Name	RT	Parent Mass	Product Mass	Collision Energy
Hexachlorobenzene	12.34	283.8	248.8	18
Hexachlorobenzene	12.34	285.81	250.83	20
Isofenphos	16.7	185	121	10
Isofenphos	16.7	213	121	14
Isofenphos	16.7	213	185	6
Methidathion	17.34	145	85	6
Methidathion	17.34	302	145	5
Nitrofen	19.3	202	139	24
Nitrofen	19.3	283	202	10
Nitrofen	19.3	283	253	10
Parathion	15.7	291	81	20
Parathion	15.7	291	109	12
Phorate	12.05	75	47	8
Phorate	12.05	121	65	8
Phorate	12.05	260	75	8
Terbufos	12.93	230.9	128.9	22
Terbufos	12.93	230.9	174.9	12
Terbufos	12.93	230.9	203	8
Triazophos	20.35	161	134.1	8
Triazophos	20.35	257	162	5
Triazophos	20.35	285	162	10

Appendix 1b. MRM transitions for LC-MS/MS

Name	RT	ESI mode	Parent Mass	Product Mass	Collision Energy
Cadusafos	6.624	Positive	271	131	20.5
Cadusafos	6.624	Positive	271	159	11.5
Carbofuran	3.820	Positive	222	123	17.5
Carbofuran	3.820	Positive	222	165	9.5
Chlorpyrifos	7.275	Positive	349.7	198	16
Chlorpyrifos	7.275	Positive	351.7	200	18
Demeton-S-methyl	3.854	Positive	231	61	25
Demeton-S-methyl	3.854	Positive	231	89	10
Diazinon	6.292	Positive	305	97	30
Diazinon	6.292	Positive	305	153	20
Diazinon	6.292	Positive	305	169	20
Dichlorvos	3.747	Positive	221	109	16
Dichlorvos	3.747	Positive	238	221	4
Dimethoate	2.667	Positive	230	125	19
Dimethoate	2.667	Positive	230	171	14
Dimethoate	2.667	Positive	230	199	8
Ethion	7.175	Positive	385	199	10
Ethion	7.175	Positive	402	199	15
Ethion	7.175	Positive	402	385	5
Ethoprophos	5.818	Positive	243.24	97	23
Ethoprophos	5.818	Positive	243.24	131	31
Fenamiphos	5.990	Positive	304	201.7	35
Fenamiphos	5.990	Positive	304	216.9	21
Fensulfothion	4.653	Positive	309	281.3	10
Fensulfothion	4.653	Positive	309	157	27
Fensulfothion	4.653	Positive	309	173	27
Fipronil	6.095	Negative	435.22	330.2	13
Fipronil	6.095	Negative	435.22	250.09	42
Fipronil-desulfinyl	6.057	Negative	387	351	10
Fipronil-desulfinyl	6.057	Negative	389	353	15
Fipronil-desulfinyl	6.057	Negative	387	282	35
Fipronil-desulfinyl	5.970	Negative	387	351	10
Fipronil-desulfinyl	5.970	Negative	389	353	15
Fipronil-desulfinyl	5.970	Negative	387	282	35
Fipronil-sulfide	6.215	Negative	419	383	10
Fipronil-sulfide	6.215	Negative	419	262	30
Fipronil-sulfide	6.215	Negative	421	385	10
Fipronil-sulfide	6.203	Negative	419	383	10
Fipronil-sulfide	6.203	Negative	419	262	30
Fipronil-sulfide	6.203	Negative	421	385	10
Fluometuron	4.729	Positive	233	72	13
Fluometuron	4.729	Positive	233	233	11
Fluquinconazole	5.698	Positive	376	307	21.5

Name	RT	ESI mode	Parent Mass	Product Mass	Collision Energy
Fluquinconazole	5.698	Positive	376	349	18
Flusilazole	6.049	Positive	316	165	23.5
Flusilazole	6.049	Positive	316	247	16.5
Mecarbam	5.749	Positive	330	97	45
Mecarbam	5.749	Positive	330	199	14
Mecarbam	5.749	Positive	330	227	8
Methamidophos	1.631	Positive	142	94	11.5
Methamidophos	1.631	Positive	142	125	12
Methomyl	2.097	Positive	163	88	8
Methomyl	2.097	Positive	163	106	9.5
Monocrotophos	2.169	Positive	224	127	12.5
Monocrotophos	2.169	Positive	224	193	7.5
Monocrotophos	2.169	Positive	224	98	10
Omethoate	1.789	Positive	214	125	20
Omethoate	1.789	Positive	214	155	14.5
Omethoate	1.789	Positive	214	183	10
Oxamyl	1.972	Positive	237	72	9.5
Oxamyl	1.972	Positive	237	90	6.5
Oxamyl	1.972	Positive	237	220	5.5
Oxydemeton-methyl	2.023	Positive	247	109	26
Oxydemeton-methyl	2.023	Positive	247	169	12
Phorate	6.435	Positive	261.02	75.3	5
Phorate	6.435	Positive	261.02	170.8	10
Phosphamidon	3.485	Positive	300	174.09	13
Phosphamidon	3.485	Positive	300	227	12
Phosphamidon	3.485	Positive	300	127	22
Quinoclamine	3.533	Positive	208.6	100.3	55
Quinoclamine	3.533	Positive	208.6	103.2	36
Quinoclamine	3.533	Positive	208.6	145.4	18
Tembotrione	4.941	Positive	458	262	27
Tembotrione	4.941	Positive	458	341	13
Topramezone	2.497	Positive	364.4	334	8
Topramezone	2.497	Positive	364.4	125	19
Topramezone	2.497	Positive	364.4	346	8
Triazophos	5.615	Positive	314	119	30
Triazophos	5.615	Positive	314	162	17
Triazoxide	4.664	Positive	248.2	68.09	37
Triazoxide	4.664	Positive	248.2	95	25

Appendix 2. Recoveries, repeatability (RSD_r), internal reproducibility (RSDR), expanded uncertainty (U) and Limit of Quantification (LOQ) for pesticides validated on rice based babyfood using QuEChERS.

		Spike level 0.0005 mg/kg					Spike level 0.001 mg/kg							
	Compound	Recovery %	RSD_r %	RSD_R %	U %	Cu %	Recovery %	RSD_r %	RSD_R, %	U %	Cu %	LOQ	Evaluation	Remarks
GC	Aldrin	59	11	14	88	15	80	9	15	50	15	0.0005	Close	Se note in the text above
GC	Cadusafos	109	6	6	22	6	102	7	8	18	9	0.0005	Pass	
LC	Cadusafos	113	10	16	41	16	100	12	20	40	20	0.0005	Pass	
GC	Carbofuran	107	4	13	30	14	103	7	11	24	12	0.0005	Pass	
LC	Carbofuran	73	7	7	56	7	92	10	10	27	11	0.0005	Pass	
GC	carbophenothion	95	6	9	21	9	88	7	13	36	13	0.0005	Pass	
GC	Chlordane	93	8	7	20	7	91	7	8	24	8	0.0005	Pass	
GC	Chlorfenvinphos	118	7	7	38	8	100	10	14	28	14	0.0005	Pass	
GC	Chlorpyrifos	95	7	7	18	7	93	8	9	24	9	0.0005	Pass	
LC	Chlorpyrifos						103	14	14	29	14	0.001	Pass	
GC	Cyhalothrin-gamma	99	8	11	22	11	94	6	7	20	8	0.0005	Pass	It is not possible to separate gamma and
GC	Cyhalothrin-lambda	109	5	6	22	6	98	7	9	19	9	0.0005	Pass	lambda isomers from each other
GC	DDD-op	99	4	6	12	6	92	3	9	24	9	0.0005	Pass	
GC	DDD-pp	99	6	6	12	6	92	3	7	22	7	0.0005	Pass	
GC	DDE-op	92	6	8	23	8	86	4	14	40	14	0.0005	Pass	
GC	DDE-pp	89	3	10	31	11	84	4	14	43	15	0.0005	Pass	
GC	DDT-op	93	5	10	25	10	85	5	13	40	13	0.0005	Pass	
GC	DDT-pp	95	4	8	20	9	82	4	11	43	11	0.0005	Pass	
GC	Demeton-S-methyl	94	14	18	38	18	107	8	16	36	16	0.0005	Pass	
LC	Demeton-S-methyl	68	13	14	70	15	86	12	11	36	12	0.0005	Pass	
GC	Diazinon	101	7	14	28	14	100	7	11	23	11	0.0005	Pass	
LC	Diazinon	116	13	17	49	18	104	20	18	39	19	0.0005	Pass	

		Spike level 0.0005 mg/kg					Spike level 0.001 mg/kg							
	Compound	**Recovery %**	**RSD_r %**	**RSD_R %**	**U %**	**Cu %**	**Recovery %**	**RSD_r %**	**RSD_R, %**	**U %**	**Cu %**	**LOQ**	**Evaluation**	**Remarks**
LC	Dichlorvos						97	14	14	29	14	0.001	Fail	See note in the text above
GC	Dicofol	114	15	16	43	16	104	19	20	42	21	0.0005	Pass	
GC	Dieldrin	90	16	19	45	20	87	13	17	43	17	0.0005	Close	See note in the text above
LC	Dimethoate	89	17	20	46	20	94	9	20	42	20	0.0005	Pass	
GC	dioxathion	104	19	18	38	19	91	20	20	44	20	0.0005	Pass	
GC	Disulfoton						94	15	24	52	25	0.001	Pass	
GC	Endrin						100	17	21	43	21	0.001	Close	See note in the text above
GC	Ethion	111	10	18	43	19	98	10	18	38	19	0.0005	Pass	
LC	Ethion	92	11	11	28	11	96	10	10	23	11	0.0005	Pass	
GC	Ethoprophos	114	6	5	31	5	100	6	8	16	8	0.0005	Pass	
LC	Ethoprophos	111	9	13	35	13	104	7	8	18	8	0.0005	Pass	
LC	Fenamiphos	105	8	18	40	19	101	17	18	38	19	0.0005	Pass	
GC	Fensulfothion	130	8	16	68	16	102	11	10	20	10	0.0005	Pass	
LC	Fensulfothion	124	17	18	61	19	113	12	11	35	11	0.0005	Pass	
LC	Fipronil						105	13	17	36	17	0.001	Close	See note in the text above
LC	Fipronil-desulfinyl	69	14	14	68	14	90	17	18	43	19	0.0005	Pass	
LC	Fipronil-sulfide	96	9	12	26	13	88	16	16	41	17	0.0005	Pass	
GC	Fluometuron	86	18	20	50	21	83	18	18	50	18	0.0005	Pass	
LC	Fluometuron	118	19	19	52	19	116	10	11	39	11	0.0005	Pass	
GC	Fluquinconazole	111	4	5	24	5	99	6	7	14	7	0.0005	Pass	
LC	Fluquinconazole	97	18	19	40	20	109	10	11	29	11	0.0005	Pass	
LC	Flusilazole	104	11	11	24	11	107	12	13	30	13	0.0005	Pass	
GC	Heptachlor	93	5	10	24	10	87	3	8	30	8	0.0005	Close	See note in the text above
GC	hexachlorobenzene	88	5	6	27	7	80	5	12	48	13	0.0005	Pass	
GC	isofenphos	102	5	6	12	6	97	4	6	13	6	0.0005	Pass	
LC	Mecarbam						101	15	18	38	19	0.001	Pass	

		Spike level 0.0005 mg/kg					Spike level 0.001 mg/kg							
	Compound	**Recovery %**	**RSD_r %**	**RSD_R %**	**U %**	**Cu %**	**Recovery %**	**RSD_r %**	**RSD_R, %**	**U %**	**Cu %**	**LOQ**	**Evaluation**	**Remarks**
LC	Methamidophos	82	8	18	53	19	79	7	19	57	19	0.0005	Pass	
GC	Methidathion						97	8	12	25	12	0.001	Pass	
LC	Methomyl						105	15	19	41	20	0.001	Pass	
LC	Monocrotophos	93	10	14	33	15	87	9	13	38	13	0.0005	Pass	
GC	Nitrofen	105	10	10	23	11	90	7	10	29	10	0.0005	Pass	
LC	Omethoate						94	10	19	42	20	0.001	Pass	
LC	Oxamyl	111	5	13	35	13	94	11	13	30	14	0.0005	Pass	
LC	Oxydemeton-methyl	92	7	19	42	19	85	10	20	51	21	0.0005	Pass	
GC	Parathion	94	17	18	40	19	94	11	19	40	19	0.001	Pass	
GC	Phorate	101	9	10	20	10	89	5	9	29	10	0.0005	Pass	
LC	Phorate	100	7	19	40	20	99	15	18	37	18	0.0005	Pass	
LC	Phosphamidon	96	18	19	40	19	104	9	15	31	15	0.0005	Pass	
LC	Quinoclamine						109	19	19	44	20	0.001	Pass	
LC	Tembotrione	114	12	15	42	16	114	11	12	37	13	0.0005	Pass	
GC	Terbufos	99	5	6	13	6	81	6	12	46	13	0.0005	Pass	
LC	Topramezone											0.01	Fail	Validation done on cereals
GC	Triazophos	123	5	12	53	13	100	9	12	25	13	0.0005	Pass	
LC	Triazophos	98	10	10	21	10	106	11	11	26	12	0.0005	Pass	
LC	Triazoxide											0.01	Fail	Validation done on cereals

Appendix 3 Pesticides on the EFSA list

Pesticide	Calculated MRL in mg/kg to be below an ADI of 0.0026 mg/kg bw	Achieved LOQ, mg/kg	Not acceptable LOQ
Aldrin	0.0004	0.0005	x
Cadusafos	0.0015	0.0005	
Carbofuran	0.0006	0.0005	
Carbophenothion	0.0019	0.0005	
Chlordane	0.0019	0.0005	
Chlorfenvinphos	0.0019	0.0005	
Chlorpyrifos	0.0038	0.0005	
Cyhalothrin-gamma	0.0046	0.0005	
Cyhalothrin-lambda	0.0096	0.0005	
DDTs	0.0077	0.0005	
Demeton-S-methyl	0.0012	0.0005	
Diazinon	0.0008	0.0005	
Dichlorvos	0.0003	0.001	x
Dicofol	0.0077	0.0005	
Dieldrin	0.0004	0.0005	x
Dimethoate	0.0038	0.0005	
Dioxathion	0.0058	0.0005	
Disulfoton	0.0012	0.001	
Endrin	0.0008	0.001	x
Ethion	0.0077	0.0005	
Ethoprophos	0.0015	0.0005	
Fenamiphos	0.0031	0.0005	
Fensulfothion	0.0012	0.0005	
Fipronil	0.0008	0.001	x
Fluometuron	0.0019	0.0005	
Fluquinconazole	0.0077	0.0005	
Flusilazole	0.0077	0.0005	
Heptachlor	0.0004	0.0005	x
Hexachlorobenzene	0.0023	0.0005	
Isofenphos	0.0038	0.0005	
Mecarbam	0.0077	0.001	
Methamidophos	0.0038	0.0005	
Methidathion	0.0038	0.001	
Methomyl	0.0096	0.001	
Monocrotophos	0.0023	0.0005	
Nitrofen	0.0011	0.0005	
Omethoate	0.0012	0.001	
Oxamyl	0.0038	0.0005	

Pesticide	Calculated MRL in mg/kg to be below an ADI of 0.0026 mg/kg bw	Achieved LOQ, mg/kg	Not acceptable LOQ
Oxydemeton-methyl	0.0012	0.0005	
Parathion	0.0023	0.0005	
Phorate	0.0027	0.0005	
Phosphamidon	0.0019	0.0005	
Quinoclamine	0.0077	0.001	
Tembotrione	0.0015	0.0005	
Terbufos	0.0023	0.0005	
Topramezone	0.0038	0.01	x
Triazophos	0.0038	0.0005	
Triazoxide	0.0008	0.01	x
SRM compounds			
1-Methyl-cyclopropene	SRM		
Abamectin	SRM		
Amitrole	SRM		
Chloropicrin	SRM		
Cyanamide	SRM		
Diclofop	SRM		
Diquat	SRM		
Emamectin	SRM		
Fenthiosulf	SRM		
Fentin acetate	SRM		
Fentin hydroxide	SRM		
Haloxyfop	SRM		
Haloxyfop-P	SRM		
Metam	SRM		
Methyl bromide	SRM		
Nicotine	SRM		
Sulcotrione	SRM		
Propineb	SRM		

Appendix 4: Principles of the QuEChERS method for extraction of babyfood

QuEChERS for babyfood

Weigh 5 g (±0.05 g) of babyfood into a 50 ml single use centrifuge tube (red cap). Add internal standard and/or spike standard (maximum 25 µl)

Add a ceramic homogenizer and 10 g of cold water and shake briefly

Add 10 ml acetonitrile and shake vigorously by hand for 1 min. (1. extraction)

Add the prepared mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3 citrate dihydrate and 0.5 g Na_2H cirate sesquihydrate. Shake for a few seconds after each addition to prevent lumps.

Shake vigorously for 1 min. (2. Extraction with phase separation)

Centrifuge for 10 min at 4500 rpm

Transfer at least 8 ml of the extract to a 15 ml single use centrifuge tube and store in the freezer (-80°C for 1 hour or over night). When the extract are almost thawed (i.e. About -40 °C) centrifugate (should be cold 5 C) for 5 min. at 4500 rpm.

Transfer 6 ml of the cold extract to a 15 ml single use centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$. Close the tube and shake vigorously for 30 seconds.

Centrifuge for 5 min. at 4500 rpm

Transfer 4 ml of the extract to a 15 ml single use centrifuge tube. Add 40 µl of 5% formic acid solution in acetonitrile (10 µl/ml extract). Dilute the extract 1:1 with acetonitrile

Transfer the final extract into auto sampler vials and analyse by GC and LC.