Impact of the different calibration approaches in the quality of the results

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1. Aim and scope

This document reports the impact that the different ways of **calibration** for quantification, described in document SANTE/11312/2021 [1], have in the quality of the results for **171** pesticides included in the European Union Multi Annual Control Program (EU-MACP) and the Working Document [2,3] using a multiresidue method by LC-MS/MS and GC-MS/MS.

2. Short description

Different ways of calibration have been tested to assess the influence they have in the **quality** of the results: multiple- and single-level matrix matched calibration using the same matrix, multiple-level calibration of a different matrix of the same group, procedural calibration, sample standard addition and extract standard addition. The different calibration approaches were tested in one representative commodity of the three main FV commodity groups: **high water** content, **high acid** and water content, and **high oil** and very low water content. The extracts were prepared using **QuEChERS** as the extraction method.

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes from 1 µL to 5000 µL and from 1 mL to 5 mL.
- Graduated 10 mL pipette.
- 50 mL and 15 mL PTFE centrifuge tubes.
- Vortex Shaker IKATM 4 Basic.
- Axial shaker Agytax SR1 CP57.
- Centrifuge Orto Alresa Consul 21, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 4000 rpm.
- Concentration workstation.
- Injection vials, 2 mL, suitable for LC and GC auto-sampler.

4. Chemicals

- Acetonitrile ultra-gradient grade
- Trisodium citrate dihydrate
- Disodium hydrogenocitrate sesquihydrate
- Sodium chloride
- Anhydrous magnesium sulphate
- Anhydrous calcium chloride
- Primary secondary amine (PSA)
- Supel QuE Z-Sep

- Ammonium formate
- Ultra-pure water
- Methanol HPLC grade
- Formic acid
- Pesticide standards

5. Procedure

5.1. Sample preparation

The main matrices used in the study were **tomato**, **orange** and **avocado**. Additionally, multiple-level calibration in **potato**, **strawberry** and **olive extract** were prepared.

5.2. Pesticide stock solutions and working mix solutions

Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile or ethyl acetate and were stored in screw-capped glass vials in the dark at -20 °C. Working mixes were prepared in 10 mL volumetric flasks by pipetting the appropriate volume of each stock solution.

5.3. Extraction procedure

QuEChERS

1. Weigh 10 g ± 0.1 g of sample in 50 mL PTFE centrifuge tube.
2. Add 10 mL of acetonitrile and 10 µL of 10 mg/L carbendazim-d3, malathion-d10 and TPP (procedure internal standards).
3. Shake the sample using an automatic axial shaker for 4 min.
4. Add 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogenocitrate sesquihydrate.
5. Shake the samples again in the automatic shaker for 4 min.
6. Centrifuge the tubes at 3700 rpm for 5 min.
7. Transfer 5 mL of the supernatant to a 15 mL PTFE tube containing:
   a. 750 mg magnesium sulphate and 125 mg PSA for matrices with high water content and high acid content and high water content.
   b. 750 mg magnesium sulphate and 175 mg Z-Sep for matrices with high oil content and very low water content.
8. Vortex the tube for 30 sec.
9. Centrifuge the tubes at 3700 rpm for 5 min.
10. Add 40 µL of formic acid 5% in acetonitrile to option a in step 7.
11. Analysis:

a. LC-MS/MS: dilute 100 mL extract with 400 mL of water containing dimethoate-d6 at 0.050 mg/L (Injection Internal Standard).
b. GC-MS/MS: recovery samples were evaporated under a gentle N_2 current and reconstituted with ethyl acetate prior to injection.
Standard calibration levels were prepared using the blank extract and reconstitute with the pesticide mix in ethyl acetate.
In both cases, add 0.002 mL of Lindane-d6 (Injection Internal Standard) and transfer the contents to a 0.20 mL spring-loaded insert.

5.4. Measurement

LC system was operated in multiple reaction monitoring mode (MRM). Selected reaction monitoring (SRM) experiments were carried out to obtain the maximum sensitivity for the detection of the target molecules. For confirmation of the studied compounds, two SRM transitions and a correct ratio between the abundances of the two optimized SRM transitions (SRM2/SRM1) were used, along with retention time matching. The mass transitions used are presented in Appendix I.

5.5. Instrumentation and analytical conditions for the LC- MS/MS system

5.5.1. 1290 UHPLC (Agilent)

- Column: Zorbax Eclipse Plus C8 2.1x100 mm and 1.8 µm particle size (Agilent)
- Mobile phase A: Water (0.1% formic acid, 5mM ammonium formate, 2% MeOH)
- Mobile phase B: Methanol (0.1% formic acid, 5mM ammonium formate, 2% H_2O)
- Column temperature: 35ºC
- Flow rate: 0.3 mL/min
- Injection volume: 5 µL.

Mobile phase gradient for pesticides analysed

min	Mobile phase A	Mobile phase B
0	100 %	0 %
1.5	75 %	25 %
10	0 %	100 %
12	0 %	100 %

Re-equilibration with initial phase: 2.5 minutes

5.5.2. 6490 triple quadrupole system (Agilent)

- Ionisation mode: Positive mode and negative mode
- Capillary (positive and negative): 3000 V
- Nebulizer: 45 psi
- Nozzle: 400 V
- Drying gas flow: 13 L/min
- Drying gas temperature: 120ºC
- Sheath gas flow: 10 L/min
- Sheath gas temperature: 375ºC
- High Pressure RF (positive): 150 V
- High Pressure RF (negative): 110 V
- Low Pressure RF (positive): 60 V
- Low Pressure RF (negative): 60 V

5.6. Instrumentation and analytical conditions for the GC- MS/MS system

5.6.1. Intuvo 9000 GC system (Agilent)

- Columns: 2 planar columns HP-5MS UI (15 m long × 0.25 mm i.d. × 0.25 µm film thickness)
- Injection mode: Splitless
- Sample injection volume: 1 µL
- Inlet temperature: 80 °C hold for 0.1 min, then up to 300 °C at 600 °C/min, hold for 5 min and then to 250 ºC at 100 °C/min
- Carrier gas: Helium at constant flow = 1.28 mL/min column 1, 1.48 mL/min column 2
- Carrier gas purity: 99.999 %
- Oven temperature: 60 °C for 0.5 min, up to 170 °C at 80 °C/min, and up to 310 °C at 20 °C/min (hold for 3.5 min)
- Post Run: 2.1 min, 310 ºC

5.6.2. 7410 triple quadrupole system (Agilent)

- Ionisation mode: electron impact ionisation
- Temperature of the transfer line: 280 °C
- Temperature of ion source: 280 °C
- Collision gas: nitrogen
- Collision gas purity: 99.999 %
- Solvent delay: 2.6 minutes

6. Results

In order to evaluate the impact that different ways of quantification had in the quality of the results, 10 µg/kg and 100 µg/kg analytical standard mixes were prepared in blank extracts of tomato, orange and avocado, as representative commodities from the three studied groups. Those were considered as the "fake samples" that should be quantified using the different quantification strategies. This way, each one of those fake samples was quantified as follows:

- Sample of **tomato** (10 and 100 µg/kg) quantified with a **calibration curve** in **tomato** extract.
- Sample of **tomato** (10 and 100 µg/kg) quantified with a **single-point calibration** using each one of the levels of the calibration curve (5 µg/kg, 10 µg/kg, 50 µg/kg, 100 µg/kg and 200 µg/kg) in **tomato** extract.
- Sample of **tomato** (10 and 100 µg/kg) quantified with a **calibration curve** in **potato** extract.
- Recovery test (10 and 100 µg/kg) in **tomato** quantified by **procedural calibration** in **tomato**.
- Sample of **tomato** (10 µg/kg) quantified by **standard addition** (10 µg/kg, 20 µg/kg, 30 µg/kg and 40 µg/kg) to the extract.
- Recovery test of **tomato** (10 µg/kg) quantified by **standard addition** (10 µg/kg, 20 µg/kg, 30 µg/kg and 40 µg/kg) to the sample.

- Sample of **orange** (10 and 100 µg/kg) quantified with a **calibration curve** in **orange** extract.
- Sample of **orange** (10 and 100 µg/kg) quantified with a **single-point calibration** using each one of the levels of the calibration curve (5 µg/kg, 10 µg/kg, 50 µg/kg, 100 µg/kg and 200 µg/kg) in **orange** extract.
- Sample of **orange** (10 and 100 µg/kg) quantified with a **calibration curve** in **strawberry** extract.
- Recovery test (10 and 100 µg/kg) in **orange** quantified by **procedural calibration** in **orange**.
- Sample of **orange** (10 µg/kg) quantified by **standard addition** (10 µg/kg, 20 µg/kg, 30 µg/kg and 40 µg/kg) to the extract.
- Recovery test of **orange** (10 µg/kg) quantified by **standard addition** (10 µg/kg, 20 µg/kg, 30 µg/kg and 40 µg/kg) to the sample.

- Sample of **avocado** (10 and 100 µg/kg) quantified with a **calibration curve** in **avocado** extract.
- Sample of **avocado** (10 and 100 µg/kg) quantified with a **single-point calibration** using each one of the levels of the calibration curve (5 µg/kg, 10 µg/kg, 50 µg/kg, 100 µg/kg and 200 µg/kg) in **avocado** extract.

- Sample of **avocado** (10 and 100 µg/kg) quantified with a **calibration curve** in **olive** extract.
- Recovery test (10 and 100 µg/kg) in **avocado** quantified by **procedural calibration** in **avocado**.
- Sample of **avocado** (10 µg/kg) quantified by **standard addition** (10 µg/kg, 20 µg/kg, 30 µg/kg and 40 µg/kg) to the extract.
- Recovery test of **avocado** (10 µg/kg) quantified by **standard addition** (10 µg/kg, 20 µg/kg, 30 µg/kg and 40 µg/kg) to the sample.

Percentage variation of concentrations from the theoretical concentrations at the 10 µg/kg level

After quantification of all the samples by the methods described above, the **percentage variation** of the concentrations with respect to the theoretical ones (10 and 100 µg/kg) was calculated using the following equation:

$$\text{Variation (\%)} = \left|\left(\frac{\text{Concentration obtained (µg/kg)}}{\text{Theoretical concentration (µg/kg)}} \times 100\right) - 100\right|$$

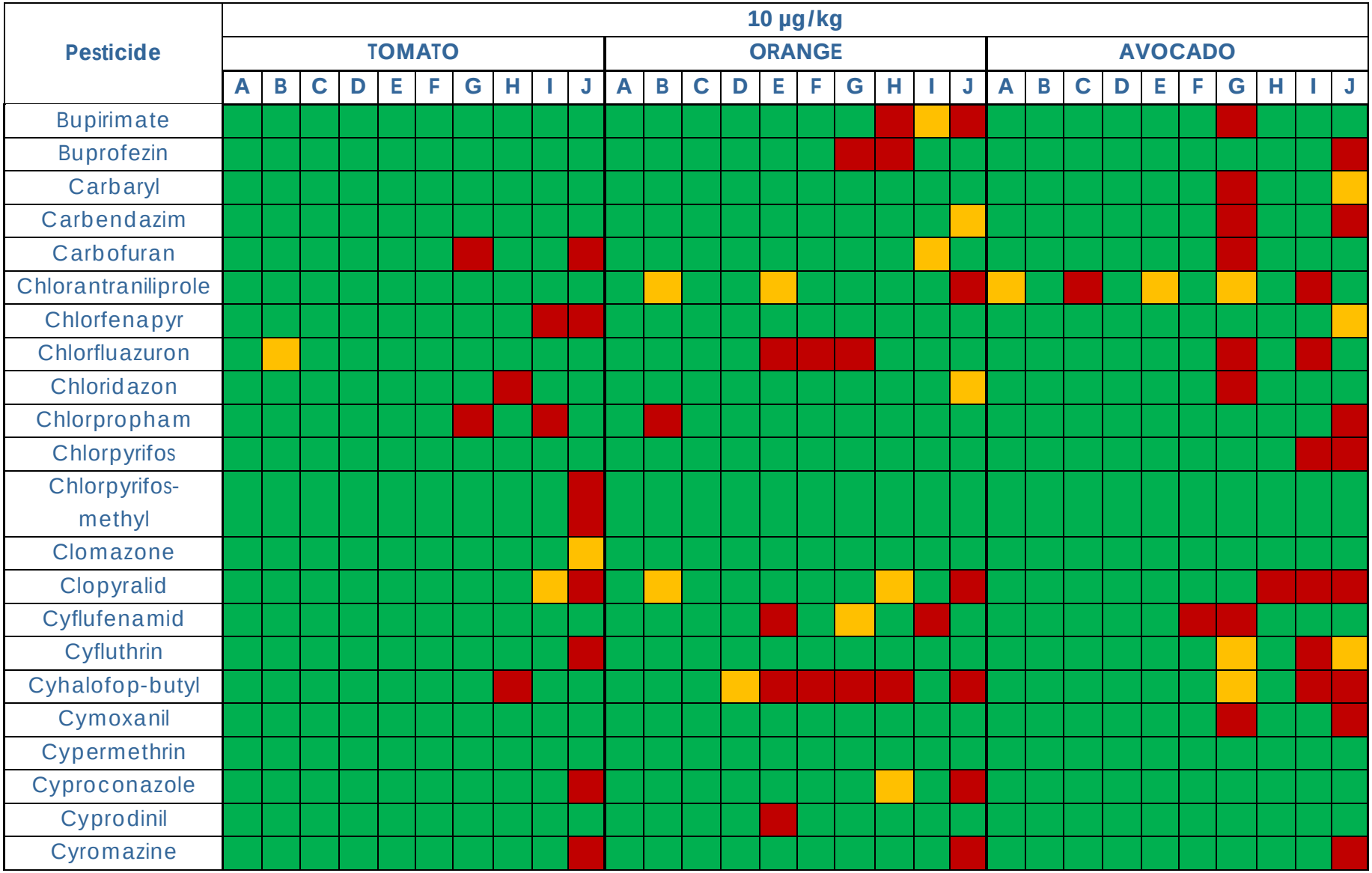
The **results** obtained for the quantification of the samples at the 10 µg/kg level are shown in **Table 1,** whereas the results for the quantification of the 100 µg/kg level are presented in **Table 2**.

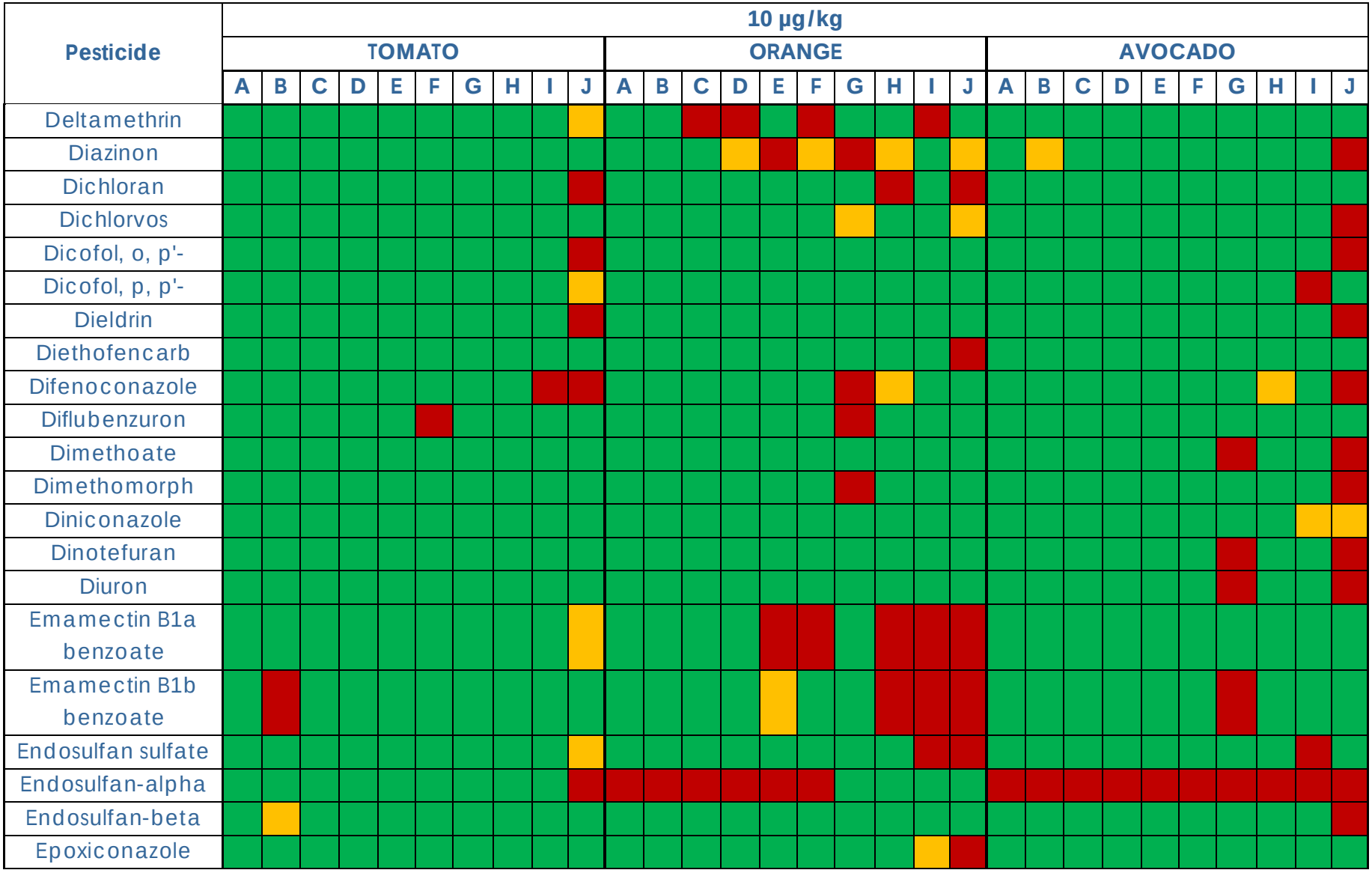
Pesticides with a variation of |0-21%| are shown in **green**, with variations of |21-25%| in **orange** and for cases with variations higher than |25%| in **red**.

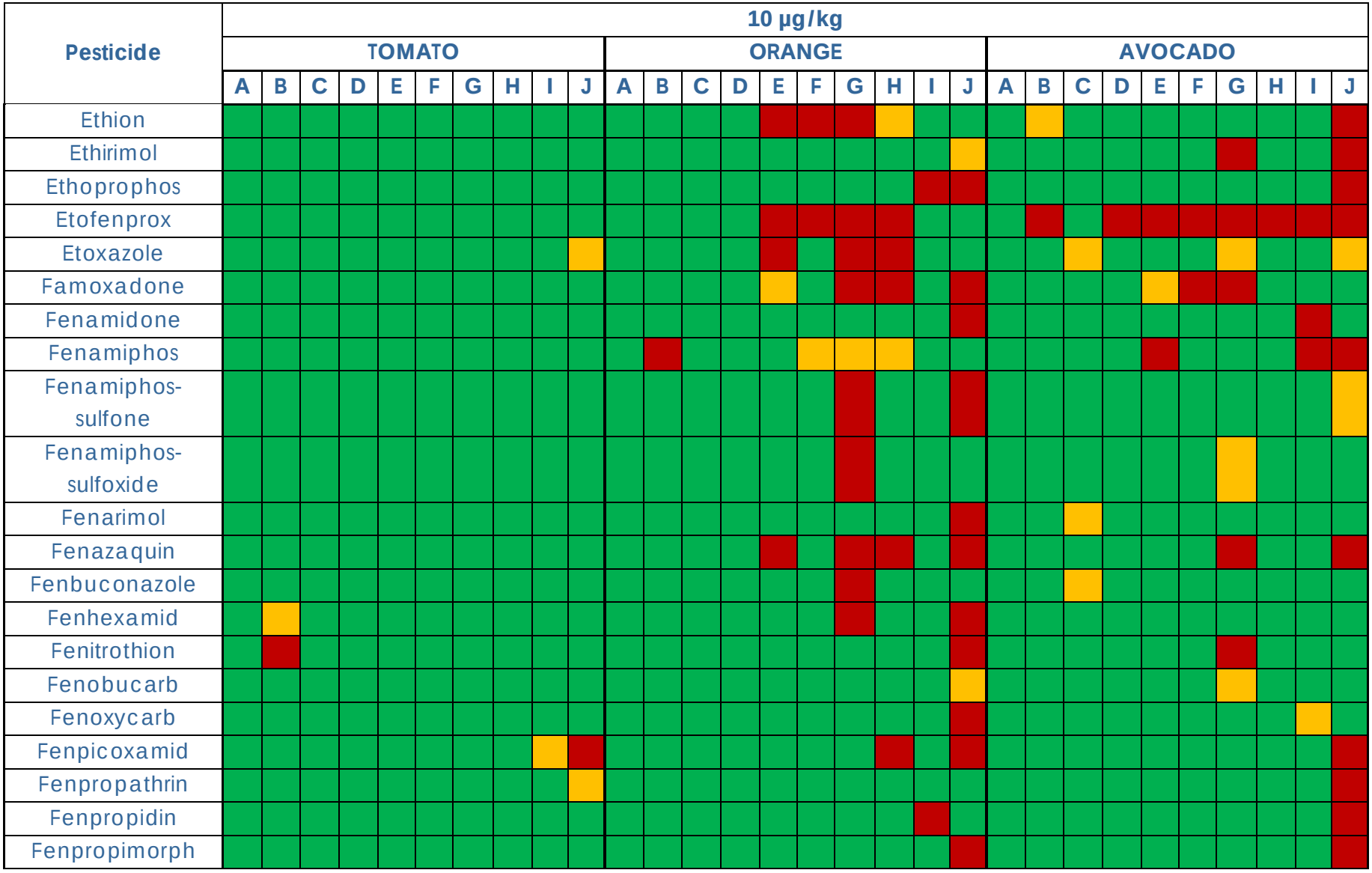
Table 3 shows the percentage of analysed compounds that fall into each of the classifications at 10 µg/kg (green, orange or red). Those tested at 100 µg/kg are displayed in **Table 4.**

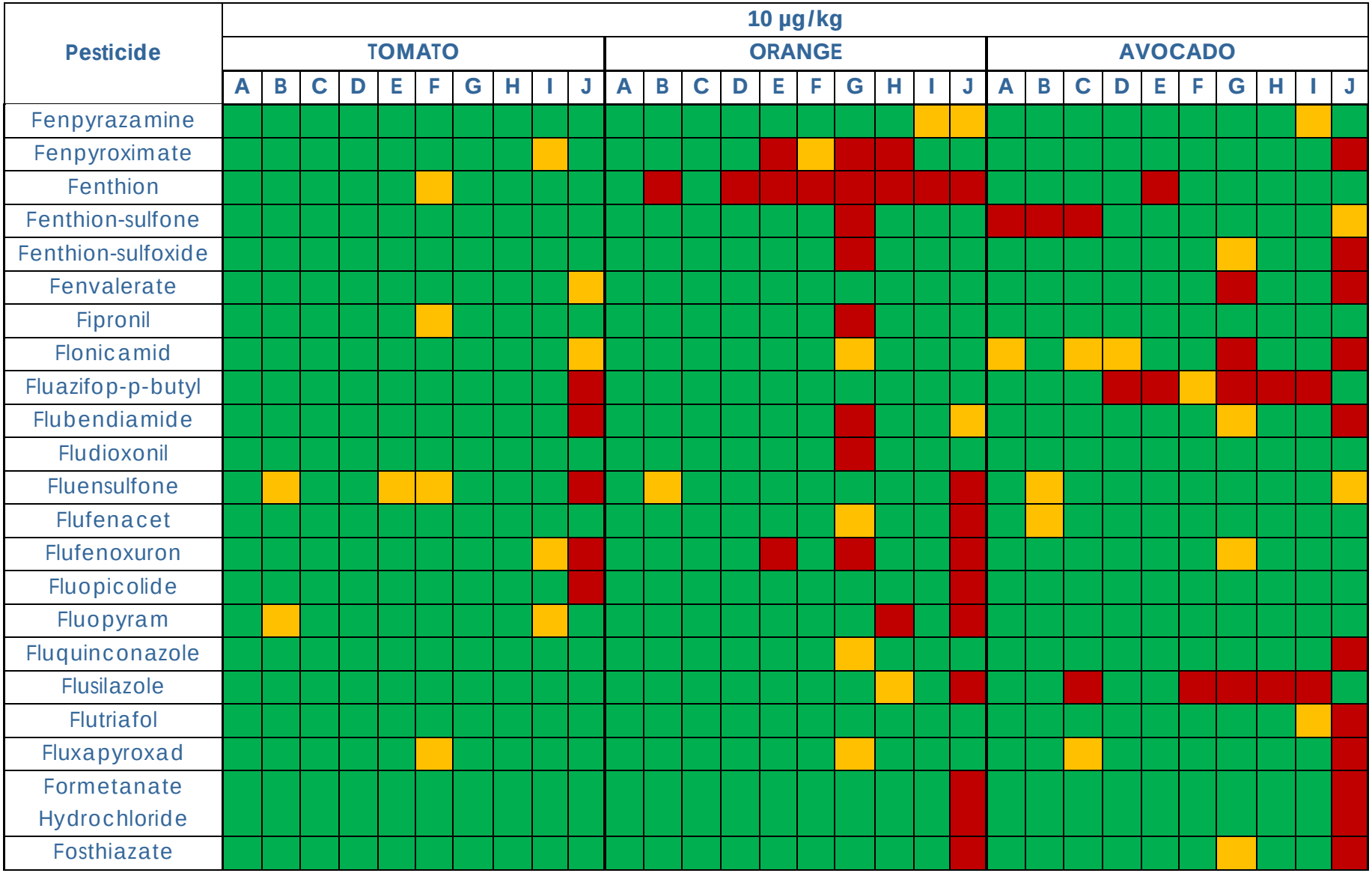
Table 1. Percentage variation of concentrations from the theoretical concentrations at the 10 µg/kg level. Pesticides with a variation of |0-21%| are shown in green, |21-25%| in orange and >|25%| in red.
A = multiple-level calibration of the same matrix (tomato, orange and avocado); single-level calibration: **B** = 5 µg/kg, **C** = 10 µg/kg, **D** = 50 µg/kg, **E** = 100 µg/kg, **F** = 200 µg/kg; **G** = multiple-level calibration of another matrix from the same group of matrices (potato, strawberry and olive); **H** = procedural calibration; **I** = sample standard addition; **J** = extract standard addition

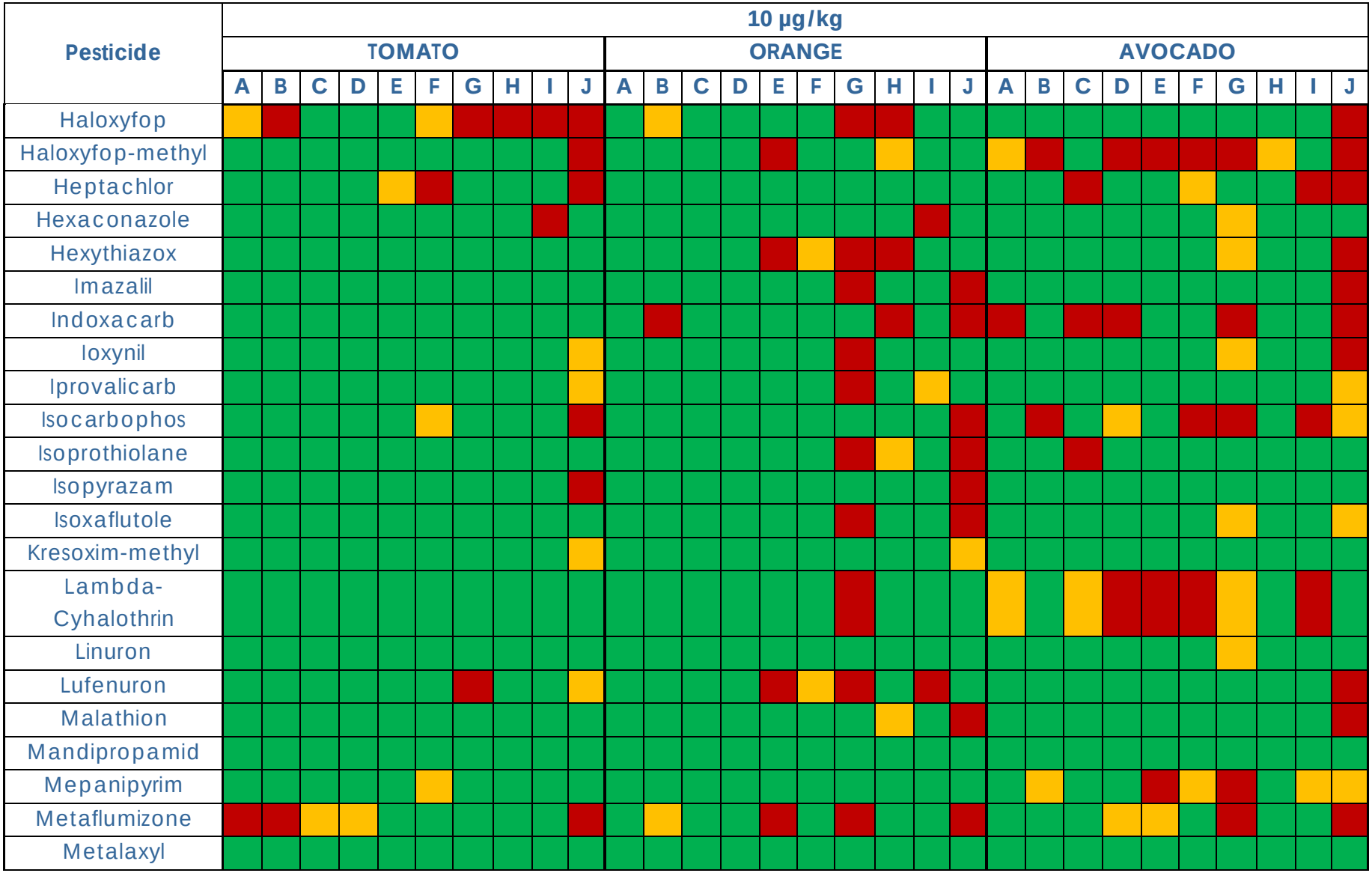
Pesticide	10 µg/kg																											
	TOMATO										ORANGE										AVOCADO									
	A	B	C	D	E	F	G	H	I	J	A	B	C	D	E	F	G	H	I	J	A	B	C	D	E	F	G	H	I	J
2,4'-DDE																														
2-Phenylphenol																														
Acephate																														
Acetamiprid																														
Acrinathrin																														
Aldicarb																														
Aldicarb-sulfone																														
Ametoctradin																														
Azinphos-methyl																														
Azoxystrobin																														
Benalaxyl																														
Benzovindiflupyr																														
Bifenthrin																														
Bitertanol																														
Boscalid																														
Bromopropylate																														

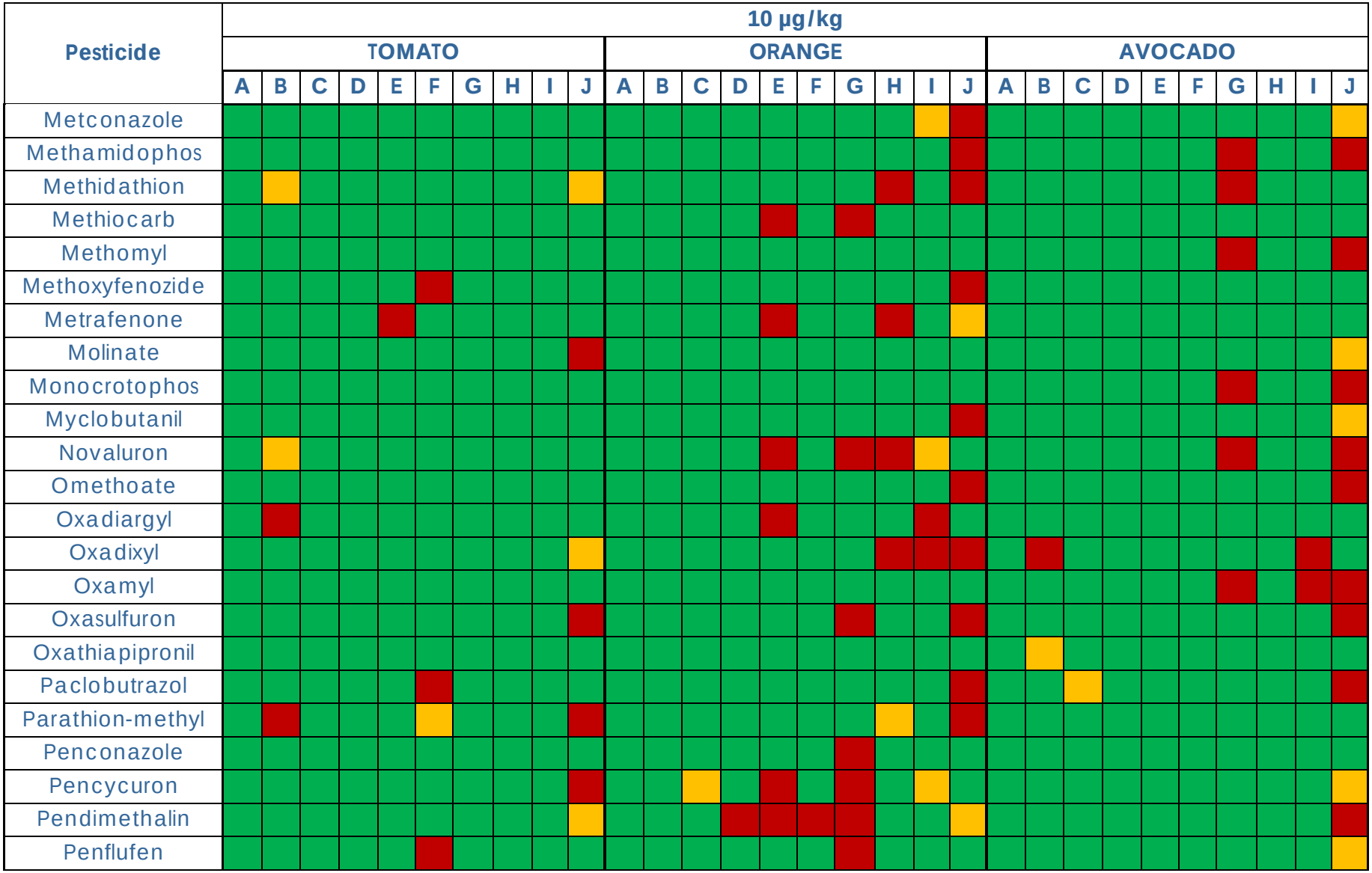

Pesticide
10 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G H I J
Bupirimate
Buprofezin
Carbaryl
Carbendazim
Carbofuran
Chlorantraniliprole
Chlorfenapyr
Chlorfluazuron
Chloridazon
Chlorpropham
Chlorpyrifos
Chlorpyrifos-methyl
Clomazone
Clopyralid
Cyflufenamid
Cyfluthrin
Cyhalofop-butyl
Cymoxanil
Cypermethrin
Cyproconazole
Cyprodinil
Cyromazine

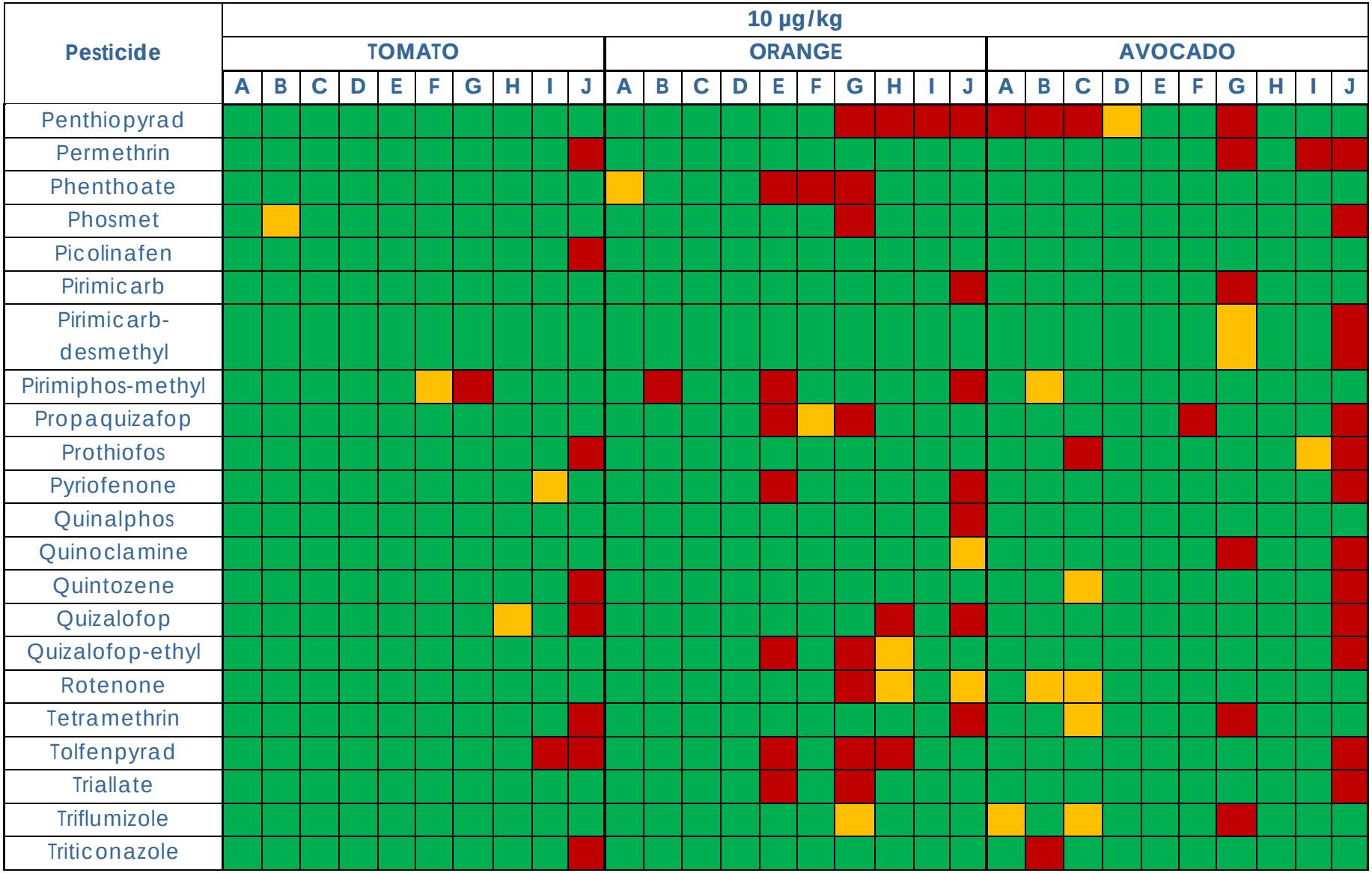

Pesticide
10 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G H I J
Deltamethrin
Diazinon
Dichloran
Dichlorvos
Dicofol, o, p'-
Dicofol, p, p'-
Dieldrin
Diethofencarb
Difenoconazole
Diflubenzuron
Dimethoate
Dimethomorph
Diniconazole
Dinotefuran
Diuron
Emamectin B1a benzoate
Emamectin B1b benzoate
Endosulfan sulfate
Endosulfan-alpha
Endosulfan-beta
Epoxiconazole


Pesticide
10 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G H I J
Ethion
Ethirimol
Ethoprophos
Etofenprox
Etoxazole
Famoxadone
Fenamidone
Fenamiphos
Fenamiphos-sulfone
Fenamiphos-sulfoxide
Fenarimol
Fenazaquin
Fenbuconazole
Fenhexamid
Fenitrothion
Fenobucarb
Fenoxycarb
Fenpicoxamid
Fenpropathrin
Fenpropidin
Fenpropimorph


Pesticide
10 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G H I J
Fenpyrazamine
Fenpyroximate
Fenthion
Fenthion-sulfone
Fenthion-sulfoxide
Fenvalerate
Fipronil
Flonicamid
Fluazifop-p-butyl
Flubendiamide
Fludioxonil
Fluensulfone
Flufenacet
Flufenoxuron
Fluopicolide
Fluopyram
Fluquinconazole
Flusilazole
Flutriafol
Fluxapyroxad
Formetanate Hydrochloride
Fosthiazate


Pesticide
10 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G H I J
Haloxyfop
Haloxyfop-methyl
Heptachlor
Hexaconazole
Hexythiazox
Imazalil
Indoxacarb
Ioxynil
Iprovalicarb
Isocarbophos
Isoprothiolane
Isopyrazam
Isoxaflutole
Kresoxim-methyl
Lambda-Cyhalothrin
Linuron
Lufenuron
Malathion
Mandipropamid
Mepanipyrim
Metaflumizone
Metalaxyl


Pesticide
10 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G H I J
Metconazole
Methamidophos
Methidathion
Methiocarb
Methomyl
Methoxyfenozide
Metrafenone
Molinate
Monocrotophos
Myclobutanil
Novaluron
Omethoate
Oxadiargyl
Oxadixyl
Oxamyl
Oxasulfuron
Oxathiapipronil
Paclobutrazol
Parathion-methyl
Penconazole
Pencycuron
Pendimethalin
Penflufen

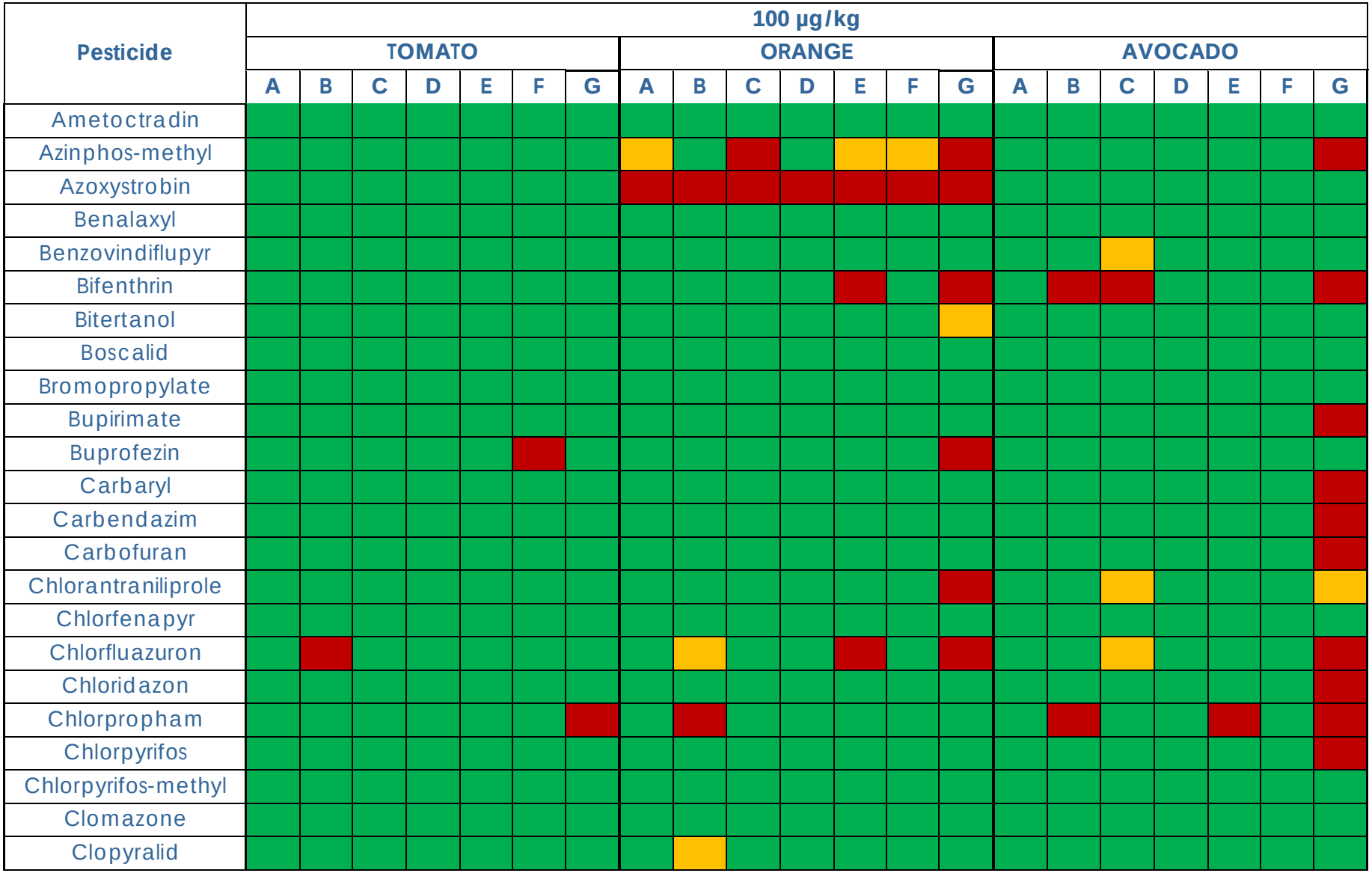

Pesticide
10 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G H I J
Penthiopyrad
Permethrin
Phenthoate
Phosmet
Picolinafen
Pirimicarb
Pirimicarb-desmethyl
Pirimiphos-methyl
Propaquizafop
Prothiofos
Pyriofenone
Quinalphos
Quinoclamine
Quintozene
Quizalofop
Quizalofop-ethyl
Rotenone
Tetramethrin
Tolfenpyrad
Triallate
Triflumizole
Triticonazole

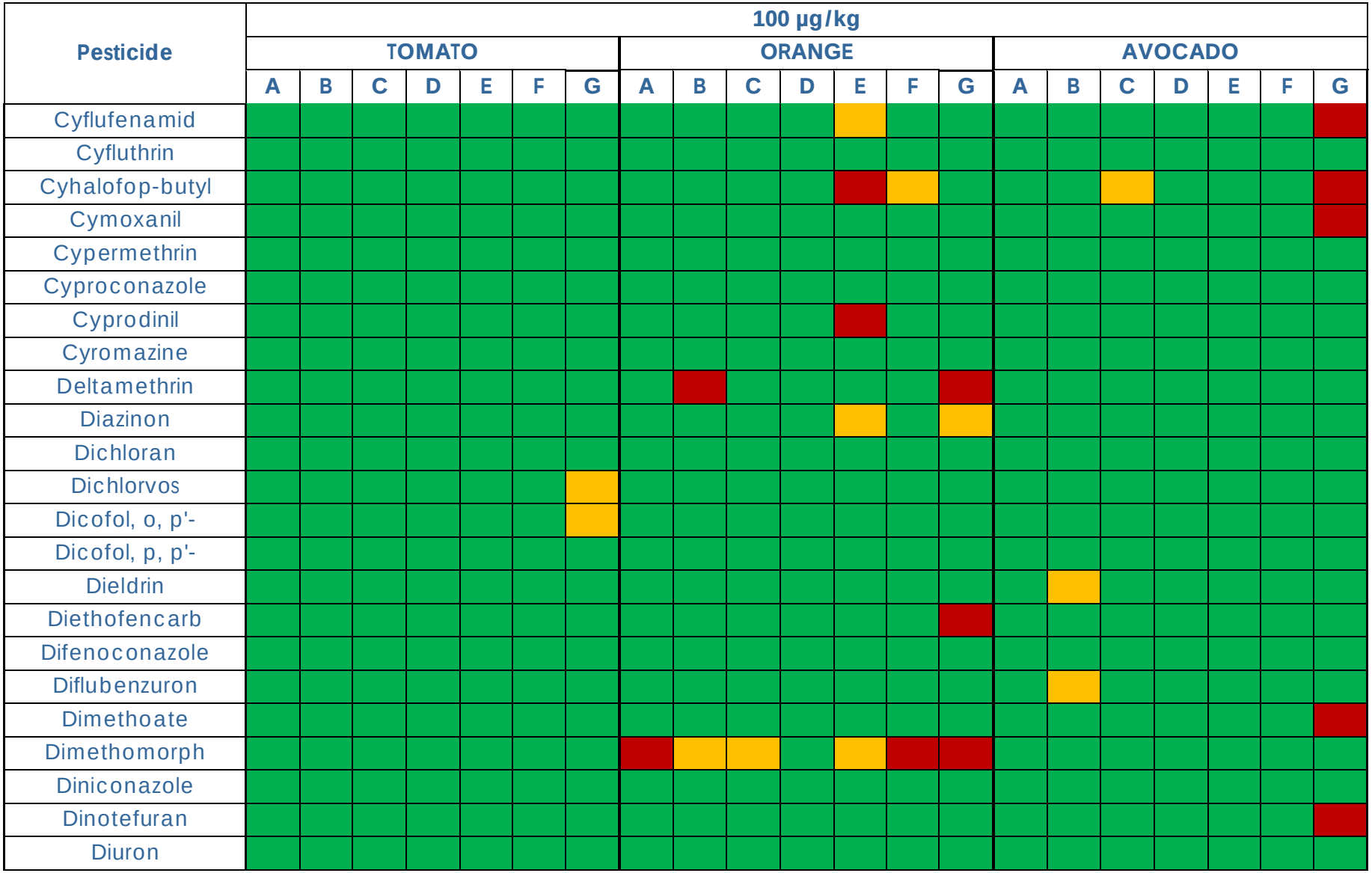
Pesticide	10 µg/kg																											
	TOMATO										ORANGE										AVOCADO									
	A	B	C	D	E	F	G	H	I	J	A	B	C	D	E	F	G	H	I	J	A	B	C	D	E	F	G	H	I	J
Tritosulfuron																														
Zoxamide																														

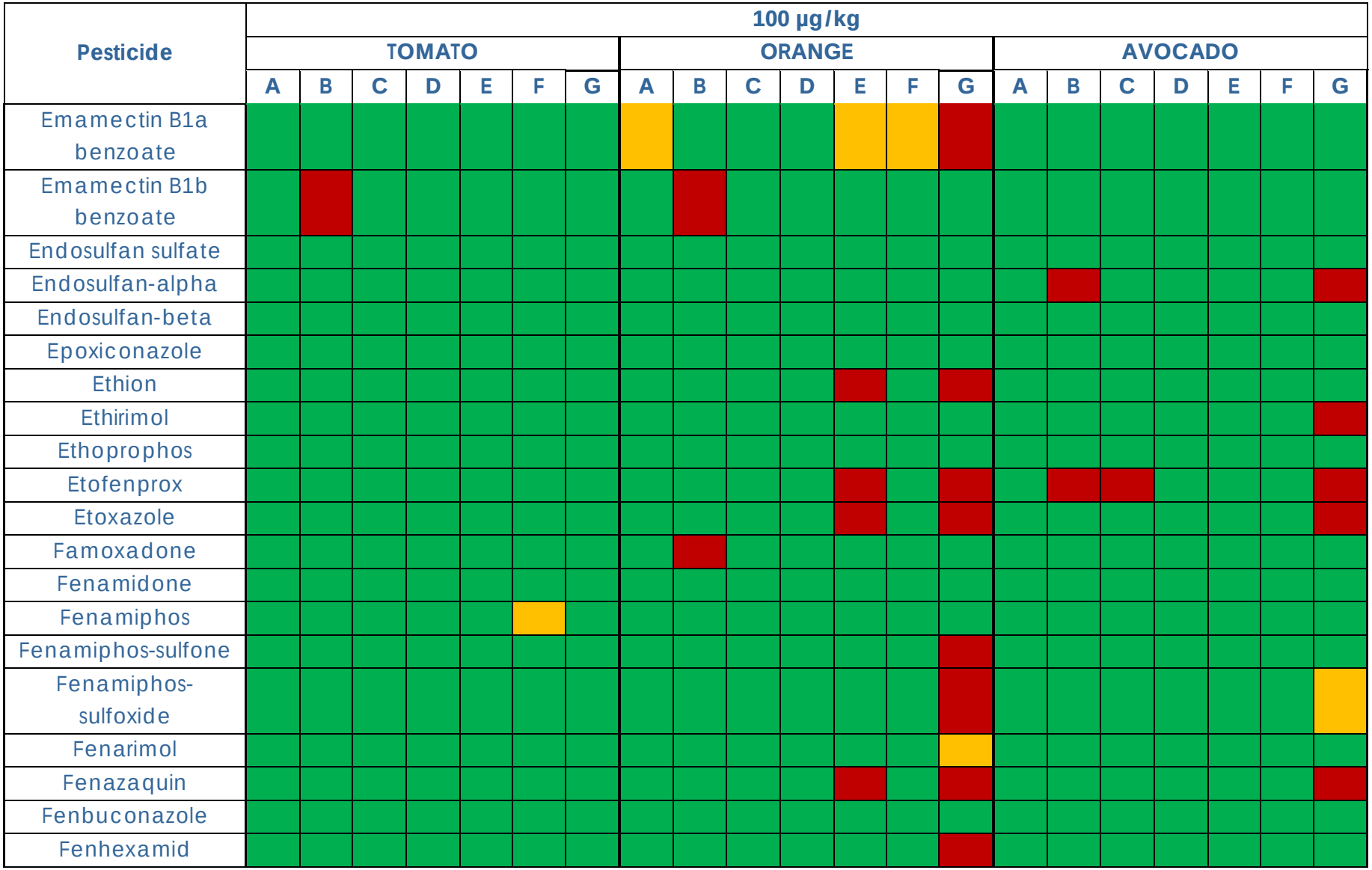
A = multiple-level calibration of the same matrix (tomato, orange and avocado); single-level calibration: **B** = 5 µg/kg, **C** = 10 µg/kg, **D** = 50 µg/kg, **E** = 100 µg/kg, **F** = 200 µg/kg; **G** = multiple-level calibration of another matrix from the same group of matrices (potato, strawberry and olive); **H** = procedural calibration; **I** = sample standard addition; **J** = extract standard addition

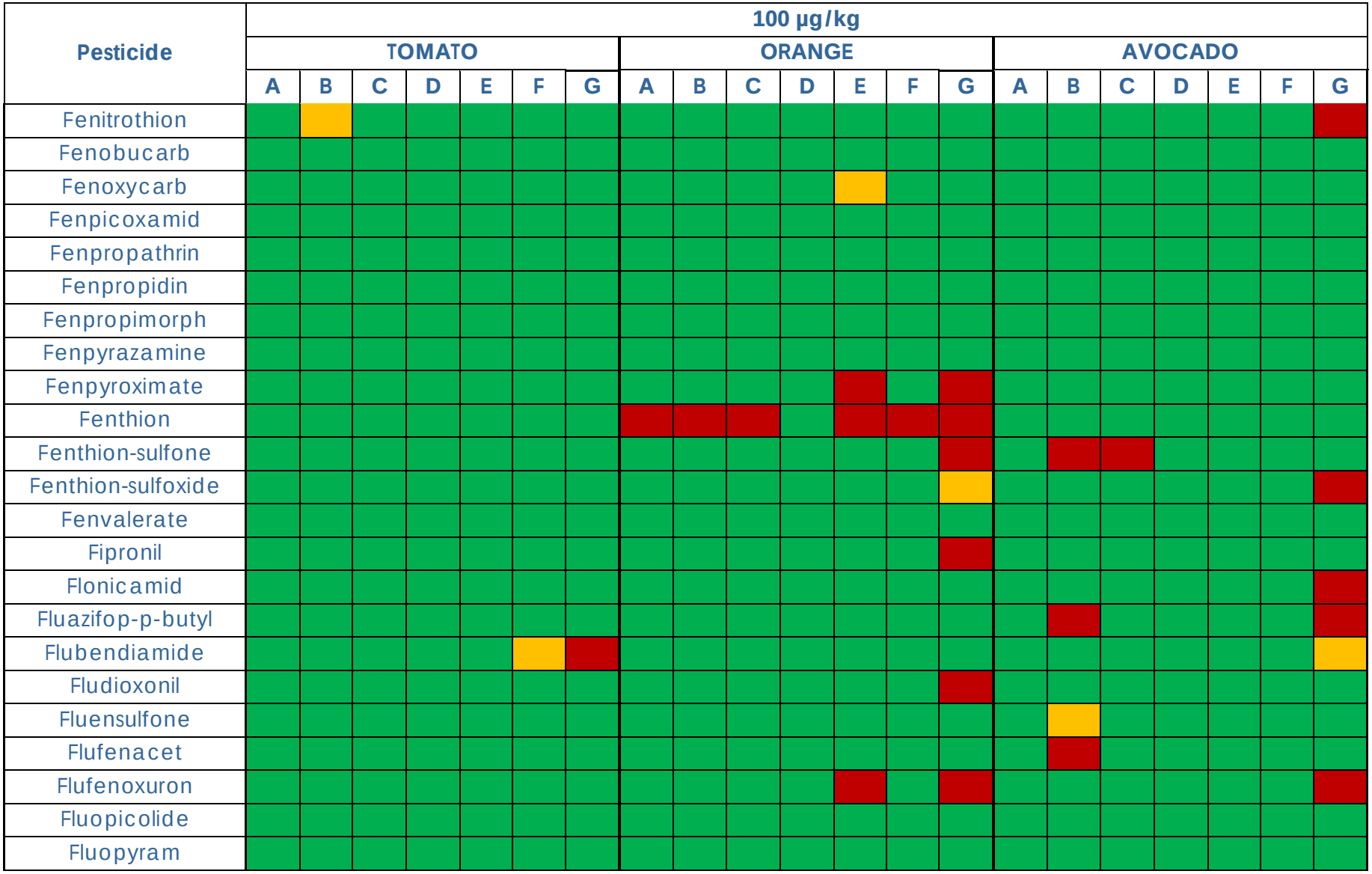
Table 2. Percentage variation of concentrations from the theoretical concentrations at the 100 µg/kg level Pesticides with a variation of |0-21%| are shown in green, |21-25%| in orange and >|25%| in red.
A = multiple-level calibration of the same matrix (tomato, orange and avocado); single-level calibration: **B** = 5 µg/kg, **C** = 10 µg/kg, **D** = 50 µg/kg, **E** = 100 µg/kg, **F** = 200 µg/kg; **G** = multiple-level calibration of another matrix from the same group of matrices (potato, strawberry and olive)

Pesticide	100 µg/kg																											
	TOMATO							ORANGE							AVOCADO													
	A	B	C	D	E	F	G	A	B	C	D	E	F	G	A	B	C	D	E	F	G							
2,4'-DDE																												
2-Phenylphenol																												
Acephate																												
Acetamiprid																												
Acrinathrin																												
Aldicarb																												
Aldicarb-sulfone																												

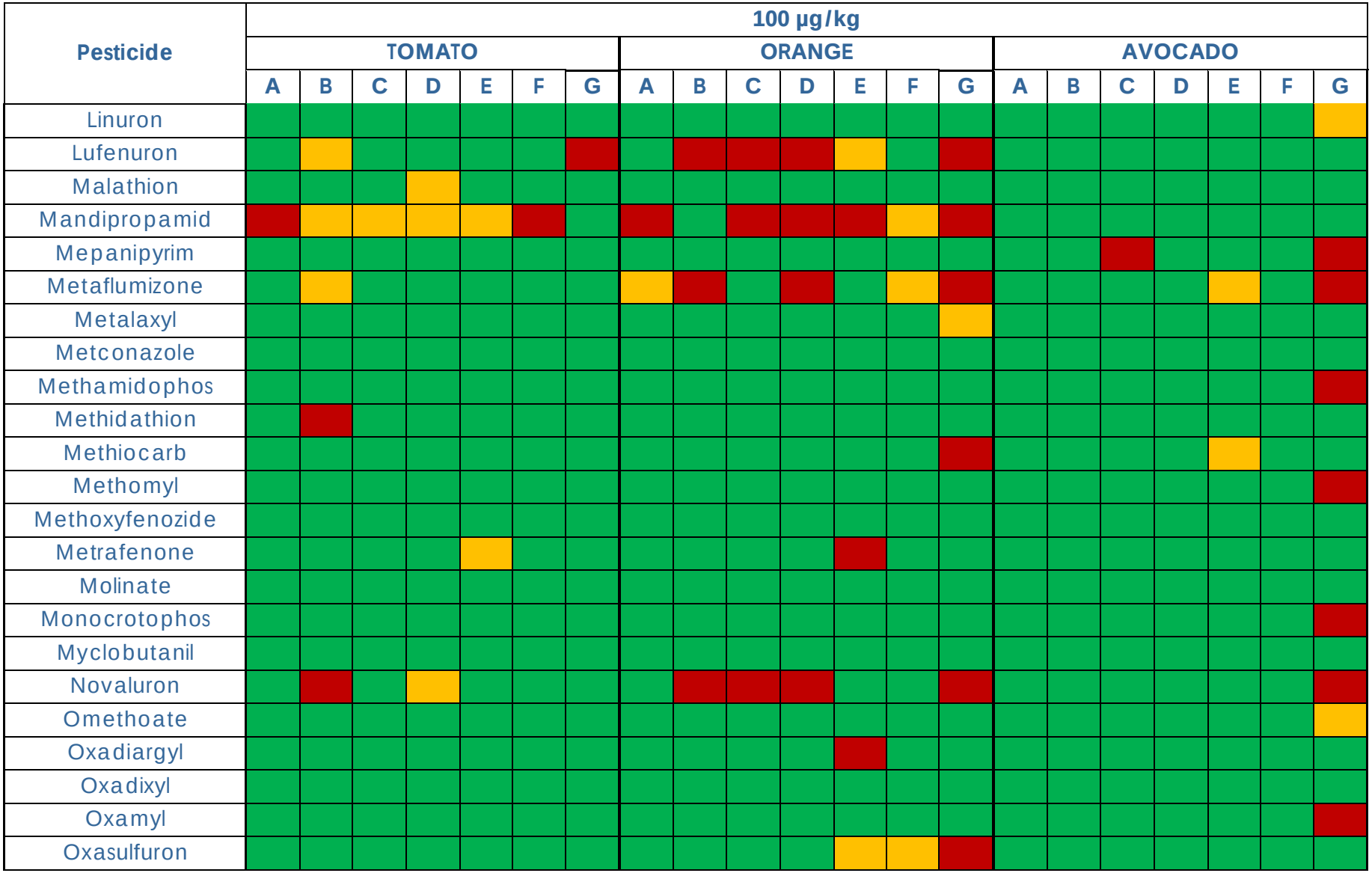

Pesticide
100 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G A B C D E F G A B C D E F G
Ametoctradin
Azinphos-methyl
Azoxystrobin
Benalaxyl
Benzovindiflupyr
Bifenthrin
Bitertanol
Boscalid
Bromopropylate
Bupirimate
Buprofezin
Carbaryl
Carbendazim
Carbofuran
Chlorantraniliprole
Chlorfenapyr
Chlorfluazuron
Chloridazon
Chlorpropham
Chlorpyrifos
Chlorpyrifos-methyl
Clomazone
Clopyralid


Pesticide
100 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G A B C D E F G A B C D E F G
Cyflufenamid
Cyfluthrin
Cyhalofop-butyl
Cymoxanil
Cypermethrin
Cyproconazole
Cyprodinil
Cyromazine
Deltamethrin
Diazinon
Dichloran
Dichlorvos
Dicofol, o, p'-
Dicofol, p, p'-
Dieldrin
Diethofencarb
Difenoconazole
Diflubenzuron
Dimethoate
Dimethomorph
Diniconazole
Dinotefuran
Diuron


Pesticide
100 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G A B C D E F G A B C D E F G
Emamectin B1a benzoate
Emamectin B1b benzoate
Endosulfan sulfate
Endosulfan-alpha
Endosulfan-beta
Epoxiconazole
Ethion
Ethirimol
Ethoprophos
Etofenprox
Etoxazole
Famoxadone
Fenamidone
Fenamiphos
Fenamiphos-sulfone
Fenamiphos-sulfoxide
Fenarimol
Fenazaquin
Fenbuconazole
Fenhexamid


Pesticide
100 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G A B C D E F G A B C D E F G
Fenitrothion
Fenobucarb
Fenoxycarb
Fenpicoxamid
Fenpropathrin
Fenpropidin
Fenpropimorph
Fenpyrazamine
Fenpyroximate
Fenthion
Fenthion-sulfone
Fenthion-sulfoxide
Fenvalerate
Fipronil
Flonicamid
Fluazifop-p-butyl
Flubendiamide
Fludioxonil
Fluensulfone
Flufenacet
Flufenoxuron
Fluopicolide
Fluopyram

Pesticide	100 µg/kg																				
	TOMATO							ORANGE							AVOCADO						
	A	B	C	D	E	F	G	A	B	C	D	E	F	G	A	B	C	D	E	F	G
Fluquinconazole																					
Flusilazole																					
Flutriafol																					
Fluxapyroxad																					
Formetanate Hydrochloride																					
Fosthiazate																					
Haloxyfop																					
Haloxyfop-methyl																					
Heptachlor																					
Hexaconazole																					
Hexythiazox																					
Imazalil																					
Indoxacarb																					
Ioxynil																					
Iprovalicarb																					
Isocarbophos																					
Isoprothiolane																					
Isopyrazam																					
Isoxaflutole																					
Kresoxim-methyl																					
Lambda-Cyhalothrin																					


Pesticide
100 µg/kg
TOMATO
ORANGE
AVOCADO
A B C D E F G
Linuron
Lufenuron
Malathion
Mandipropamid
Mepanipyrim
Metaflumizone
Metalaxyl
Metconazole
Methamidophos
Methidathion
Methiocarb
Methomyl
Methoxyfenozide
Metrafenone
Molinate
Monocrotophos
Myclobutanil
Novaluron
Omethoate
Oxadiargyl
Oxadixyl
Oxamyl
Oxasulfuron

Pesticide	100 µg/kg																				
	TOMATO							ORANGE							AVOCADO						
	A	B	C	D	E	F	G	A	B	C	D	E	F	G	A	B	C	D	E	F	G
Oxathiapipronil																					
Paclobutrazol																					
Parathion-methyl																					
Penconazole																					
Pencycuron																					
Pendimethalin																					
Penflufen																					
Penthiopyrad																					
Permethrin																					
Phenthoate																					
Phosmet																					
Picolinafen																					
Pirimicarb																					
Pirimicarb-desmethyl																					
Pirimiphos-methyl																					
Propaquizafop																					
Prothiofos																					
Pyriofenone																					
Quinalphos																					
Quinoclamine																					
Quintozene																					
Quizalofop																					

Pesticide	100 µg/kg																				
	TOMATO							ORANGE							AVOCADO						
	A	B	C	D	E	F	G	A	B	C	D	E	F	G	A	B	C	D	E	F	G
Quizalofop-ethyl																					
Rotenone																					
Tetramethrin																					
Tolfenpyrad																					
Triallate																					
Triflumizole																					
Triticonazole																					
Tritosulfuron																					
Zoxamide																					

A = multiple-level calibration of the same matrix (tomato, orange and avocado); single-level calibration: **B** = 5 µg/kg, **C** = 10 µg/kg, **D** = 50 µg/kg, **E** = 100 µg/kg, **F** = 200 µg/kg; **G** = multiple-level calibration of another matrix from the same group of matrices (potato, strawberry and olive)

Table 3. Percentage of analysed compounds that fall into each of the classifications at 10 µg/kg (green, orange or red).

10 µg/kg									
Quantification	**TOMATO**			**ORANGE**			**AVOCADO**		
	|0-21%|	**|21-25%|**	**>|25%|**	**|0-21%|**	**|21-25%|**	**>|25%|**	**|0-21%|**	**|21-25%|**	**>|25%|**
Multiple-level calibration	98%	1%	1%	98%	1%	1%	93%	4%	3%
Single-level calibration: 5 µg/kg	92%	5%	3%	93%	3%	4%	89%	5%	6%
Single-level calibration: 10 µg/kg	99%	1%	0%	98%	1%	1%	86%	6%	8%
Single-level calibration: 50 µg/kg	99%	1%	0%	97%	1%	2%	93%	2%	5%
Single-level calibration: 100 µg/kg	98%	1%	1%	79%	2%	19%	92%	2%	6%
Single-level calibration: 200 µg/kg	91%	5%	4%	90%	4%	6%	91%	3%	6%
Multiple-level calibration of another matrix of the	96%	1%	3%	64%	5%	31%	64%	10%	26%

10 µg/kg									
Quantification	TOMATO			ORANGE			AVOCADO		
	|0-21%|	|21-25%|	>|25%|	|0-21%|	|21-25%|	>|25%|	|0-21%|	|21-25%|	>|25%|
same group of matrices									
Procedural calibration	97%	1%	2%	78%	8%	14%	95%	1%	4%
Sample standard addition	92%	4%	4%	86%	5%	9%	83%	4%	13%
Extract standard addition	66%	11%	23%	55%	8%	37%	45%	12%	43%

Table 4. Percentage of analysed compounds that fall into each of the classifications at 100 µg/kg (green, orange or red).

100 µg/kg									
Quantification	TOMATO			ORANGE			AVOCADO		
	|0-21%|	|21-25%|	>|25%|	|0-21%|	|21-25%|	>|25%|	|0-21%|	|21-25%|	>|25%|
Multiple-level calibration	97%	2%	1%	94%	2%	4%	98%	1%	1%
Single-level calibration: 5 µg/kg	93%	4%	3%	89%	3%	8%	88%	3%	9%

100 µg/kg									
Quantification	TOMATO			ORANGE			AVOCADO		
	|0-21%|	|21-25%|	>|25%|	|0-21%|	|21-25%|	>|25%|	|0-21%|	|21-25%|	>|25%|
Single-level calibration: 10 µg/kg	98%	1%	1%	94%	1%	5%	90%	4%	6%
Single-level calibration: 50 µg/kg	97%	2%	1%	95%	0%	5%	98%	1%	1%
Single-level calibration: 100 µg/kg	99%	1%	0%	79%	6%	15%	97%	2%	1%
Single-level calibration: 200 µg/kg	95%	2%	3%	92%	4%	4%	97%	1%	3%
Multiple-level calibration of another matrix of the same group of matrices	93%	4%	3%	71%	5%	24%	64%	4%	30%

7. Conclusions

The evaluation of the results obtained for different matrix groups, taking into account the workload of the laboratory, leads us to the conclusion that the best way to quantify is with a **multiple-level calibration** in the same type of matrix. With this method, quantifying the **10 µg/kg** sample, **98%** of the compounds analysed in tomato and orange showed a variation with respect to the theoretical concentration of less than |21%|, and with a complicated matrix such as avocado, **93%**.

Quantification with **single-level calibration** is in general valid, obtaining in most of the cases variations above **90%**, although better quantification is obtained when the level used to quantify is **very close** in concentration to the fake sample. As this cannot be known in advance, quantification may be somewhat affected.

Quantification using a multiple-level calibration prepared with **another matrix** from the same commodity group may be acceptable in simpler matrix groups. However, in more complex matrices it seems to be less advisable, as the differences in quantification can increase. In the case of tomato quantified with the potato multiple-level calibration by GC-MS/MS, 100% of the pesticides presented a variation of less than |21%|. For the compounds analysed by LC-MS/MS, similar results were found, 94%. In the case of **avocado**, 70% of the compounds analysed by GC-MS/MS obtained values lower than |21%| quantified with olive multiple-level calibration, and for LC-MS/MS the percentage of compounds analysed below this value was 62%. The most remarkable case is **orange**, where the percentage of compounds analysed by GC-MS/MS with a percentage of variation lower than |21%| quantified with strawberry multiple-calibration was 96%, and 52% for the compounds analysed by LC-MS/MS.

Analysis by **procedural calibration** is very acceptable: 97% of the compounds analysed in tomato, 78 % in orange and 95% in avocado showed a variation of less than |21%| quantifying the 10 µg/kg level. In addition, this method of quantification can **compensate differences in recoveries**. However, quantifying with this method requires the use of significant **amounts** of analytical standards.

Quantification by **standard addition to the sample** gives generally acceptable results, obtaining variations between **92%-83%** in the case of the 10 µg/kg level, and may be a perfectly applicable option when the laboratory does not have an acceptable **blank sample** to prepare a matrix-matched calibration curve.

Results obtained in this study by **standard addition to the extract** are generally **lower**, as the percentage of compounds that showed a variation below |21%| at the 10 µg/kg level were below **70%** in all matrices evaluated.

8. References

[1] Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. Document Nº SANTE/11312/2021. EUROPEAN UNION REFERENCE LABORATORIES.

[2] COMMISSION IMPLEMENTING REGULATION (EU) 2022/741 of 13 May 2022 concerning a coordinated multiannual control programme of the Union for 2023, 2024 and 2025 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin.

[3] Working document on pesticides to be considered for inclusion in the national control programmes to ensure compliance with maximum residue levels of pesticides residues in and on food of plant and animal origin (SANCO/12745/2013).

APPENDIX I: MASS TRANSITIONS

Table A. Detection parameters for the selected compounds analysed by LC-MS/MS.

Compound	Precursor Ion (1)	Product ion (1)	Precursor Ion (2)	Product ion (2)	Ret. Time (min)
Acephate	184	143	184	125	2,793
Acetamiprid	223	126	223	56	6,076
Aldicarb	213	89	213	116	7,472
Aldicarb-sulfone	239,9	223	239,9	86	3,545
Ametoctradin	276,2	176,1	276,2	149	12,978
Azinphos-methyl	318	132,1	318	261	10,331
Azoxystrobin	404	372	404	344	10,727
Benzovindiflupyr	398	377,9	398	342	12,441
Bifenthrin	440,1	181	440,1	198,2	15,151
Bitertanol	338,2	269,2	338,2	99,1	12,655
Buprofezin	306	201	306	116	13,568
Carbaryl	202	145	202	127	9,002
Carbendazim	192	160	192	132	4,213
Carbofuran	222	165	222	123	8,674
Chlorantraniliprole	483,9	452,9	483,9	285,9	10,345
Chlorfluazuron	540	382,9	540	158,1	14,224
Chloridazon	222,1	104,1	222,1	92	6
Chlorpropham	214	123,9	214	96	8,581
Clomazone	240,1	124,9	240,1	127,8	10,498
Clopyralid	192	146	192	110	3,101
Cyflufenamid	413	294,9	413	240,8	12,81
Cyhalofop-butyl	375,1	256	375,1	120,1	13,061
Cymoxanil	199,1	128	199,1	110,9	6,416
Cyprodinil	226,2	92,9	226,2	76,9	11,634
Cyromazine	167	59,9	167	125	1,761
Diazinon	305	169	305	153	12,535
Dichlorvos	220,8	108,8	220,8	78,9	8,512
Diethofencarb	268	226	268	180	10,639
Difenoconazole	406	251	406	337	12,883
Diflubenzuron	311	158	311	141	11,881
Dimethoate	230	199	230	171	6,054
Dimethomorph	388	301	388	165	11,053
Diniconazole	326,1	70	326,1	159	12,987
Dinotefuran	203,1	129,1	203,1	114,1	3,321
Diuron	233	72,1	233,03	160	10,035
Emamectin B1a benzoate	886,5	158,1	886,5	302,2	13,431
Emamectin B1b benzoate	872,5	157,9	872,5	82	13,164
Epoxiconazole	330,1	121	330,1	101,2	11,761

Compound	Precursor Ion (1)	Product ion (1)	Precursor Ion (2)	Product ion (2)	Ret. Time (min)
Ethion	385,1	199	385,1	171	13,713
Ethirimol	210,2	140,1	210,16	43,1	7,35
Ethoprophos	243,1	130,9	243,1	97	11,87
Etofenprox	394,2	177,3	394,2	359,1	14,887
Etoxazole	360	140,9	360	304	14,047
Famoxadone	392	331	392	238	12,486
Fenamiphos	304,1	217,1	304,1	234	12,078
Fenamiphos-sulfone	336,1	266	336,1	188	9,012
Fenamiphos-sulfoxide	320,1	108,1	320,11	292,1	8,805
Fenarimol	331	268	331	259	11,761
Fenazaquin	307,3	161,3	307,3	147,2	14,367
Fenbuconazole	337,1	70	337,1	125,1	11,917
Fenhexamid	302	97	302	55	11,645
Fenobucarb	207,8	95	207,8	152,1	10,832
Fenoxycarb	302,2	88,2	302,2	116,2	12,033
Fenpicoxamid	615,3	515	615,3	238,9	13,24
Fenpropidin	274,3	147,1	274,3	85,8	10,362
Fenpropimorph	304,3	147,1	304,3	130	10,645
Fenpyrazamine	332,2	272,1	332,2	230,2	11,508
Fenpyroximate	422,2	366,2	422,21	107	13,951
Fenthion	279	247,1	279	169,1	12,264
Fenthion-sulfone	310,7	125	310,7	108,8	9,271
Fenthion-sulfoxide	295	280	295,02	109	8,988
Fipronil	434,9	329,9	434,9	249,9	12,195
Flonicamid	230,1	202,6	230,1	173,9	4,378
Flubendiamide	680,9	254	680,9	273,9	12,396
Fludioxonil	265,9	228,9	265,9	158	11,101
Flufenacet	364,1	152	364,1	194,1	11,835
Flufenoxuron	489,1	158	489,1	140,9	13,988
Fluquinconazole	376	307,1	376	108	11,49
Flutriafol	302,1	70,1	302,1	95	9,839
Fluxapyroxad	381,9	362	381,9	342	11,244
Formetanate Hydrochloride	222,1	165,1	222,13	65,1	2,895
Fosthiazate	284	227,8	284	103,8	9,475
Haloxyfop	362,1	316,2	362,1	288,1	12,135
Haloxyfop-methyl	375,9	316	375,9	287,9	12,957
Hexaconazole	314,1	70,1	314,1	159	12,712
Hexythiazox	353,1	228,2	353,1	168,2	13,896
Imazalil	297	159	297	255	9,503
Ioxynil	369,8	126,8	369,8	214,8	10,039
Iprovalicarb	321,2	119	321,2	202,9	11,806

Compound	Precursor Ion (1)	Product ion (1)	Precursor Ion (2)	Product ion (2)	Ret. Time (min)
Isoprothiolane	291	230,7	291	189,1	11,22
Isoxaflutole	360	250,9	360	219,7	10,096
Linuron	249	160,1	249,02	133	10,73
Lufenuron	508,9	339	508,9	325,9	13,678
Malathion	331	127,1	331	285	11,307
Mandipropamid	412,1	328,1	412,13	356,1	11,095
Metaflumizone	505	302	505	328	13,402
Metalaxyl	280,3	220	280,3	192,4	10,073
Metconazole	320,1	70,1	320,1	125	12,663
Methamidophos	142,1	94,1	142,1	125	2,33
Methiocarb	226	169	226,1	121,1	10,903
Methomyl	163,1	106	163,1	88	4,137
Methoxyfenozide	369,3	149	369,3	133	11,518
Metrafenone	409,1	209,1	409,1	226,9	12,769
Monocrotophos	224,2	127	224,2	193,1	4,739
Myclobutanil	289,2	70,2	289,2	125,1	11,478
Novaluron	490,8	470,7	490,8	305,1	13,215
Omethoate	214,1	125	214,1	183	3,128
Oxadiargyl	341,1	222,9	341,05	150,9	12,68
Oxamyl	237	72	237	90	3,839
Oxasulfuron	407,1	150,1	407,1	209,7	8,158
Oxathiapipronil	540,2	500	540,2	522	11,228
Paclobutrazol	294,1	70,1	294,1	125,2	11,26
Penconazole	284	70	284	159	12,377
Pencycuron	329,1	125,1	329,1	89,1	12,901
Pendimethalin	282,1	212,1	282,1	194,1	13,806
Penflufen	318,1	234	318,1	141	12,327
Penthiopyrad	357,9	149	357,9	207,6	12,476
Phenthoate	321	247,1	321	79,1	12,216
Phosmet	318	160	317,99	133	10,331
Pirimicarb	239,2	72,2	239,2	182,1	7,603
Pirimicarb-desmethyl	225,1	72,1	225,1	168,1	5,294
Pirimiphos-methyl	306,2	164,2	306,2	108,2	12,583
Propaquizafop	444,1	99,9	444,1	371	13,376
Pyriofenone	366,1	183,9	366,1	209	12,787
Quinalphos	299,1	270,8	299,1	242,8	12,079
Quinoclamine	208	105,1	208	77	7,866
Quizalofop	345	299	345	254,9	11,76
Quizalofop-ethyl	373,1	271,2	373,09	255,1	13,186
Rotenone	395	213,1	395	192,1	11,875
Tolfenpyrad	384,1	197	384,1	170,9	13,47
Triallate	306	145	306,01	86	13,85

Compound	Precursor Ion (1)	Product ion (1)	Precursor Ion (2)	Product ion (2)	Ret. Time (min)
Triflumizole	346,1	277,8	346,1	72,9	13,243
Triticonazole	318,1	70,2	318,1	125,2	11,719
Tritosulfuron	446	195	446	145	10,515
Zoxamide	336	187	336	159	12,52

Table B. Detection parameters for the selected compounds analysed by GC-MS/MS.

Compound	Precursor Ion (1)	Product ion (1)	Precursor Ion (2)	Product ion (2)	Ret. Time (min)
2,4'-DDE	246	176	246	211	7,112
2-Phenylphenol	170	115	170	141	4,462
Acrinathrin	208	181	289	93	9,187
Benalaxyl	204	176	148	105	8,105
Boscalid	140	112	140	76	10,341
Bromopropylate	341	185	341	155	8,66
Bupirimate	273	193	273	108	7,528
Chlorfenapyr	247	227	247	200	7,667
Chlorpyrifos	314	258	314	286	6,499
Chlorpyrifos-methyl	286	271	288	93	6,077
Cyfluthrin	163	127	226	206	10,051
Cypermethrin	163	127	209	141	10,275
Cyproconazole	222	125	139	111	7,759
Deltamethrin	253	172	181	152,1	11,462
Dichloran	206	176	206	148	5,457
Dicofol, o, p'-	251	139	139	111	8,37
Dicofol, p, p'-	251	139	139	111	7,726
Dieldrin	279	243	345	263	7,48
Endosulfan sulfate	272	237	387	289	8,278
Endosulfan-alpha	239	204	195	160	7,335
Endosulfan-beta	207	172	240,9	205,9	7,858
Fenamidone	268	180	238	103	8,834
Fenitrothion	277	260	277	109	6,352
Fenpropathrin	265	210	265	89	8,696
Fenvalerate	167	125	125	89	10,828
Fluazifop-p-butyl	282	91	282	238	7,637
Fluensulfone	119	92	226	206	4,738
Fluopicolide	209	182	173	109	8,269
Fluopyram	173	145	223	196	6,928
Flusilazole	233	165	233	152	7,565
Heptachlor	272	237	272	143	6,155
Indoxacarb	203	134	264	148	11,48

Compound	Precursor Ion (1)	Product ion (1)	Precursor Ion (2)	Product ion (2)	Ret. Time (min)
Isocarbophos	136	108	230	212	6,633
Isopyrazam	159	139	359	303	9,478
Kresoxim-methyl	206	116	206	131	7,566
Lambda-Cyhalothrin	197	141	197	161	9,187
Mepanipyrim	222	207	222	158	7,259
Methidathion	145	85	145	58	7,132
Molinate	187	126	126	55	4,492
Oxadixyl	163	132	163	117	7,984
Parathion-methyl	263	109	233	124	6,124
Permethrin	163	127	183	153	9,666
Picolinafen	376	238	238	145	8,709
Prothiofos	309	239	309	221	7,33
Quintozene	295	237	295	265	5,589
Tetramethrin	164	107	164	77	8,645