

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 9100 A

Product name: HYCOP

Chemical active substance:

Copper hydroxide, 500 g/kg (as Cu)

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

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Version history

When	What
August 2020	Updated by applicant
January 2021	RMS finalised the assessment
August 2021	The final version of the RR after commenting

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are:

- none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gaps are:

- none

A letter of access to protected data for copper compound allowing the renewal of approval is submitted by applicant to support the application for HYCOP.

Commodity/crop	Supported/ Not supported
Berries and small fruits (Grapevine)	Supported
Root and tuber vegetables (Potato)	Supported
Solanaceous fruits (Tomato, aubergine)	Supported
Pome fruit (apple, pear, quince)	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of Copper hydroxide in plant protection product is provided as follows:

Comments of zRMS:	<p>The proposed analytical method is suitable for the determination of copper hydroxide in plant protection product Copper Hydroxide 50 % WP.</p> <p>The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. Proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance.</p> <p>The validation of the analytical method has been accepted.</p>
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Reference: KCP 5.1.1

Report Copper Hydroxide 50 % WP: Method development and validation for determination of the content of active substance in the formulation, Małgorzata XXX, 2016, Code of study: BA-07/16

Guideline(s): SANCO/3030/99 rev. 4
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Test item

Trade name : **Copper Hydroxide 50% WP**
Active substance: Copper hydroxide
Chemical name: Copper (II) hydroxide
CAS No: [20427-59-2]
Molecular mass : 97.56 g/mol
Molecular formula: CuH_2O_2
Batch No: SCL – 150192

Reference item

Reference item used for validation was:
Copper oxychloride, Supelco, 97.0 %, Lot no 4308600

Reagents and equipment

Apparatus and materials

Titrometer TitroLine *alpha*, SCHOTT
Analytical balance Mettler, AT261 DeltaRange
Beaker, 150 mL volume
Volumetric flask, 100 mL volume
Pipette, 10 mL volume

Reagents

Sodium thiosulphate, standardized solution, (0,1M), POCh
Sodium fluoride, pure for analysis, POCh (aqueous saturated solution ~ 48 g/l)
Potassium iodide, pure for analysis, POCh
Sulphuric acid, pure for analysis (water solution 1 M) - water solution of sulphuric acid, (5.5 mL of concentrated sulphuric acid in 100 mL solution of distilled water).
Distilled water

Validation - Results and discussions

Specificity

Iodometric determination of copper is hindered by the presence of substances that can liberate iodine from iodides – as, for example, iron (III). Sodium fluoride was added to mask small quantities of iron (III).

Linearity

The linearity of the detector response was assessed using five standard solutions at the mass range of copper oxychloride from 0.0826 g to 0.1797 g, which corresponds to the concentration range of 67 % to 134 % of copper content in copper hydroxide in the preparation.
Results showed that the analytical method is linear over the range tested for the active ingredient (Correlation coefficient $R^2 > 0.99$)

Repeatability (Precision)

The test was performed on the basis of six independent determinations of active substance content in the test item.

The relative standard deviation obtained was 0.13 0.19% . Since this value was lower than the acceptable relative standard deviation for main ingredient ($\sim 50\%$) $RSDr \leq 2.97$ 1.49% , the repeatability test for the active ingredients was considered acceptable.

Recovery (Accuracy)

Accuracy of active substance determination in Copper Hydroxide 50 % WP was assessed by recovery value at two levels of concentration. Each of twelve 150 mL beakers were charged with appropriate amount of sample and weighed. About 0.05 g of the copper oxychloride standard was added to the each of the first six flasks. To the each of remaining six flasks 0.01 g of the copper oxychloride standard was added. Determination of Cu (II) content was performed according to the method described above. Obtained final results were examined and the nominal and calculated contents were compared.

The obtained result was 99.35 %, for main ingredient at concentration $>10\%$ the average recovery value should be $100 \pm 2\%$, therefore accuracy of the analytical method can be considered acceptable.

Table 5.2-1: Methods suitable for the determination of Copper hydroxide in plant protection product Copper hydroxide 50% WP

	Copper hydroxide
Author(s), year	Małgorzata XXX, 2016,
Principle of method	Potentiometric titration with platinum electrode method
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Range of copper oxychloride from 0.0826 g to 0.1797 g (corresponds to the concentration range of 67 % to 134 % of copper content in copper hydroxide in the preparation) $R^2 = 1$
Precision – Repeatability Mean $n = 6$ (%RSD)	$RSD = 0.13$ 0.19% Acceptable relative standard deviation for main ingredient ($\sim 50\%$) is $RSDr \leq 2.97$ 1.49% .
Accuracy $n = 12$ (% Recovery)	Average recovery : 99.35 %
Interference/ Specificity	No interference, the method is specific
Comment	None

Conclusion

The method for determination of copper hydroxide in Copper Hydroxide 50 % WP preparation is specific. The validation parameters for linearity, instrument precision, repeatability and accuracy are within the acceptance range.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

Acceptable methods for analysis of relevant impurities in plant protection product are ongoing and will be provided as soon as possible.

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

Comments of zRMS:	<p>The proposed analytical method is suitable for the determination of relevant impurities As, Cd, Ni, Pb, Co, Cr, Sb, and Hg in plant protection product Copper Hydroxide 50% WP.</p> <p>The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. The LOQs were determined.</p> <p>In the case of Sb, Pb, Cr, Co, As, Ni, the initial concentrations in the unfortified samples are more than 10% of the concentrations added and thus, according to SANCO/3030/99 rev. 5, the marginal recovery should be calculated instead of total recovery. In this case, the calculated marginal recoveries for lower levels for some of the impurities are outside the 70-130% limit and the Horrat value is between 1 and 2. However, as the determination of concentrations of the relevant impurities in the PPP performed with 5 repetitions indicate good precision and these concentrations are below the limits set in Commission Implementing Regulation (EU) 2018/1981 for these impurities, the validation can be accepted.</p> <p>The validation of the analytical method has been accepted.</p>
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Reference: KCP 5.1.1/2

Report Determination of As, Cd, Ni, Pb, Co, Cr, Sb, and Hg in Copper Hydroxide 50% WP. J. A. XXX XXX, 2020. Report No. 19-4150-16

Guideline(s): SANCO/3030/99 rev. 5

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

As, Cd, Ni, Pb, Co, Cr and Sb have been determined simultaneously in a sample of test item by ICP-OES. Hg has been determined in a sample of test item by Direct Mercury Analyzer.

As, Cd, Ni, Pb, Co, Cr and Sb

Preparation of test item solutions

Weigh (to the nearest 0.1 mg) 475-525 mg of test item into a 50 ml Digitube, add 5 ml of nitric acid and add 1 ml of perchloric acid. Weigh five independent samples of test item. Put all the tubes in the block digestion system and heat at 110°C for 40 minutes. Refrigerate and bring to volume (25 ml) with MilliQ water. Filter with paper filter on a 50 ml falcon tube and centrifuge by 5 min at 3000 rpm. Transfer the content on a plastic tube before analysis.

Hg

Preparation of test item solutions

Weigh 0.09-0.11 g of test item into a quartz boat and the sample weigh is transferred from the analytical balance to the DMA-80. Sample boats are loaded onto the instrument autosampler. Weigh two independent samples of test item.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) HYCOP/SHA 9100 A

	Sb	Pb	Cr	Co	As	Cd	Ni	Hg
Author(s), year	J. A. XXX XXX, 2020							
Principle of method	ICP-OES							Direct Mercury Analyzer
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	9 points 0.02-4.0 mg/L y=200.489 75x + 83 R = 0.99996	9 points 0.02-4.0 mg/L y=50.9890 1x + 5.0791179 R = 0.99991	10 points 0.005-2.0 mg/L y=1533.09 252x + 48.005837 R = 0.99991	10 points 0.005-2.0 mg/L y=2892.67 589x + 7.0689480 R = 1.0000	9 points 0.02-4.0 mg/L y=126.848 29x + 7.4457034 R = 0.99996	10 points 0.005-2.0 mg/L y=10522.8 0007x - 96 R = 1.0000	9 points 0.02-4.0 mg/L y=320.093 66x + 17.735017 R = 0.99994	8 5 points 0.5-50 ng Hg (5-500 mgHg/kg) y=0.91110 x + 0.0835009 R = 0.99965
Precision – Repeatability Mean n = 5 (%RSD)	0.02 mg/L %RSD = 7% 0.5 mg/L %RSD = 3%	0.1 mg/L %RSD = 5% 0.5 mg/L %RSD = 2%	0.01 mg/L %RSD = 8% 0.5 mg/L %RSD = 2%	0.006 mg/L %RSD = 9% 0.5 mg/L %RSD = 1%	0.1 mg/L %RSD = 17% 0.5 mg/L %RSD = 1%	0.1 mg/L %RSD = 1% 0.5 mg/L %RSD = 1%	0.1 mg/L %RSD = 8% 0.5 mg/L %RSD = 3%	1 ng Hg %RSD = 4% 20 ng Hg %RSD = 1%
Accuracy n = 5 (% Recovery)	0.02 mg/L Mean recovery: 90 ± 6% 0.5 mg/L Mean recovery: 76 ± 2%	0.1 mg/L Mean recovery: 81 ± 4% 0.5 mg/L Mean recovery: 75 ± 2%	0.01 mg/L Mean recovery: 107 ± 9% 0.5 mg/L Mean recovery: 127 ± 2%	0.006 mg/L Mean recovery: 91 ± 9% 0.5 mg/L Mean recovery: 76 ± 1%	0.1 mg/L Mean recovery: 87 ± 15% 0.5 mg/L Mean recovery: 100 ± 1%	0.1 mg/L Mean recovery: 82 ± 1% 0.5 mg/L Mean recovery: 79 ± 1%	0.1 mg/L Mean recovery: 106 ± 9% 0.5 mg/L Mean recovery: 109 ± 3%	1 ng Hg Mean recovery: 96 ± 3% 20 ng Hg Mean recovery: 92 ± 1%
Interference/ Specificity	No interference							
LOQ	LOQ = 1 mg/kg (FP) formulated product	LOQ = 5 mg/kg (FP)	LOQ = 0.5 mg/kg (FP)	LOQ = 0.3 mg/kg (FP)	LOQ = 5.0 mg/kg (FP)	LOQ = 5.0 mg/kg (FP)	LOQ = 5.0 mg/kg (FP)	LOQ = 0.01 mg/kg (FP)

Conclusion

The method for determination of relevant impurities Sb, Pb, Cr, Co, Cd, Ni, As and Hg in formulation Copper Hydroxide 50% WP was successfully validated and is suitable for determination of relevant impurities in the test item.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No. 44.305 is available for Copper hydroxide.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

All information and validation data were provided in the EU review of Copper compounds and were considered adequate.

Please refer to post-registration methods.

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of Copper hydroxide (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Total copper	5 mg/kg	Reg. (EC) No 149/2008
Plant, high acid content		5 mg/kg	Reg. (EC) No 149/2008
Plant, high protein/high starch content (dry commodities)		10 mg/kg	Reg. (EC) No 149/2008
Plant, high oil content		20 mg/kg	Reg. (EC) No 149/2008
Plant, difficult matrices (hops, spices, tea)		40 mg/kg	Reg. (EC) No 149/2008
Muscle	Total copper	5 mg/kg	Reg. (EC) No 149/2008
Milk		2 mg/kg	Reg. (EC) No 149/2008
Eggs		2 mg/kg	Reg. (EC) No 149/2008
Fat		5 mg/kg	Reg. (EC) No 149/2008
Liver, kidney		30 mg/kg	Reg. (EC) No 149/2008

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Soil (Ecotoxicology)	Total copper	5 mg/kg	NOEC (<i>Eisenia andrei</i>) = 8.4 mg Cu/kg soil
Drinking water (Human toxicology)	Dissolved Copper	2 mg/L	EU Directive 98/83
Surface water (Ecotoxicology)	Dissolved Copper	NOEC: 15.5 µg Cu (total)/L	EFSA, 2018 Lowest NOEC from aquatic toxicity study on <i>Oncorhynchus mykiss</i>
Air	Total copper	24.0 µg/m ³	AOEL sys/AOEL inhal: 0.08 mg/kg bw/d
Tissue (meat or liver)	Total copper	0.1 mg/kg	SANCO/825/00 rev 8.1; general limit
Body fluids		0.05 mg/L	

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Copper hydroxide in plant matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Total copper				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	2 mg/kg	AAS (for total copper content)	XXX F. (2002b) report n°00119 EU agreed dRAR Annex B5.1.2, France, 2017
	Primary	0.2 mg/kg	AAS	XXX F., XXX S., (2010) report n° RA.09.23 EU agreed dRAR Annex B.5.1.2, France, 2017
	Primary	3.0 mg/kg in tomato juice 1.1 mg/kg in melon pulp 0.8 mg/kg melon peel	AAS (measurement at 324.8 nm)	XXX S., (2016) report n° RA.16.08 EU agreed dRAR Annex B.5.1.2, France, 2017
	Primary (matrix effect evaluation)	Not applicable	FAAS (measurement at 324.8 nm)	KCP5.2.1/02, XXX S., (2017) report n° RA.17.02 Appendix 2
	Primary EN 13805	Not applicable	Atomic absorption (flame, electrothermal (ET), hydride, cold-	Anon., (2014) report n° EN 13805 EU agreed

Component of residue definition: Total copper				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
			vapour) techniques and ICP-MS	dRAR Annex B5.2.1, France, 2017
	Primary	1 mg/kg	AAS (for total copper content)	KCP 5.2.1/01
	ILV	-	Not required.	EU agreed dRAR Annex B5.2.1, France, 2017
	Confirmatory (if required)	-	-	-
High acid content	Primary	5 mg/kg	AAS (for total copper content)	XXX F. (2002a) report n°00123 EU agreed dRAR Annex B.5.2.1, France, 2017
	Primary	0.2 mg/kg	AAS	XXX F., XXX S., (2010) report n° RA.09.23 EU agreed dRAR Annex B.5.1.2, France, 2017
	Primary (matrix effect evaluation)	Not applicable	FAAS (measurement at 324.8 nm)	KCP5.2.1/02, XXX S., (2017) report n° RA.17.02 Appendix 2
	ILV	-	Not required.	EU agreed dRAR Annex B5.2.1, France, 2017
	Confirmatory (if required)	-	Not required.	-
High oil content	Primary	1.0 mg/kg in oilseed rape	AAS (measurement at 324.8 nm)	XXX F., XXX S., (2010) report n° RA.09.23 EU agreed dRAR Annex B5.1.2, France, 2017
	Primary	5.5 mg/kg	FAAS (measurement at 324.8 nm)	XXX S., (2017) report n° RA.17.02 Appendix 2
	Primary EN 13805	Not applicable	Atomic absorption (flame, electrothermal (ET), hydride, cold-vapour) techniques and ICP-MS	Anon., (2014) report n° EN 13805 EU agreed dRAR Annex B5.2.1, France, 2017
	ILV	-	Not required.	EU agreed dRAR Annex B5.2.1, France, 2017
	Confirmatory (if required)	-	Not required.	-
High	Primary	7.5 mg/kg	FAAS (measurement	XXX S., (2017)

Component of residue definition: Total copper				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
protein/high starch content (dry)			at 324.8 nm)	report n° RA.17.02 Appendix 2
	Primary EN 13805	Not applicable	Atomic absorption (flame, electrothermal (ET), hydride, cold-vapour) techniques and ICP-MS	Anon., (2014) report n° EN 13805 EU agreed dRAR Annex B5.2.1, France, 2017
	ILV	-	Not required.	EU agreed dRAR Annex B5.2.1, France, 2017
	Confirmatory (if required)	-	Not required.	-
Difficult (if required, depends on intended use)	Primary	0.5 mg/kg in hops	AAS (measurement at 324.8 nm)	XXX F., XXX S., (2010) report n° RA.09.23 EU agreed dRAR Annex B5.1.2, France, 2017
	ILV	-	Not required.	EU agreed dRAR Annex B5.2.1, France, 2017
	Confirmatory (if required)	-	Not required.	-

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	The methods of analysis do not rely on an extraction process but utilises acid digestion with AAS or ICP-MS detection.

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable method for analysis of Total copper in animal matrices is given in the following table.

Table 5.3-4: Validated methods for food and feed of animal origin

Component of residue definition: Total Copper				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk, eggs, muscle, fat, kidney and liver	Primary EN14082:2003	Not applicable	Atomic absorption spectrometry (AAS)	Anon., (2003) report n° EN14082:2003 EU agreed dRAR Annex B5.2.2, France, 2017
	ILV	-	Not required	EU agreed dRAR Annex B5.2.1, France, 2017
	Confirmatory (if required)	-	Not required.	-

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	The methods of analysis do not rely on an extraction process but utilises acid digestion with AAS or ICP-MS detection.

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Copper hydroxide in soil is given in the following tables.

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: Total copper			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	5.0 mg/kg	ICP-AES (bio-available copper)	XXX, (2003) report n°20031084/02-UVX EU agreed dRAR Annex B5.2.3, France, 2017
Primary	20 mg/kg	AAS	XXX, (1989) report n° 88-003 EU agreed dRAR Annex B5.2.3, France, 2017
Primary	Soil: 15 mg/kg (following SANCO/825/00) 0.52 mg/kg (following	ICP-AES	XXX, (2004) report n° 20031084/01-UVX EU agreed

Component of residue definition: Total copper			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	DIN 32645)		dRAR Annex B5.2.3, France, 2017
Confirmatory	-	Not required.	-

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Copper in surface and drinking water is given in the following tables.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: Dissolved Copper				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water and Surface water	Primary	0.1 µg/L	Method DIN38406	EU agreed
	Primary	100 µg/L	ICP-AES	XXX A., (2000 and 2001) EU agreed dRAR Annex B.5.2.4, France, 2017
	Primary	30 µg/L	ICP-AES	XXX D.O., (1989) EU agreed dRAR Annex B.5.2.4, France, 2017
	Primary	0.3 µg/L	ICP-MS	XXX XXX, M. (2016) EU agreed dRAR Annex B.5.2.4, France, 2017
	Primary DIN 38406 Part 7	2 µg/L	AAS	Anon., (1991) EU agreed dRAR Annex B.5.2.4, France, 2017
	Primary ISO 15586:2003	Not applicable	AAS	Anon., (2004) EU agreed dRAR Annex B.5.2.4, France, 2017
	ILV	-	Not required.	EU agreed dRAR Annex B.5.2.4, France, 2017
	Confirmatory	-	Not required.	-

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Copper hydroxide in air is given in the following tables.

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: Total Copper			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.5 ng/m ³ 0.3 ng/m ³	AAS (for total copper) ICP/OES (for total copper)	Anon., (1999) EU agreed dRAR Annex B.5.2.5, France, 2017
Primary	0.3 µg/m ³	ICP-MS	KCP5.2.5/01, XXX XXX M., (2018) report n° CH-657/2017 Appendix 2
Confirmatory	-	Not required.	-

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Copper in body fluids and tissues is given in the following table.

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Total copper			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	3.0 mg/kg for plasma, 13.9 mg/kg for bile, 359 mg/kg for liver, 841 mg/kg for faeces and 46 mg/kg for carcass	ICP-AES (emission wave- length 324.752 nm)	XXX M.W., (2004) EU agreed dRAR Annex B.5.2.6, France, 2017
Confirmatory	-	Not required.	-

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

5.3.2.8 Other studies/ information

No new or additional studies have been submitted.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	XXX M.	2016	Copper Hydroxide 50 % WP: Method development and validation for determination of the content of active substance in the formulation, Institute of industrial organic chemistry, Code of study: BA-07/16 GLP/Unpublished	N	Sharda Cropchem. Ltd.
KCP 5.1.1/2	J. A. XXX XXX	2020	Determination of As, Cd, Ni, Pb, Co, Cr, Sb and Hg in Copper Hydroxide 50% WP. Laboratorios Munuera Report No. 19-4150-16 GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.2.1/01	XXX D.	2018	Determination of residues of 10% tribasic copper, 4% dimethomorph and 25% fosetyl aluminium in courgettes, melons and potatoes. Report no. B18_041 GLP Unpublished	N	Sharda Cropchem. Ltd.
KCP 5.2.1/02	XXX S.	2017	Method Validation for the determination of copper in/on dry and oily matrices and Matrix Effect evaluation on dry, oily, high water and acid matrices Company Report No RA.17.02 Isagro – Centro di Saggio BPL GLP Published	N	European Union Copper Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.5/01	XXX XXX M.	2018	Validation of the Analytical Method for the determination of Copper residues in Air Company Report No: CH-657/2017 ChemService GLP Unpublished	N	European Union Copper Task Force

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Please note that all data mentioned as part of DAR, RAR, or EFSA journals are considered as relied on.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2 and KCP 5.2	XXX, F.	2002a	Analytical method validation for the determination of copper in/on grapes and their processed fractions Company Report No: 00123 Isagro Ricerca S.r.l GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2 and KCP 5.2	XXX, F.	2002b	Analytical method validation for the determination of copper in/on tomatoes, their processed fractions and leaves. Company Report No: 00119 Isagro Ricerca S.r.l GLP Unpublished	N	European Union Copper Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2 and KCP 5.2	XXX, F., XXX S.	2010	Method validation for the reduction of the Limit of Quantification for copper in representative matrices of plant origin. Company Report No: RA.09.23 Isagro Ricerca S.r.l GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, R.J.	2008a	Magnitude of residues of copper in field melons (cucurbits-inedible peel) following applications of metallic copper (as copper oxychloride)/Cymoxanil (DPX-KK807) 44WP (9.5:1)-southern Europe, season 2007 Company Report No: DuPont-22565 Charles River Laboratories (UK) GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, R.J.	2008b	Magnitude of residues of copper in protected melons (cucurbits – inedible peel) following applications of metallic copper (as copper oxychloride) / cymoxanil (DPX-KK807) 44WP (9.5:1) – Southern Europe, season 2007 Company Report No: DuPont 22564 DuPont GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, A.C.	2006b	Magnitude of residues of copper and cymoxanil in field melons (fruiting vegetables) following applications of metallic copper (as copper oxychloride)/cymoxanil (DPX-KK807) 44WG (9.5:1) under maximum label rates - southern Europe, season 2005 Company Report No: DuPont-16970 Charles River Laboratories (UK) GLP Unpublished	N	European Union Copper Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2	XXX, AC	2006c	Magnitude of residues of copper and cymoxanil in protected melons (fruiting vegetables) following applications of metallic copper (as copper oxychloride)/cymoxanil (DPX-KK807) 44WG (9.5:1) under maximum label rates – southern europe, 2004 Company Report No: DuPont 14536 DuPont GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, A.C.	2006a	Magnitude of residues of copper and cymoxanil in field melons (fruiting vegetables) following applications of metallic copper (as copper oxychloride)/cymoxanil (DPX-KK807) 44WG (9.5:1) under maximum label rates - southern Europe, 2004 Company Report No: DuPont-14542, Revision No. 1 Charles River Laboratories (UK) GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, O	2005	Residue determination of copper in melon after 6 applications of ATOFAP02 (WG 20%) or ATOFAP17NC (WG 40%) Company Report No: B_05RFLME01 Staphyt GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, O	2006	Residue determination of copper in melon after 6 applications of ATOFAP02 (Copper - 20% WG) or ATOFAP17NC (Copper - 40% WG) Company Report No: B_06RFLME01 GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, O.	2015	A Field Study to Evaluate the Effects of Copper on the Earthworm Fauna in Central Europe Company Report No: 20031343/G1-NFEw Eurofins GLP Unpublished	N	European Union Copper Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2	XXX R., XXX S	2015	Kinetics and speciation of copper in copper based fungicide formulations used in crop protection (Update February 2016) F-Cu 2016-2 Department of Biology, University of Antwerp, Belgium Non-GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, C.	2000	Community level study with Copper hydroxide 50% WP in aquatic microcosms Company Report No: URA-001/4-50 Fraunhofer - Institut für Umweltchemie und Okotoxikologie - IUCT GLP: Yes Publised: No	N	European Union Copper Task Force
KCP 5.1.2	XXX W.J.M., XXX C.	2015	In vitro percutaneous absorption of copper, formulated as Copper hydroxide 50 WP or Copper oxychloride SC, through human and rat skin Company Report No: V20600/19 + Amendment 01 TNO, Zeist, the Netherlands GLP Unpublished	N	European Union Copper Task Force
KCP 5.1.2	XXX, A., de XXX H.P.M.	2016	Validation of the determination of ⁶⁵ Cu in receptor fluid, stripped skin, tape strips, receptor/donor wash solution and skin wash used in the ‘In vitro percutaneous absorption test of copper through human and rat skin’, using a double-focusing high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) Company Report No: V20801 Triskelion BV GLP Unpublished	N	European Union Copper Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2	XXX WJM	2016	In vitro dermal absorption of copper (Cu) from 8 formulations through human skin Company Report No: V9062 + Amendment 01 TNO, Zeist, the Netherlands GLP Unpublished	N	European Union Copper Task Force
KCP 5.2	XXX S.	2016	Method Validation for the determination of copper in/on tomato juice and melon (pulp and peel) Company Report No: RA.16.08 Isagro GLP Unpublished	N	European Union Copper Task Force
KCP 5.2	Anon.	2014	Foodstuffs – Determination of trace elements – Pressure digestion Report No.: DIN EN 13805 Deutsches Institut für Normung Non-GLP Published:	N	Public
KCP 5.2	Anon.	2003	Foodstuffs – Determination of trace elements – Determination of lead, cadmium, zinc, copper, iron and chromium by atomic absorption spectrometry (AAS) after dry ashing Report No.: EN 14082 European committee for standardization Non-GLP Published	N	Public
KCP 5.2	XXX, R.	2003	Validation of an analytical method for the determination of bioavailable copper in soil samples Company Report No: 20031084/02-UVX GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH GLP Unpublished	N	European Union Copper Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	XXX, D. O.	1989	Method validation report for terrestrial outdoor field dissipation study with copper-containing pesticides Company Report No: 88-003 Biospherics Inc. GLP Unpublished	N	European Union Copper Task Force
KCP 5.2	XXX, R.	2004	Validation of an analytical method for the determination of total copper in soil samples Company Report No: 20031084/01-UVX GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH GLP Unpublished	N	European Union Copper Task Force
KCP 5.2	XXX, A.	2001	Assessment of side effects of URA-13900-F-0-WP on the larvae of the midge, <i>Chironomous riparius</i> with the laboratory test method. Company Report No: 99520/01-ASCr GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH GLP Unpublished	N	European Union Copper Task Force
KCP 5.2	XXX, A.	2000	Assessment of side effects of URA-08740-F-0-WP on the larvae of the midge, <i>Chironomous riparius</i> with the laboratory test method Company Report No: 99507/01-ASCr GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH GLP Unpublished	N	European Union Copper Task Force
KCP 5.2	XXX M. P.	2016	Validation of the analytical method for the determination of copper in surface water Company Report No.: CH-157/2016 ChemService S.r.l. Controlli e Ricerche GLP Unpublished	N	European Union Copper Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	Anon.	1991	German standard methods for the examination of water, waste water and sludge; Cations (group E); Determination of copper by atomic absorption spectrometry (AAS) (E 7) Report No.: DIN 38406 Part 7, September 1991 Deutsches Institut für Normung Non-GLP Published	N	Public
KCP 5.2	Anon.	2004	Water quality. Determination of trace elements using atomic absorption spectrometry with graphite furnace Report No.: DIN EN ISO 15586 Deutsches Institut für Normung Non-GLP Published	N	Public
KCP 5.2	Anon.	1999	Determination of suspended matter in ambient air. Measurement of the concentration by mass of As, Be, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sb, Tl, Zn by atomic absorption spectrometry (AAS) after sampling on filters and digestion in an oxidising acid mixture. Report No.: VDI 2267, Part 1 Verein Deutscher Ingenieure Non-GLP Published	N	Public
KCP 5.2	Anon.	1997	Determination of suspended matter in ambient air. Determination of the mass concentration of Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, V, Zn by optical emission spectrometry (ICPOES) after sampling on filters and digestion in an oxidising agent. Report No.: VDI 2267, Part 5 Verein Deutscher Ingenieure Non-GLP Published	N	Public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2	XXX, M. W.	2004	Five copper substances: Absorption, distribution, and excretion in male rats. Company Report No: DuPont-11784 E.I. du Pont de Nemours and Company GLP Unpublished	N	European Union Copper Task Force

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Copper hydroxide

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.1.2.1.1 Analytical method 1

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	Method is accepted as pre-registration method
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Reference: KCP 5.2.1/01

Report Determination of residues of 10% tribasic copper, 4% dimethomorph and 25% fosetyl aluminium in courgettes, melons and potatoes. XXX D., Report no. B18_041

Guideline(s): Yes

- The Principles of Good Laboratory Practice (GLP) as described in the Italian D.L. N° 50 of the 2nd March 2007 (Directive 2004/09/CE and Directive 2004/10/CE), in the OECD guidelines 1997 (with updates) and D.L. 194/95. OECD SERIES ON PRINCIPLES OF GOOD LABORATORY PRACTICE AND COMPLIANCE MONITORING Number 1 (ENV/MC/CHEM(98)17) 1998
- Guidance document on pesticide residue analytical methods (ENV/JM/MONO(2007)17)
- OECD/OCDE 509 (2009) - OECD GUIDELINE FOR THE TESTING OF CHEMICALS
- Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 (SANCO/3029/99 rev.4 11/07/00)
- ASSIGNING VALUES TO NONDETECTED/NON-QUANTIFIED PESTICIDE RESIDUES IN HUMAN HEALTH FOOD EXPOSURE ASSESSMENTS, Office of Pesticide Programs, U.S. Environmental Protection Agency, 2000

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

For copper determination, an aliquot of homogenized sample was mineralized by acidic digestion and microwave heating and then the residues were quantified analyzing the filtered mineralized sample by a Flame Atomic Absorption Spectrometer (FAAS).

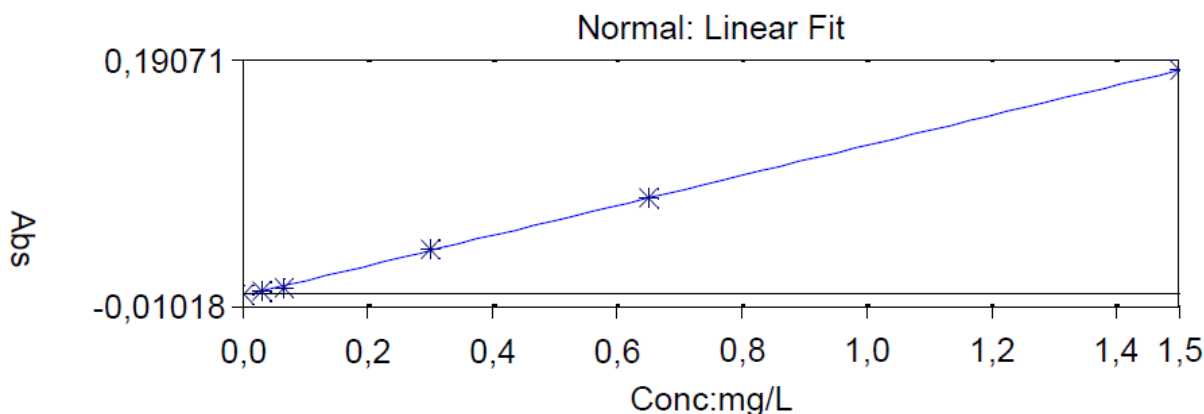
The analytical method adopted was validated within the present Analytical Phase; as all samples belong to the same commodity group (high water content), a full validation was conducted for courgettes while for the other two matrices (potatoes and melons) a limited set of validation tests was carried out to confirm the method performance. Samples deriving from untreated plots were used to be fortified for the validation tests.

Results and discussions

Linearity

Calibration curve was obtained by injecting calibration standards prepared in ultrapure water at five concentration levels in the range from 0.03 to 1.50 mg/L of copper. The response was shown to be linear in all the concentration range with a very good correlation factor of 0.9998, in compliance with the validation requirements.

The calibration curve was generated automatically by the instrumental software and every value of absorbance reported was the mean of three successive readings of the sample.



Recoveries and repeatability

A complete validation was carried out for courgettes, while for potatoes and melons a confirmation of validation was performed.

Recovery and repeatability data were calculated analyzing fortified untreated blank samples. The copper fortification were done directly in vessel, after sample weighting and before digestion. Recoveries were calculated as the copper residue found after the subtraction of mean residue found in the related untreated blank sample.

Two fortification levels were tested: at the expected LOQ at 1.00 mg/kg and at 10xLOQ, corresponding to 10.00 mg/kg, with five replicates for each level.

Recoveries were within the range 70÷110% and every relative standard deviation percentage (RSD%) obtained was lower than 20%, as required by SANCO/3029/99 rev.4.

Specificity

The analytical method is suitable of determining copper in the tested matrices and the specificity derives from its relative freedom from interferences, due to preliminary mineralization and operating at a defined wavelength characteristic for the analyte.

Limit of Quantification (LOQ) and Limit of Detection (LOD) - Statistical Evaluation

The LOQ tested was 1.00 mg/kg of copper in courgettes and melons (flesh and peel) while for potatoes the LOQ tested was 1.50 mg/Kg; recovery and repeatability data at these concentration levels were in compliance to SANCO/3029/99 rev.4 requirements.

As an amount of copper naturally occurring was expected in the interesting matrix it was also conducted a statistical evaluation of expected LOD and LOQ, to take in account the natural background copper level, according to EPA guideline (Office of Pesticides Programs – U.S. Environmental Protection Agency – Washington DC 20460 “Assigned Values to non-detected/non-quantified in human health food exposure assessments”, March 23, 2000).

The LOQ statistically evaluated confirmed the results experimentally obtained, being lower than the tested levels.

Table A 1: Recovery results from method validation of copper using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Courgettes	Copper	1.00 mg/kg	97%	6%	
	Copper	10.00 mg/kg	95%	1%	

Table A 2: Characteristics for the analytical method used for validation of copper residues in courgettes

	Copper
Specificity	The analytical method is suitable of determining copper in the tested matrices and the specificity derives from its relative freedom from interferences, due to preliminary mineralization and operating at a defined wavelength characteristic for the analyte.
Calibration (type, number of data points)	Calibration curve was obtained by injecting calibration standards prepared in ultrapure water at five concentration levels in the range from 0.03 to 1.50 mg/L of copper. The response was shown to be linear in all the concentration range with a very good correlation factor of 0.9998, in compliance with the validation requirements. The calibration curve was generated automatically by the instrumental software and every value of absorbance reported was the mean of three successive readings of the sample
Calibration range	Calibration curve: $Y=0.12266x -0.0023$ $R^2 = 0.9998$
Limit of quantification	1 mg/kg

Conclusion

A complete validation was conducted for courgettes in compliance to Document SANCO/3029/99 rev.4, paragraph 3 “Residues analysis in Plants, Plant products, food stuffs (of plant and animal origin) and

feeding stuffs”, with the exception of the blank values exceeding the 30% of LOQ level because of the natural contribution of copper occurring in plants. Only a confirmation of validation, consisting in the assessment of one blank and five recoveries at LOQ, was conducted for melons flesh, melons peel and potatoes.

A 2.1.2.1.2 Analytical method 2

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	Metod is accepted
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Reference:	KCP 5.2.1/02
Report	Method Validation for the determination of copper in/on dry and oily matrices and Matrix Effect evaluation on dry, oily, high water and acid matrices. XXX S.: 2017: RA.17.02
Guideline(s):	Yes REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99. Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection; 2010-11-16. OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007); 2007-08-13
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of this study is to determine the matrix effect in dry, oily, high water and acid matrices and to validate the method in dry and oily matrices at limits of quantification (LOQ) established.

Level of copper are determined from homogenized samples by acidic digestion and microwave heating. The solution containing the mineralized sample was analysed by reading of its absorbance at 324.8 nm, after calibration of the Flame Atomic Absorption Spectrometer (FAAS) with standard solutions in solvent.

The matrices reported in the following table cover the categories object of the study:

Commodity categories	Representative RAC commodities
High water content	Lettuce
High acid content	Grape
High oil content	Oilseed rape seed

Dry matrix	Wheat grain
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A full validation method was carried out only on oily matrix and dry matrix, while the matrix effect was evaluated for all matrices tested.

Effects of matrix constituents present in the final mineralized sample were assessed by comparing the concentration (mg/L) of Copper obtained from standard solutions in neat solvent with those observed for standards added in mineralized sample of untreated matrices.

Stock, standard solutions and fortification solutions were prepared in water solution of nitric acid 1%.

Results and discussions

- Determine the matrix effect in dry matrix (wheat grain), oily matrix (oilseed rape seed), matrix with high water content (lettuce) and matrix with high acid content (grape):

Matrix Effect was calculated for two levels of the calibration curve: one at a level near to the LOQ level and another at a higher level. The mean matrix effect for each matrix tested is reported below.

Matrix	Mean Matrix Effect (%)
Lettuce	-19.5
Grape	-11.5
Oilseed rape seed	-0.8
Wheat grain	-2.4

The mean effects of matrix on response were not significant for all matrices (<20%).

- Validation of a copper method in dry matrix (wheat grain) and oily matrix (oilseed rape seed) by FAAS:

The validation of this method was successful and has met the criteria reported in the Guideline Requirements. Method validation data are summarised in Table A 1 and Table A 2.

All mean recoveries were within the required 70-110% range for both matrices. The repeatability found has acceptable %RSD as all values were below the required 20%.

Table A 3: Recovery results from method validation of total copper using this analytical method

Crop group	Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
dry matrices	Wheat grain	7.5	7	88.6	4.6	-
		75	5	104.3	2.6	-
oily matrices	Oilseed rape seed	5.5	7	87.4	10.7	-
		55	5		3.4	-

Table A 4: Characteristics for the analytical method used for validation of total copper residues in wheat grain (dry matrices) and oilseed rape seed (oily matrices)

	Total copper
Specificity	The specificity of the method is given by using the F AAS analytical technique at the Copper specific wavelength of 324.8 nm. No

	Total copper
	additional confirmatory method is required. Blank values in control samples of the oilseed rape seed, used for method validation, were below 30% of the LOQ. Blank values in control samples of the wheat grain, used for method validation, were 37.8% of the set LOQ.
Calibration (type, number of data points)	The regression equation was generated by the calibration curve in solvent (type 1/x) using the absorbance responses versus the respective concentrations of the calibration standards. Number of data points = 6
Calibration range	Accepted calibration range: Standards prepared in solvent. For grain: 0.01 – 10.0 mg/L corresponding to 0.2 - 200 mg/kg. $y = 0.000509 + 0.110782 x$, $R^2 = 0.9999$ For grain: 0.0125 – 10.0 mg/L corresponding to 0.4 - 400 mg/kg. $y = -0.000391 + 0.101070 x$, $R^2 = 1.0000$
Assessment of matrix effects is presented	Yes. Matrix Effect was calculated for two levels of the calibration curve: one at a level near to the LOQ level and another at a higher level. The mean effects of matrix on response were not significant for all matrices (<20%).
Limit of determination/quantification	For oilseed rape seed (<i>oily matrix</i>) the LOQ tested have been first estimated on the basis of the natural background copper level in the untreated samples to establish the lowest possible LOQ, based on the background not exceeding 30% of the LOQ. For wheat grain (<i>dry matrix</i>) the tested LOQ was chosen to support a suggested MRL for the cereals at 15 mg/kg. To support an MRL the LOQ should not exceed~ of the proposed MRL <u>Tested LOQs:</u> Oilseed rape seed: 5.5 mg/kg Wheat grain: 7.5 mg/kg

Conclusion

The analytical method was successfully validated and meets all guideline criteria to determine residues of total copper in dry and oily matrices. Also, the matrix effect in dry, oily, high water and acid matrices was assessed as not significant for all matrices (<20%).

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

A 2.1.2.5.1 Analytical method 1

A 2.1.2.5.1.1 Method validation

Comments of zRMS:	Method is accepted
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Reference:	KCP 5.2.5/01
Report	Validation of the Analytical Method for the determination of Copper residues in Air. XXX XXX M.: 2018: CH-657/2017
Guideline(s):	Yes SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1
GLP:	Yes
Acceptability:	Yes

Materials and methods

Residues of copper in air are adsorbed with a membrane filter. The filter holder, 37 mm two-piece cassette, was assembled, preloaded with the GN-4 0.8 um Metrical® membrane filter and the support pad in MCE (mixed cellulose ester). A measured volume of air is drawn through the membrane filter. The membrane filter is dissolved with nitric acid (65-71% % v/v for ultratrace metal analysis) and the determination of Copper residues is performed by Inductively Coupled Plasma-Mass Spectrometry using an external standard.

Its quantification is achieved by comparing the Copper analytical standard intensity signal versus the intensity signal in air samples.

Instrumental conditions

Detector	:	ICP/MS
Power	:	1550 W
Carrier gas	:	0.85 L/min
Replicates	:	3 times
Sample introduction setting (peristaltic pump)		
Pump rate	:	0.1 rps
Copper (Cu)	:	m/z 63 (quantitative) m/z 65 (qualitative)
Germanium (Ge)	:	m/z 72 (used as internal standard correction) ⁽¹⁾

⁽¹⁾ Internal standard for Copper quantification.

Experiment:

- 1) For accuracy and precision: The air sampling was performed for 30 minutes at a 10 L/min air flow rate in order to sample a total 0.30 m³ volume of air and therefore to reach the required L.O.Q. of 0.30 µg/m³.
- 2) For retention capacity: The air sampling was performed at temperature of 35°C and relative humidity of 80% on a 0.30 µg/m³ fortified sample for 6 hours with a 10 L/min air flow rate in order to sample a total volume of air higher than 100 L, according to the guideline SANCO/825/00 rev. 8.1.

Results and discussions

Specificity:

Since the analysis performed by ICP/MS is highly specific, and gave both quantification and identification data, a confirmatory test using another instrumental technique was not necessary and specificity and therefore confirmatory were verified with the same injections and instrumental technique. Any interference from the control sample were < 30 % LOQ.

Linearity:

The Copper nominal concentration tested in injected solutions ranged from 1.00 to 50.00 µg/L, corresponding to a Copper concentration ranging from 0.17 µg/m³ to 8.33 µg/m³ in the air sample and was found to be linear for both ⁶³Cu quantifier and ⁶⁵Cu qualifier isotopes (correlation coefficient > 0.99).

No significant memory effect was detected in the washing dilution medium injected after the highest working standard solution and the range tested for Copper was found to be linear (each correlation coefficient > 0.99).

Limit of quantification:

The limit of quantification (L.O.Q.) was the low fortification level at 0.30 µg/m³ for Copper in air samples, corresponding to a final injected solution of 1.80 µg/L.

The limit of detection (L.O.D.), defined as half of the lowest calibration level, was 0.50 µg/L, corresponding to 0.08 µg/m³ for Copper in air samples.

Accuracy and Precision:

The SANCO/825/00 rev. 8.1 guideline requires mean recoveries for each level in the range from 70 to 110% and a RSD% lower than 20 % for each level. From the data obtained, these criteria were fulfilled and therefore precision and accuracy of the analytical method can be considered acceptable for both ⁶³Cu quantifier and ⁶⁵Cu qualifier isotopes.

Table A 5: Recovery results from method validation of copper using the analytical method

Matrix	Qualifier isotopes	Fortification level	n	Mean recovery (%)	RSD (%)
Air	⁶³ Cu	0.30 µg/m ³	6	83.7%	2.69%
		2.99 µg/m ³	6	83.0%	1.90%
	⁶⁵ Cu	0.30 µg/m ³	6	84.2%	3.32%
		2.99 µg/m ³	6	84.2%	1.50%

Retention capacity:

The obtained recovery value of 87% was in the acceptable range which demonstrates that the retention capacity was considered sufficient and no significant breakthrough occurred.

Conclusion

The analytical method for copper in air was successfully validated under SANCO/825/00 rev 8.1.

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitte