FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: FEL02

Product name: Cuprofix C/Cuprofix C Disperss

Chemical active substances:

Copper, 200 g/kg
Cymoxanil, 40 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT/

(Art. 33 new authorization)

Applicant: UPL Holdings Coöperatief U.A.

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Version history

When	What
March 2023	Part B-Section 5 -Core assessment, Version 01 of applicant
November 2023	zRMS assessment of dRR
April 2024	The final version of the RR after the commenting period

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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

Commodity/crop	**Supported/ Not supported**
Plant: high water, high acid, high oil, high protein/high starch content (dry) and difficult matrices	**Supported**
Animal: Muscle, milk, eggs, fat, liver, kidney	**Supported**
Soil	**Supported**
Water	**Supported**
Air	**Supported**
Body fluids and tissues	**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)

The plant protection product FEL02 was not the representative formulation in the active substance authorization or renewal of copper compounds or cymoxanil. There are no previously evaluated studies available for this application in the Central zone.

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

Copper

Comments of zRMS:	Applicant submitted the method of analysis of a.s. copper in formulation Bouillie Bordelaise RSR ~~Dispress~~ Disperss. Formulation Bouillie Bordelaise RSR ~~Dispress~~ Disperss contains the same amount of copper as formulation Cuprofix C (200 g/kg). The proposed analytical method is suitable for the determination of active substances copper in the plant protection product Cuprofix C. The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. The specificity of the method in the presence of another active

	substance (cymoxanil) was not determined; it was addressed in the study Re-validation of SOP DLA-249.1 Version 2 "Copper-cymoxanil mixed formulations (ATOFELnn) determination of content of active ingredients", Diepenhorst, P. C. (2010), Report no: DL 09-102 (see KCP 5.1.1/03). The proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.

Reference:	KCP 5.1.1/01
Report	Validation of draft SOP DLA-060 Copper Compounds determination of copper content in formulations, Diepenhorst, P. C. (2000), Report no: DL 99-065
Guideline(s):	No guideline specified in the study report, but method used is comparable to SANCO/3030/99 rev. 5 (2019) According to data requirements: Directive 96/46/EC, Annex I, 4. Analytical methods, 4.1
Deviations:	Yes, the specificity of the method in the presence of another active substance (cymoxanil) is not established.
GLP:	Yes
Acceptability:	Yes, the deviation does not have impact on study.

The aim of the study was to validate the method for the determination of copper in plant protection formulations containing Bordeaux mixture.

Materials and methods

Principle of the method

The analytical method SOP DLA-060 is an iodometric titration method after acid digestion. It involves the titration of iodine, liberated from potassium iodide by copper (II) ions, with sodium thiosulphate solution.

Materials

Test formulation:

Name: Bouillie Bordelaise RSR Disperss
Batch: 9045-3
Active substance content: 77% Bordeaux mixture technical (20% copper)

Copper Primary Reference substance:

Name: Copper
CAS: 7440-50-8
Purity: 99.9%

Sample preparation

Test formulation was dispersed in sulphuric acid and nitric acid and decomposed by boiling and the excess of nitric acid was removed. All organic material has been decomposed in this step. By boiling with water the eventually formed nitrosulphuric acid was hydrolyzed into nitrous acid and sulphuric acid. The excess of nitrous acid was decomposed with urea, the copper (II) was complexed with an excess of ammonia and the solution was neutralized with acetic acid. Sodium fluoride was added to prevent potential interferences by iron (III)-, arsenic (V)-, molybdenum (VI)- and antimony (V) ions.

Validation

In this study DL 99-065 the SOP DLA-060 has been validated for linearity, repeatability, accuracy, non-analyte interference, specificity, LOD and LOQ. Validation was performed with the copper reference substance. Accuracy/precision was determined from Bouillie Bordelaise RSR Disperss samples fortified with the copper reference substance. Validation data have been determined both on manual titration with visual endpoint indication and on automatic titration with potentiometric endpoint indication.

Validation - Results and discussions

Linearity

The analytical method SOP DLA-060 appears to be linear either by manual titration with endpoint determination by starch indicator or by automatic titration with potentiometric endpoint determination.

The calculated intercepts for manual titration and automatic titration (0.044 and 0.0075 mL, respectively) do not differ significantly from 0, so they are negligible.
The calculated slopes for both manual titration and automatic titration (156.95 and 157.87 mL/g, respectively) do not differ significantly from the theoretical value based on the reaction equations (157.38 mL/g). The correlation coefficients are 0.99998 and 0.99995. The average recoveries are not significantly different from 100%.

Reagent blanks
For both endpoint determinations no consumption of titrant for the reagent blank was found.

Repeatability
The average (n=10) copper content result in Bouillie Bordelaise RSR Disperss for the manual endpoint titration is 201.2 g/kg with a standard deviation of 0.287 g/kg. The precision (relative standard deviation, coefficient of variation) is 0.143% and is within the limits defined by the Horwitz curve (RSDr=1.71). For the automatic titration an average copper content of 200.3 g/kg is found with a standard deviation of 1.35 g/kg. The precision is 0.67% and is within the limits defined by the Horwitz curve (RSDr=1.71). The results are not significantly different according to the Student t-test. According to the F-test the variabilities of the two titration methods are significantly different. A cause-related explanation, why the automatic titration shows more variability than the manual titration, is not found.

Accuracy
The accuracy for added copper standard to Bouillie Bordelaise RSR Disperss is very good:
- for the manual titration 100.6% recovery is found with a standard deviation of 0.85%
- for the automatic titration 99.7% recovery is found with a standard deviation of 0.91%. These recoveries are not significantly different from 100%, so no effect on the analytical result caused by the matrix of Bouillie Bordelaise RSR Disperss is found.

The recoveries calculated for the total of copper present (Bouillie Bordelaise RSR Disperss and copper Standard) are 100.3% and 99.9% with standard deviations of 0.417% and 0.465%.

The accuracy for Bouillie Bordelaise RSR Disperss and the dyestuff Prussian Blue is very good too:
- for the manual titration 99.9% recovery is found with a standard deviation of 0.124%.
- for the automatic titration 99.7% recovery is found with a standard deviation of 0.235%.

Striking is the difference in variability of the repeatability testing and the accuracy testing. The large variability in the repeatability testing compared to the small variability in the accuracy testing can only be explained by possible micro inhomogeneity of the Test Item.

Linearity of recovery of Bouillie Bordelaise RSR Disperss
The linearity for copper in Bouillie Bordelaise in presence of a constant ratio of formulation agents with a range for copper from 60 - 160% of the target value (linearity of recovery) appears to be very good. For the manual titration the copper content in g (calculated with the average result in the repeatability testing) versus mL sodium thiosulphate solution 0.1 mol/L have a linear relation with a correlation coefficient of 0.99995, an intercept of - 0.099 mL and a slope of 158.41 g/mL. According to the standard error the intercept is negligible (0 within the upper and lower interval of confidentiality) and the slope is not significantly different from the theoretical value (157.38).

For the automatic titration the copper content in g (calculated with the average result in the repeatability testing) versus mL sodium thiosulphate 0.1 mol/L a linear relation is found with a correlation coefficient of 0.99998, an intercept of - 0.0036 mL and a slope of 157.30. According to the standard error the intercept is negligible (0 within the upper and lower interval of confidentiality) and the slope is practically the same as the theoretical value.

LOD/LOQ
For Bouillie Bordelaise the LOD is assessed to 0.76 g/kg and the LOQ to 2.5 g/kg on both titration methods.

As the automatic titration with potentiometric endpoint indication is more objective, needs less handling and is less sensitive for chemical interference, this method is preferred above the manual titration with visual endpoint indication.

Table 5.2-1: Method suitable for the determination of active substance copper in plant protection product Bouillie Bordelaise RSR Disperss

	Copper
Author(s), year	Diepenhorst, P. C., 2000
Principle of method	Iodometric titration after acid digestion
Linearity **n = 11**	Two outliers were identified and discarded from calculations in the manual titration method. Linear between 30 mg and 160 mg Cu (30, 130, 160 mg) Correlation coefficients (r) = 0.99998 (manual titration) and 0.99995 (automatic titration) The calculated slopes: 156.95 (manual titration) and 157.87 mL/g (automatic titration)
Precision – Repeatability Mean **n = 10** **(%RSD)**	Manual titration: an average copper content result in Bouillie Bordelaise RSR Disperss: 201.2 g/kg with a standard deviation of 0.287 g/kg. The precision (relative standard deviation, coefficient of variation) is 0.143%. Automatic titration (n=9, one outlier identified): an average copper content of 200.3 g/kg is found with a standard deviation of 1.35 g/kg. The precision is 0.67%. RSDr=1.71 Horrat value for manual titration: 0.08 Horrat value for automatic titration: 0.39
Accuracy **n =5** **(% Recovery)**	Added copper standard to Bouillie Bordelaise RSR Disperss (marginal recovery): Manual titration: 100.6% recovery; SD = 0.85% Automatic titration: 99.7% recovery; SD = 0.91%.
Interference/ Specificity	Specific for analysis of copper
Comment	The formulation used in the validation study was Bouillie Bordelaise RSR Disperss, which has a comparable composition as FEL02 in terms of Bordeaux mixture content.

Deviations from the study plan:

In several determinations for the linearity of detection with visual endpoint indication sodium fluoride is added, while according to the study plan these additions were not necessary because the analyses were done with pure coper and the interfering compounds could not be present. The analysis of copper standard at the levels, 30 mg, 130 mg and 160 mg were carried out in triplicate for suspected outliers.

For the same test with potentiometric endpoint indication several determinations have been done without yielding an equivalence point. After solving the problem by addition of potassium thiocyanate as in the endpoint determination, the lacking data point at 30 mg was determined at the end of the study in duplicate, while already one data point was achieved at that level in the beginning of the study. For the same reason for the linearity of recovery in both test items several extra mineralisations have been carried out for the potentiometric endpoint determination. For the repeatability of the copper content in Cuprofix 30 by manual titration only 8 determinations were carried instead of 10, since no more sample was available. For the linearity of recovery, the copper content in Cuprofix 30 by automatic titration at the level of 500 mg was carried out in quadruplicate determinations instead of duplicate, as the cure for the problems was found later in the study. For the reagent blanks both in the visual titration and the potentiometric titration only 2 determinations for each titration method have been carried out there was no titrant consumption at all. For the non-analyte interference titration for Cuprofix 30 with potentiometric endpoint determination 3 instead of 5 determinations were carried out as no titrant was consumed.

These changes have no impact on the study.

Conclusion

The analytical method SOP DLA-060 (iodometric titration after acid digestion) is suitable for the determination of the active substance copper in plant protection products containing Bordeaux mixture. Validation complies with SANCO/3030/99 rev. 5 (2019) for specificity, linearity, recovery and precision. The formulation used in the validation study was Bouillie Bordelaise RSR Disperss. Because of the selective character of the titration method and the comparable compositions of Bouillie Bordelaise RSR Disperss and FEL02 in terms of Bordeaux mixture content, it is concluded that the validation results obtained with Bouillie Bordelaise RSR Disperss are representative for FEL02 as well. The specificity of the method in the presence of the second active substance (cymoxanil) was not addressed.

Cymoxanil

Comments of zRMS:	Applicant submitted the method of analysis of a.s. cymoxanil in formulation Nautile WG containing cymoxanil TC (51 g/kg), mancozeb TC (790 g/kg), and surfactants and diluents (159 g/kg). The proposed analytical method is suitable for the determination of active substances cymoxanil in the plant protection product Cuprofix C. The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and accuracy. The specificity of the method in the presence of another active substance (copper) was not determined; it was addressed in the study Re-validation of SOP DLA-249.1 Version 2 "Copper-cymoxanil mixed formulations (ATOFELnn) determination of the content of active ingredients", Diepenhorst, P. C. (2010), Report no: DL 09-102 (see KCP 5.1.1/03). The proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.

Reference:	KCP 5.1.1/02
Report	Validation of draft SOP DLA-229.2 Mancozeb/cymoxanil WG determination of active ingredients and suspensibility, Diepenhorst, P. C. (1999), Report no: DL 99-024
Guideline(s):	Directive 96/46/EC, Annex I, 4. Analytical methods, 4.1
Deviations:	Yes, the specificity of the method in the presence of another active substance (cymoxanil) is not established.
GLP:	Yes
Acceptability:	Yes, the deviation does not have an impact on the study.

The aim of the study was to validate the method for the determination of cymoxanil and mancozeb in WG formulations.

Materials and methods

Materials
Test formulation:
Name: Nautile WG
Batch: 9904-1482/011
Active substance content: cymoxanil 51 g/kg

Cymoxanil analytical standard:
Name: Cymoxanil analytical standard:
CAS: 57966-95-7
Purity: 98.5%

Method:
HPLC-UV with external standard

Validation - Results and discussions
Linearity
The test solutions for linearity testing of cymoxanil have been made by weighting about 21 mg of cymoxanil working standard and 25 mg of acetophenone into a 50 mL volumetric flask. Then about 30 mL of acetonitrile was added, followed by dissolution by mixing, making up to the mark with acetonitrile at 20°C and mixing thoroughly (solution A). Subsequently, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL and 8 mL of solution A were pipetted into 100 mL volu-

metric flasks, the volume was made up to the mark with eluent and the contents of the flask were mixed well. Of these solutions as well as a blank solution (eluent) 10 µL has been injected and the peak areas of both cymoxanil and internal standard have been correlated with the contents in mg/10 mL.

Repeatability
10 replicate determinations of the formulation were made.

Interference
5 determinations of cymoxanil 50 g/kg blank formulation were made to determine non-analyte interference.

Accuracy
To determine the accuracy of the method, determinations with additions of about 25 mg (to the nearest 0.01 mg) of cymoxanil working standard to the blank formulation have been carried out 5 times.

Table 5.2-2: Method suitable for the determination of active substance cymoxanil in WG formulation containing 51 g/kg of cymoxanil.

	Cymoxanil
Author(s), year	Diepenhorst, P. C., 1999
Principle of method	HPLC-UV with external standard
Linearity **n = 7**	Linear between 0 mg/10 mL and 0.32 mg/10 mL Correlation coefficients (r) = 0.99997 Y = 2 603 180 * X – 19.35
Precision – Repeatability Mean **n = 10** **(%RSD)**	The average cymoxanil content found in the test substance was 48.23 g/kg with a standard deviation of 0.564 g/kg. The precision (relative standard deviation, coefficient of variation) was 1.17%. RSDr=2.12% Horrat value: 0.55
Accuracy **n =5** **(% Recovery)**	Marginal recovery: 102.05
Interference/ Specificity	Specificity and interference were studied using UV spectra of the analyte peak and the spectral peak purity. The purity index for cymoxanil was 1.000 and for the internal standard acetophenone 0.996, so no significant interference was found. The retention times and the UV spectra of both cymoxanil and internal standard peak in the samples show no significant difference from those of the standard. There was no non-analyte interference observed.
Comment	The formulation used in the validation study was Nautile WG containing cymoxanil TC (51 g/kg), mancozeb TC (790 g/kg), and surfactants and diluents (159 g/kg).

Conclusion

The analytical method SOP DLA-229.2 is suitable for the determination of the active substance cymoxanil in plant protection products in the form of WG. Validation complies with SANCO/3030/99 rev. 5 (2019) for specificity, linearity, recovery and precision.

Copper and Cymoxanil

~~Comments of zRMS:~~	~~Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information~~

Reference:	KCP 5.1.1/02
Report	Validation of draft SOP DLA 251 Version 0 Solution Disperss (ATOFDH01), determination of famoxadone cymoxanil and copper content, Heugens, R. (2003), Report no: DL 03 034
Guideline(s):	SANCO/3030/99 rev.4 (2000)
Deviations:	Yes, but no impact on study
GLP:	Yes
Acceptability:	Yes

The aim of the study was to validate the method for the determination of copper and cymoxanil in plant protection formulations containing Bordeaux mixture.

Materials and methods

Principle of the method

The copper content is determined after digestion with nitric acid and sulphuric acid to copper (II) ions, followed by reaction with potassium iodide to form iodine. The amount of iodine formed is determined by titration with sodium thiosulphate solution and the endpoint is determined by potentiometry. The method for analysis of copper is the same as described in KCP 5.1.1/01.

The cymoxanil content is determined by extraction with acetonitrile, filtration, dilution, followed by HPLC UV (228 nm) analysis using a reversed phase C18 column. The amount of active ingredient is assessed using an internal standard (ethyl benzoate).

Materials
Test formulation: ATOFDH01 (Solution Disperss); it contains in addition to the active substances cymoxanil (40 g/kg) and copper (200 g/kg) also famoxadone (20 g/kg).
Batch: 1.297.2
Type of formulation: WG

Validation - Results and discussions
Copper
The analytical procedure has been validated in the study DL 99 065 (KCP 5.1.1/01) for linearity, accuracy and precision for Bouillie Bordelaise RSR Disperss (Bordeaux mixture WG formulation containing 200 g/kg copper). The method was concluded to be suitable for the determination of copper in FEL 02 as well. None of the possible interfering inorganic ions but iron (III) will be present in the formulation Solution Disperss as was also in the case in the test items Bouillie Bordelaise RSR Disperss and Cuprofix 30. Therefore, the data on linearity, accuracy, precision are also valid for FEL02 formulation. Only the repeatability for the copper determination and the linearity of recovery were determined in this study

Linearity
Solutions of copper with an amount range of 0.0310 to 0.1625 g results in good linearity with a correlation coefficient of 0.99995. These concentrations are equivalent to a range from 62 to 325 g/kg copper in a sample Solution Disperss under the described analytical conditions. That range covers 31 to 163% of the target concentration.

Linearity of recovery
Increasing amounts of the test item Solution Disperss from 0.2025 till 0.8041 g are analyzed and the absolute copper concentration correlated with the sample weight results in good linearity. From 41% – 161% of the target sample weight no intercept is found with a correlation coefficient of 0.999989. A sample amount of 1.01136 g (203% of the target sample weight) is clearly beyond the range of the determination.

Repeatability
Repeatability was performed with titration immediately after the addition of the potassium iodide solution. The average copper content in Solution Disperss is 197.2 g/kg (n=10) with a standard deviation of 1.11 g/kg. The precision (RSD) is 0.56% and is within the limits defined by the Horwitz curve. (RSDr=1.71). The %RSD is considered acceptable (Hr value <1) and therefore the precision of the analytical method is considered acceptable.

Accuracy
The accuracy is calculated from the linearity of recovery. Samples of 0.2, 0.4, 0.6, 0.8 and 1 g were used and titration was done directly after addition of the potassium iodide and potassium thiocyanate. The highest sample was beyond the range of the method. The mean accuracy of the other samples was calculated to be 100.7% with a standard deviation of 0.57% compared to the repeatability results. According to the F test the variability in the accuracy is not significantly different from the variability in the repeatability. According to the Student t test the accuracy is adequate.

Specificity and interference
The blank sample and used chemicals for decomposing the sample show no interference. Potential interferences by iron (III) ions, arsenic (V) ions, molybdenum (VI) ions and antimony (V) ions were prevented by additional of fluoride. The method is therefore considered specific for copper in the investigated formulation showing no interference of importance for the adjuvants.

Cymoxanil

Linearity
Solutions of cymoxanil with a concentration range of 0 to 0.2013 mg/mL result in good linearity with a correlation coefficient of 0.99997. These concentrations are equivalent to a range from 0 to 72 g/kg cymoxanil in a sample Solution Disperss under the described analytical conditions. This range convers 0 to 181% of the target concentration.

Linearity of recovery
Increasing amounts of Solution Disperss from 0.1011 till 0.5010 g are analyzed and the absolute cymoxanil amount correlated with the sample weight results in good linearity. From 40 – 200% of the target sample weight no significant intercept was found with a correlation coefficient of 0.99999.

Repeatability
The average cymoxanil content in Solution Disperss is 45.48 g/kg (n=10) with a standard deviation of 0.126 g/kg. The precision (RSD) is 0.28% and is within the limits defined by the Horwitz curve (RSDr=2.13). The acceptability of the %RSD is considered acceptable (Hr value <1) and therefore the precision of the analytical method is considered acceptable.

Accuracy
The accuracy is calculated from the linearity of recovery. The mean accuracy is determined to be 100.2% with a precision of 0.48% compared to the repeatability results. According to the F test the variability in the accuracy is not significantly different from the variability in the repeatability. According to the Student t test the accuracy is adequate.

Specificity
Specificity and interference are studied using UV spectra of the analyte peak and the spectral peak purity. The retention times and the UV spectra of both cymoxanil peak and internal standard peak in the samples show no significant difference of those of the standards and thus no indications for interference for the investigated matrix are found. Student t test and F test result in no significant difference between repeatability and accuracy. Therefore, it can be concluded there is no systemic error shown.

Table 5.2-3: Methods suitable for the determination of active substances copper and cymoxanil in plant protection product Solution Disperss

	Copper	**Cymoxanil**
Author(s), year	Heugens, R., 2003	Heugens, R., 2003
Principle of method	Iodometric titration after acid digestion	HPLC-UV
Linearity **(Linear between mg/L / % range of the declared content)** **(Correlation coefficient, expressed as r)**	0.0310 – 0.1625 g Correlation coefficient: 0.99995	0 - 0.2013 mg/mL Correlation coefficient: 0.99997
Precision – Repeatability **Mean (n = 10, %RSD)**	0.56% RSDr = 1.71 Horrat value*: 0.33	0.28% RSDr = 2.13

	~~Copper~~	~~Cymoxanil~~
		~~Horrat value*: 0.13~~
~~Accuracy~~ ~~Mean (n = 6, %Recovery)~~	~~Recovery: 100.7%~~ ~~Precision: 0.56%~~	~~Recovery: 100.2%~~ ~~Precision: 0.48%~~
~~Interference/ Specificity~~	~~Specific for copper in the presence of cymoxanil~~	~~Specific for cymoxanil in the presence of copper~~
~~Comment~~	~~The formulation used in the validation study can be regarded as a worst case as it contains additional active substance (famoxadone). Therefore, the method is also considered acceptable for FEL02 formulation.~~	~~The formulation used in the validation study can be regarded as a worst case as it contains additional active substance (famoxadone). Therefore, the method is also considered acceptable for FEL02 formulation.~~

~~* Calculated by the applicant~~

~~Conclusion~~

~~The analytical methods presented above are acceptable for the determination of the active substances copper, cymoxanil and famoxadone in the presence of each other in the product Solution Disperss according to SANCO/3030/99 rev. 5. It is also concluded that the validation results obtained with Solution Disperss are representative for FEL02 as the presence of additional active substance, famoxadone, in the formulation can be regarded as a worst case~~

Comments of zRMS:	The proposed analytical methods are suitable for the determination of active substances copper and cymoxanil in the plant protection product Cuprofix C. The proposed analytical methods have been validated in terms of specificity, repeatability, and accuracy. The linearity for copper was validated in the study described in KCP 5.1.1/01 and for cymoxanil in the study described in KCP 5.1.1/02. The proposed methods fulfil the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical methods has been accepted.

Reference:	KCP 5.1.1/03
Report	Re-validation of SOP DLA-249.1 Version 2 “Copper-cymoxanil mixed formulations (ATOFELnn) determination of content of active ingredients”, Diepenhorst, P. C. (2010), Report no: DL 09-102
Guideline(s):	Directive 96/46/EC, Annex I, 4. Analytical methods, 4.1
Deviations:	Yes. 1. In this study only the repeatability, accuracy, specificity and interference of the copper determination in Cuprofix C Disperss (ATOFEL02) are assessed. The linearity, accuracy, specificity and interference of the copper determination have been validated in the study “Validation of draft SOP DLA-060 Copper Compounds determination of copper content in formulations” (Report no: DL 99-065) – see KCP 5.1.1/01. 2. In this study the repeatability, linearity of recovery, specificity and interference of the cymoxanil determination in Cuprofix C Disperss (ATOFEL02) have been determined. The linearity of detection for the analyte cymoxanil and internal standard acetophenone has been validated in the study “Validation of draft SOP DLA-229.2, Mancozeb/cymoxanil WG determination of active ingredients and suspensibility” (Report no: DL 99-024) – see KCP 5.1.1/02.
GLP:	Yes
Acceptability:	Yes, in combination with studies described in KCP 5.1.1/01 and KCP 5.1.1/02.

The aim of the study was to re-validate the method for the determination of copper and cymoxanil in copper-cymoxanil formulations.

Materials and methods

Principle of the method

Copper:

The analytical method SOP DLA-249.1 consists of an iodometric titration method after acid digestion for the determination of copper and the HPLC method with an external standard for the determination of cymoxanil.

The copper content is determined after mineralisation with sulfuric acid and nitric acid, dissolving the copper as copper (II) ions and oxidising the organic compounds. The copper content is determined by titration with sodium thiosulfate of the iodine, evolved after the addition of potassium iodide.

Cymoxanil:

In the analytical method under test, cymoxanil is extracted from the formulation with acetonitrile and determined by HPLC using acetophenone as the internal standard.

Materials

Test formulation:

Name: Cuprofix C ~~Dispress~~ Disperss (ATOFEL02)
Batch: 8.335.3
Active substance content: 810 g/kg Bordeaux mixture TC (200 g/kg as copper) and 42 g/kg cymoxanil TC (40 g/kg a.i.)

Copper Primary Reference substance:

Name: Copper
Origin: Aldrich 349216
Batch no: MKAA0260
Purity: 99.9%

Cymoxanil working standard

Name: cymoxanil
Origin: Dupont
Batch no: 9312040
Purity: 98.6%

Method

Copper:

Test test item was dispersed in sulphuric acid and nitric acid and decomposed by boiling and the excess nitric acid was removed. All organic material has been decomposed in this step. By boiling with water the eventually formed nitrosulphuric acid was hydrolyzed into nitrous acid and sulphuric acid. The excess of nitrous acid was decomposed with urea, the copper (II) was complexed with an excess of ammonia and the solution was neutralized with acetic acid. Sodium fluoride was added to prevent potential interferences by iron (III)-, arsenic (V)-, molybdenum (VI)- and antimony (V) ions.

On the addition of potassium iodide, the copper (II) is quantitatively reduced to copper (I), liberating iodine, and precipitated as copper (I) iodide. The iodine formed was titrated with sodium thiosulfate. The equivalence point has been determined by potentiometric titration using a platinum indicator electrode.

Cymoxanil:

The determination has been carried out by extraction with acetonitrile containing acetophenone as internal standard, filtration over a 0.45 filter, dilution and injection of the filtrate into the HPLC, equipped with UV-detection at 240 nm and a reversed-phase C18 column.

Validation - Results and discussions

Copper:

In this study, only the repeatability, accuracy, and specificity and interference of the copper determination in Cuprofix C Disperss (ATOFEL02) have been determined.

The linearity, accuracy, specificity and interference of the copper determination have been validated in the study "Validation of draft SOP DLA-060 Copper Compounds determination of copper content in formulations" (Report no: DL 99-065) – see KCP 5.1.1/02.

Repeatability
Repeatability was performed by running the analytical method five times on the test item Cuprofix C Disperss (ATOFELO2).

Accuracy
To about 250 mg of the test item Cuprofix C Disperss (ATOFELO2) about 50 mg of the copper standard was added and the content of copper according to SOP DLA-249.1 was analysed. This test was done in fivefold.

Cymoxanil:
In this study, the repeatability, linearity of recovery, and specificity and interference of the cymoxanil determination in Cuprofix C Disperss (ATOFEL02) have been determined.
The linearity of detection for the analyte cymoxanil and internal standard acetophenone has been validated in the study "Validation of draft SOP DLA-229.2, Mancozeb/cymoxanil WG determination of active ingredients and suspensibility" (Report no: DL 99-024) – see KCP 5.1.1/03.

Repeatability
The test substance Cuprofix C Disperss (ATOFELO2) has been analysed for cymoxanil according to the proposed SOP DLA-249.1 against the working standard cymoxanil as a reference. The repeatability has been tested by performing 6 replicate determinations.

Accuracy
About 200 mg of the mortared test item was weighed into a 30 ml screwcap vial. About 50 mg of the cymoxanil working standard was weighed into a 50 ml volumetric flask and dissolved in about 40 ml acetonitrile. After dilution to 50 ml with acetonitrile and homogenation, 5 ml of this solution was pipetted into the screwcap vial. After the addition of 2 ml water and 20 ml of internal standard solution ultrasonication was applied for 5 min. The cymoxanil content was measured according to the proposed SOP DLA-249.1. This procedure has been done sixfold.

Table 5.2-4: Method suitable for the determination of active substances copper and cymoxanil in plant protection product Cuprofix C Disperss

	Copper	**Cymoxanil**
Author(s), year	Diepenhorst, P. C., 2010	
Principle of method	Iodometric titration after acid digestion	HPLC with external standard
Linearity	Validated in the study described in KCP 5.1.1/01	Validated in the study described in KCP 5.1.1/02
Precision – Repeatability Mean n = 5 for copper n=6 for cymoxanil (%RSD)	Manual titration: an average copper content in Cuprofix C Disperss: 200.0 g/kg with a standard deviation of 0.14 g/kg. RSD=0.07%. RSDr=1.71% Horrat value: 0.04	HPLC: an average cymoxanil content in Cuprofix C Disperss: 38.3 g/kg with a standard deviation of 0.39 g/kg. RSD=1.01%. RSDr=2.19% Horrat value: 0.46
Accuracy n = 5 for copper n=6 for cymoxanil (% Recovery)	The mean marginal recovery for copper was 99.7% with a standard deviation of 0.46 %.	The mean marginal recovery for cymoxanil was 99.1% with a standard deviation of 1.47%.
Interference/ Specificity	The used chemicals for decomposing the sample show no interference. Potential interferences by iron (III) ions, arsenic (V) ions, molybdenum (VI) ions and antimony (V) ions are prevented by the addition of fluoride. The addition of sodium thiocyanate solution is to release the iodine adsorbed to the copper (I) iodide and improve the determination of the equivalence point. Therefore the method is specific for the	Interferences have been studied using the data of accuracy and repeatability tests and by injections of a blank eluent solution, a blank sample solution containing no internal standard and the internal standard solution. The method is specific. No significant interference was observed.

	Copper	Cymoxanil
	copper content in the test item.	
Comment		

Conclusion

The analytical method SOP DLA-249.1 is suitable for the determination of the active substances copper and cymoxanil in plant protection products Cuprofix C. The linearity for copper was validated in the study described in KCP 5.1.1/01 and for cymoxanil in the study described in KCP 5.1.1/02. Validation complies with SANCO/3030/99 rev. 5 (2019) for specificity, recovery and precision.

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

Copper

According to the Regulation (EU) 2018/1981[1] the relevant impurities to be taken into consideration for active substances copper compounds: arsenic, cadmium, lead, nickel, cobalt, mercury, chromium and antimony. Relevant impurities in the preparation result from impurities in the technical active substance. Methods for the determination of these impurities in the technical material are summarised in the RAR (France, 2017). Other relevant impurities will not be formed during the formulation process or during storage.

However, as the lack of method in formulation has been considered as a data gap of the RAR (France, 2017) and EFSA conclusion (2018),[2] a validation for the determination of these impurities in formulation is provided in this dossier and summarized below.

Cymoxanil

There are no relevant impurities in Cymoxanil.[3]

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product FEL02 is provided as follows:

Comments of zRMS:	The analytical method for the determination of relevant impurities (arsenic, cadmium, lead, nickel, cobalt, mercury, chromium and antimony) in plant protection product CUPROFIX C is suitable for the determination of the content of each of the relevant impurity in the presence of each other, active substance and other components. The proposed analytical methods have been fully validated in terms of the interference, specificity, linearity, accuracy (recovery and repeatability) and LOQ values. Proposed method fulfils the requirements of SANCO/3030/99 rev. 5 guidance. The validation of the analytical methods has been accepted.

[1] COMMISSION IMPLEMENTING REGULATION (EU) 2018/1981 of 13 December 2018 renewing the approval of the active substances copper compounds, as candidates for substitution, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and amending the Annex to Commission Implementing Regulation (EU) No 540/2011.
[2] EFSA Journal 2018;16(1):5152.
[3] Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/ 2009 of the European Parliament and of the Council as regards the list of approved active substances

Reference:	KCP 5.1.1/~~03~~ 04
Report	FEL02: Validation of the Analytical Method for the Determination of the Metallic Impurities Content (Arsenic, Cadmium, Lead, Nickel, Antimony, Chromium, Cobalt and Mercury), Pardo Martinez, M. (2019), Report no: CH - 204/2019
Guideline(s):	SANCO/3030/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with the validation requirements stated in the current guideline SANCO/3030/99, rev. 5 (2019)

Materials and method

Principle of the method

The determination of the eight metal impurities (Arsenic, Cadmium, Lead, Nickel, Antimony, Chromium, Cobalt and Mercury) content is performed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using external standards.

Materials

Test Item

Test item identification: FEL02
Nominal active ingredient: Copper 20 % w/w; Cymoxanil 4 % w/w
Active ingredient content: Copper 19.0 % w/w; Cymoxanil 4.2 % w/w (from the Certificate of Analysis)
Batch number: 0718322
Storage condition: stored at room temperature

Reference Materials

Multi-element analytical standard

Supplier: UltraScientific Analytical solutions
Lot number: CS-0167
Certified value: Arsenic: 100.1 ± 0.5 µg/mL
Cadmium: 100.2 ± 0.5 µg/mL
Lead: 100.2 ± 0.5 µg/mL
Nickel: 100.2 ± 0.5 µg/mL
Cobalt: 100.1 ± 0.5µg/mL
Antimony: 100.0 ± 0.5 µg/mL
Chromium: 100.0 ± 0.5 µg/mL
Storage condition: stored at room temperature (ca. 22°C) in the dark

Mercury (Hg) ICP Standard

Supplier: UltraScientific Analytical solutions
Lot Number: CP-5666
Certified value: 1002 ± 2 µg/mL
Storage condition: stored at room temperature (15° to 30°C)

Certified Reference Material

A trace element fortified sample (filtered and diluted Lake Ontario water preserved with 0.2% nitric acid), supplied by Environment and Climate Change Canada.
Lot number: 0618
Certified value: Arsenic: 28.0 ± 1.9 µg/L
Cadmium: 24.0 ± 1.5 µg/L
Lead: 27.0 ± 2.4 µg/L
Nickel: 15.4 ± 1.1 µg/L
Antimony: 23.5 ± 3.1 µg/L
Chromium: 24.0 ± 1.3 µg/L
Cobalt: 27.7 ± 1.6 µg/L
Storage condition: Refrigerated (about 4°C) in the dark

ICP-MS Internal Standard
Mix solution 100 µg/mL (Li, Sc, Ge, Rh, In, Tb, Lu, Bi) 10% HNO_3, Agilent Technologies

Reference standards as well as test item were diluted using dilution medium (nitric acid at 0.5% v/v with gold at 1 µg/mL) to the predefined concentrations after digestion with acid. Test samples were boiled with nitric acid with gold standard added, cooled down to the room temperature, washed several times with dilution medium and made to volume with the dilution medium. The analytical conditions were the following:

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

Validation - Results and discussions
Specificity
The analytical method was shown to be specific for each metal present in the test item, using the ICP-MS instrument and, for quantification analysis, a response ratio between a specific isotope and an internal standard.

Metal	Symbol	Quantifier Isotope	Internal Standard
Arsenic	As	^{75}As	^{72}Ge
Cadmium	Cd	^{114}Cd	^{103}Rh
Lead	Pb	^{208}Pb	^{175}Lu
Mercury	Hg	^{202}Hg	^{175}Lu
Antimony	Sb	^{121}Sb	^{103}Rh
Chromium	Cr	^{52}Cr	^{72}Ge
Cobalt	Co	^{59}Co	^{72}Ge
Nickel	Ni	^{58}Ni	^{72}Ge

Linearity
Data and results, from a single analysis of six working standard solutions and blank solution, were used to determine the linearity range for each metal.

Metal	Concentration range (ng/mL), n=6	Slope	Intercept	Correlation coefficient (r)
Arsenic	0 – 1001.00	0.0615	0.1258	0.99986
Cadmium	0 – 1002.00	0.0089	0.0151	0.99988
Lead	0 – 1002.00	0.0766	0.1548	0.99980
Mercury	0 – 1002.00	0.0104	0.0307	0.99987
Antimony	0 – 1000.00	0.0110	-0.0022	0.99995
Chromium	0 – 1000.00	0.3142	0.4192	0.99992
Cobalt	0 – 1001.00	0.4890	1.3109	0.99970
Nickel	0 – 1002.00	0.3232	0.1499	0.99998

No significant memory signal was detected in the washing injected after the highest working standard solution and the range tested for each metal was found to be linear (each correlation coefficient $r > 0.99$).

Limit of quantification (LOQ) and limit of detection (LOD)
Data from linearity test were used to calculate the LOD whereas data from accuracy test were used to calculate the LOQ.

LOQ, defined as the lowest fortification level tested at which an acceptable mean recovery with an acceptable RSD% is obtained, was a final injected solution of ca. 2.50 ng/mL (corresponding to ca. 0.25 mg/kg in the test item) for all metallic impurities except for Lead and Nickel which was a final injected solution of ca. 25.0 ng/mL (corresponding to about 2.50 mg/kg in the test item).

LOD, defined as half the lowest working standard solution, was a final injected solution of 0.50 ng/mL, corresponding to 0.05 mg/kg for each metal in the test item.

Metal	LOD (ng/mL)[1]	LOD (mg/kg)[2]	LOQ (ng/mL)[1]	LOQ (mg/kg)[2]
Arsenic	0.50	0.05	2.50	0.25
Cadmium	0.50	0.05	2.50	0.25
Lead	0.50	0.05	25.00	2.50
Nickel	0.50	0.05	25.00	2.50
Chromium	0.50	0.05	2.50	0.25
Cobalt	0.50	0.05	2.50	0.25
Antimony	0.50	0.05	2.50	0.25
Mercury	0.50	0.05	2.50	0.25

[1] Injected concentration
[2] Calculated with respect to the test item

Repeatability (Precision)
Data and results, from 6 determinations of the test item, were used to determine the precision.

Metal	Mean value (n=6) (mg/kg)[1]	RSD%	Horwitz %RSDr[2]	Hr[3]
Arsenic	1.07	7.62	10.60	0.72
Cadmium	<0.25	-	-	-
Lead	19.77	5.23	6.84	0.76
Mercury	<0.25	-	-	-
Antimony	1.53	5.86	10.05	0.58
Chromium	1.19	5.75	10.44	0.55
Cobalt	<0.25	-	-	-
Nickel	4.80	5.92	8.46	0.70

[1] Calculated with respect to the weighed test item
[2] % RSDr = % RSD_R x 0.67; where $\%RSD_R = 2^{(1-0.5 \log C)}$, based on the Horwitz equation
[3] Hr = %RSD/%RSDr (calculated by the applicant)
< 0.25: Lower than LOQ (0.25 mg/kg) but higher than LOD (0.05 mg/kg)

Since the cadmium, mercury and cobalt contents were not detectable or quantifiable in repeatability test, the precision was determined via the accuracy test using the lowest fortification level. The result is presented below.

Metal	Spike level (mg/kg)	Total conc. (mg/kg)[1]	Mean (n=6) (mg/kg)	RSD%	Horwitz %RSDr[2]	Hr[3]
Cadmium	0.25	0.25	0.30	3.38	13.20	0.26
Mercury	0.25	0.25	0.29	4.97	13.20	0.38
Cobalt	0.25	0.25	0.26	7.72	13.21	0.58

[1] Total concentration in the test item after spiking (as the test item does not contain any metal)
[2] Calculated in the total concentration found in the test item
[3] Hr = %RSD/%RSDr (calculated by the applicant)

The acceptability of the %RSD, assessed using the modified Horwitz equation, is considered acceptable (Hr value <1) and therefore the precision of the analytical method is considered acceptable.

Accuracy
For the accuracy, the mean recovery values obtained from three fortification levels comply with the SANCO/3030/99 rev. 5 guideline's requirement, as below: in the range 75 to 125% for metal content lower than 0.1 % w/w (1000 mg/kg).

From data obtained, these criteria were fulfilled for each metal and therefore accuracy of the analytical method can be considered acceptable.

Metal	Level	No. of determination	Added amount (mg/kg)	Mean Recovery value (%)
Arsenic	Low	6	0.25	108.9
	Medium	6	2.50	104.1
	High	6	25.03	109.4
Cadmium	Low	6	0.25	118.0
	Medium	6	2.51	105.1
	High	6	25.05	106.8
Lead	Low	6	0.25	-[1]
	Medium	6	2.51	109.2
	High	6	25.05	109.1
Chromium	Low	6	0.25	111.5
	Medium	6	2.50	100.3
	High	6	25.00	108.4
Cobalt	Low	6	0.25	105.0
	Medium	6	2.50	90.2
	High	6	25.03	106.7
Antimony	Low	6	0.25	99.3
	Medium	6	2.50	104.6
	High	6	25.00	110.6
Mercury	Low	6	0.25	116.7
	Medium	6	2.51	119.6
	High	6	25.05	107.3
Nickel	Low	6	0.25	-[1]
	Medium	6	2.51	108.1
	High	6	25.05	105.5

[1] The mean recovery value was not in compliance with the SANCO/3030/99 rev. 5 guideline's requirement (in the range 75 to 125 %); therefore, it was not considered as the LOQ of the validation method.

Conclusion

PARAMETER	SPECIFICATION ACCORDING SANCO/3030/99 REV. 5	RESULTS
Specificity / interference	Where the formulation contains more than one relevant impurity the method(s) must be capable of determining each in the presence of other impurities and in the presence of the active substance.	The analytical method was shown to be specific for each of 8 metals in test item sample. The method is suitable for the determination of the content of each of the relevant impurities in the plant protection product FEL02 in the presence of each other, active substances and other components.
Linearity	Calibration appropriate to the nominal concentration (range ±at least 20% in relevant analytical solutions). • duplicate determinations at 3 concentrations, or	For each metal: - Six working standard solutions in the concentration range of 1.00 – 1002.00 ng/mL (or 0.1 – 100.2 mg/kg with respect to the test item) and blank solution

PARAMETER	SPECIFICATION ACCORDING SANCO/3030/99 REV. 5	RESULTS
	• single determinations at 5 concentrations	- Linear correlation coefficient r > 0.99
LOD and LOQ	LOQ must be at an appropriate level.	- LOQ = 0.25 mg/kg for As, Cd, Cr, Co, Sb, Hg - LOQ = 2.50 mg/kg for Pb, Ni - LOD = 0.05 mg/kg for all 8 metals
Confirmatory	Required for active substance and significant/relevant impurities unless primary method is highly specific	Since the analysis by ICP-MS gave quantification and identification data, the confirmatory test using another instrumental technique was not necessary.
Repeatability	Minimum 5 independently prepared samples determination at the same concentration. RSDr > RSD% (Hr <1)	- Six independently prepared samples for each metal - Acceptable repeatability (Hr <1) for all metals
Accuracy	At least 2 different fortification levels appropriate to material specification. Mean recovery values in range of 75 - 125 % for impurity content lower than 0.1 %w/w (1000 mg/kg).	- For each metal: 3 fortification levels, 6 replicates at each level - Acceptable accuracy results for As, Cd, Cr, Co, Sb, Hg in the target concentration range of 0.25-25 mg/kg - Acceptable accuracy results for Pb, Ni in the target concentration range of 2.5-25 mg/kg

The analytical method presented above is acceptable for the determination of the relevant impurities Arsenic, Cadmium, Lead, Nickel, Cobalt, Mercury, Chromium and Antimony in the formulation FEL02 and is fully validated according to SANCO/3030/99 rev. 5.

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

This information is not required under Regulation (EC) 1107/2009.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Copper

The following two CIPAC methods are available for the determination of total copper in formulations:

- CIPAC method 44/WP/M/ (copper in wettable powders)
- CIPAC method 44/DP/M/ (copper in dustable powders)

Each of the methods noted above is based on two alternative procedures, titration or electrolytic, which are applicable to the different copper matrices.

These CIPAC methods have been collaboratively tested and are, therefore, applicable for the determination of total copper in relevant preparations.

These methods are suitable for total copper determination but not specific to copper variant. The active substance is determined as total copper, while the identity of variant is determined by spectroscopic characterisation. The total amount of the variant is then calculated stoichiometrically.

There is no CIPAC method available for the determination of copper as Bordeaux Mixture in plant protection products.

Cymoxanil

CIPAC MT 419 (Handbook volume J) is available for the determination of cymoxanil in technical material as well as formulations.
No separate validation is required for FEL02.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of copper and cymoxanil for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new or additional studies it is referred to **Błąd! Nie można odnaleźć źródła odwołania.**.

Table 5.2-5: Validated methods for the generation of pre-authorization data – Copper

Component of residue definition: Total Copper				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Grape Must Wet pomace Dry pomace Stems Raisins Wine	Primary: **Method MR029/RES**	Grapefruit 5.0 mg/kg Must 1.5 mg/kg Wet pomace 5.0 mg/kg Dry pomace 25.0 mg/kg Stems 40.0 mg/kg Raisins 13 mg/kg Wine 0.28 mg/kg	FAAS (measurement at 324.8 nm)	Sicbaldi, F. (2002a) report n°00123 EU agreed RAR Annex B.5.1.2, France, 2017 **Refer to the post-registration method**
Blanching water Tomato fruit and canned tomato Wet pomace Tomato leaves Juice	Primary: **Method MR029/RES**	Blanching water 0.3 mg/kg Tomato fruit and canned tomato 2.0 mg/kg Wet pomace 6 mg/kg Tomato leaves 10 mg/kg Juice 12 mg/kg	FAAS (measurement at 324.8 nm)	Sicbaldi, F. (2002b) report n°00119 EU agreed RAR Annex B.5.1.2, France, 2017 **Refer to the post-registration method**
Cucumber	Primary: acid digestion with a 6:1:2 (v/v) mixture of 65% nitric acid, 30% hydrogen peroxide and distilled water with microwave heating	calculated LOQ: 0.2 mg/kg	AAS (measurement at 324.8 nm)	Sicbaldi, F. and Riccelli, S. (2010) report n°RA.09.23 EU agreed RAR Annex B.5.1.2, France, 2017 **Refer to the post-registration method**

Melon	Primary: acid digestion with nitric acid	calculated LOQ: 0.2 mg/kg (Melon peel) and 0.7 mg/kg (Melon)	ICP-AES (measurement at 324.8 nm).	Hansford, R.J. (2008a, b) report n° DuPont-22565 and DuPont-22564 Foster, A.C. (2006) report n° DuPont-16970 EU agreed RAR Annex B.5.1.2, France, 2017
	Primary: acid digestion with nitric acid and heating under reflux	2 mg/kg	ICP-AES	Foster, A.C. (2006a, b) report n° DuPont-14536 and DuPont-14542, revision n°1 EU agreed RAR Annex B.5.1.2, France, 2017
Melon Whole fruit Pulp Peel Leaves	Primary: acid digestion with 65% nitric acid in the heat	calculated LOQ: 10 mg/kg in melon peel, whole fruit, pulp and leaves calculated LOQ: 3 mg/kg in melon peel and 5 mg/kg in melon whole fruit and pulp	ICP-AES (measurement at 327.8 nm)	Goebel, O. (2008a, b) report n° B-05RFLME01 and B-06RFLME01 EU agreed RAR Annex B.5.1.2, France, 2017
Carrot Plum Sugar Beet Apple Kiwi Fruit Grape Field Strawberries Flowering Brassica	Primary	Carrot whole plant: 1.547 mg/kg Carrot tops: 23.182 mg/kg Carrot roots: 1.111 mg/kg Plum: 0.911 mg/kg Sugar Beet tops: 1.100 mg/kg Sugar Beet roots: 0.832 mg/kg Sugar Beet whole plant: 0.744 mg/kg Apple: 0.893 mg/kg Kiwi Fruit: 1.503 mg/kg Grape Berries: 0.898 mg/kg Field Strawberries: 0.933 mg/kg Broccoli whole plant: 0.848 mg/kg Broccoli inflorescence: 0.816 mg/kg Cauliflower whole plant: 0.791 mg/kg Cauliflower inflorescence: 0.798 mg/kg	ICP-MS	Falconer, D. (2019), report n° 41027 Appendix 2, KCP 5.1.2/03
Potato	Primary: acid digestion with nitric acid and heating using microwave. Method: Renolab MA RES 002	2 mg/kg	FAAS at 324.8 nm	Maccaferri, L. (2009), report n° RA.08.26 Appendix 2, KCP 5.1.2/04
Animal products, food of animal origin (Residues)	Not required			

Soil, grass and earthworm	Primary: treated with nitric acid, hydrofluoric acid and perchloric acid	Grass: 40 mg/kg Soil: 20 mg/kg	ICP-OES (emission wavelengths 324.754 nm)	Klein, O. (2015) report n° 20031343/G1-NFEw EU agreed RAR Annex B5.1.2, France, 2017
	Primary: treated with Antifoam B Silicone Emulsion and a mixture of HCl / HNO_3 (75:25 v:v) and heated under reflux or with microwave	Soil: 4 mg/kg Earthworm: 15 mg/kg	ICP-OES (emission wavelengths 324.754 nm)	
Water	Primary: Total and dissolved copper analysis	4 µg/L	ICP-MS	Blust, R. and Steven Joosen, S. (2016) report n° F-Cu 2016-2 EU agreed RAR Annex B5.1.2, France, 2017
	Primary: Copper ion selective electrode potentiometry	25.3 µg/L	Metrohm 692 pH/Ion Meter fitted with a Metrohm Cupric ion selective electrode and Ag/AgCl reference electrode	
	Primary	1 µg/L for copper	AAS (GF-AAS) for low concentration levels	Schafers, C. (2000) report n° URA-001/4-50 EU agreed RAR Annex B5.1.2, France, 2017
Water, media (Ecotoxicology)	Primary	1 ppb w/v	ICP-MS	Brotherhood, A. (2013) report n°1323923 and Guzman, M.H. (2011) report n° TM1192-Issue No. 2 Appendix 2, KCP 5.1.2/05
	Primary	1.314 mg/L	AAS	Ruhland, S. (2018) report n° 17 35 CRB 0157 Appendix 2, KCP 5.1.2/06
	Primary	140.2 mg/L	AAS	Scheller, K. (2018a and 2018b) report n° 17 35 CRB 0150 and 17 35 CRB 0149 Appendix 2, KCP 5.1.2/07

	Primary	Water stock solution: 70 mg/L Sugar feeding solution: 1.8 mg/kg	ICP-MS	Colli, M. (2018) report n° BT215/17 Appendix 2, KCP 5.1.2/08
Air	No analytical method provided			
Body fluids and tissues (Toxicology)	VDI, 1997	Receptor fluid: 0.1 µg/L Receptor/donor wash: 0.25 µg/L Skin membrane: 0.02 µg Tape strip: 0.02 µg Skin wash: 0.05 µg	HR-ICP-MS	Maas, W.J.M. and Kunne, C. (2015) report n° 20600/19 and Schouten A. and de Haan, H.P.M. (2016) report n° V20801 EU agreed RAR Annex B5.1.2, France, 2017
	Primary	Receptor fluid: 5.57 µg/L	ICP-AES/ICP-MS for dose solutions, skin wash, tape strips. ICP-MS (field dilutions)/GF-AAS (concentrate) for skin membrane. GF-AAS for receptor fluid, compartment wash and donor compartment wash.	Maas, W.J.M. (2016) report n° V9062/final and amendment 01 EU agreed RAR Annex B5.1.2, France, 2017
	Primary: Digestion or dilution with nitric acid	Receptor fluids: 0.05 µg/L Receptor wash: 0.05 µg/L Tape strip: 0.0198 µg/L Cotton swab: 1.487 µg/L Membrane: 0.0173 µg/L	ICP-MS	Maas, W.J.M., Brufau Dones, G. (2016) report n° DuPont-42821 Appendix 2, KCP 5.1.2/01
	Primary:	Residual carcass: 5 ppb Tape strips: 1 ppb Skin: 5 ppb Urine: 10 ppb Blood: 10 ppb Plasma: 10 ppb Feces: 150 ppb	ICP-MS	Maas, W.J.M., Bogaards, J.J.P., de Bie, A.Th. (2016) report n° DuPont-42648 Appendix 2, KCP 5.1.2/02

Table 5.2-4: Validated methods for the generation of pre-authorization data – Cymoxanil

Component of residue definition: Cymoxanil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or**	**Author(s), year / missing / EU agreed**

			HPLC-UV)	
Plants, plant products (Residues)	Primary, extraction with QuEChERS	0.02 mg/kg	LC-MS/MS	Richter, S. (2009) report n° P/B 1668 G Appendix 2, KCP 5.1.2/09
	Primary, extended and revised version of DFG S19 method	0.01 mg/kg	LC-MS/MS	Lakaschus, S., Gizler, A. (2013) report n° DuPont-35769 **Refer to the post-registration method** (Appendix 2, KCP 5.2/03)
	Primary, extended and revised version of DFG S19 method	0.01 mg/kg	LC-MS/MS	Weber, H. (2008) report n° GAB-0703V Appendix 2, KCP 5.1.2/10
	Primary	0.01 mg/kg	GC-NDP (primary) LC-MS/MS (confirmatory)	Lakaschus, S. (2004) report n° DuPont-15026 Appendix 2, KCP 5.1.2/11
	Primary, extraction with acetonitrile/2% potassium bicar-bonate aqueous solution (80/20, v/v) mixture and QuEChERS	0.01 mg/kg	LC-MS/MS	Tetuan, B. (2011) report n° 10 F PT GW P/A (PROMO/ZOX CM/10.01) Appendix 2, KCP 5.1.2/12
	Confirmatory (if required)	-	-	Not relevant since no study was required to be provided.
Animal products, food of animal origin (Residues)	Primary	-	-	Not relevant since no study was required to be provided.
	Confirmatory (if required)	-	-	Not relevant since no study was required to be provided.
Soil, water, sediment (Environmental fate)	Primary	-	-	Not relevant since no study was required to be provided.
	Confirmatory (if required)	-	-	Not relevant since no study was required to be provided.
Soil, water (Efficacy)	Primary	-	-	Not relevant since no study was required to be provided.
	Confirmatory (if required)	-	-	Not relevant since no study was required to be provided.
Feed, body fluids (Toxicology)	Primary	-	-	Not relevant since no study was required to be provided.
	Confirmatory (if required)	-	-	Not relevant since no study was required to be provided.
Body fluids, air (Exposure)	Primary	-	-	Not relevant since no study was required to be provided.
	Confirmatory (if required)	-	-	Not relevant since no study was required to be provided.
Soil, water	Primary	0.12 µg/L	HPLC-MS	Hutchinson, K.A. and Sharpe, A.D. (2012a) report No. BR0587/B

(Ecotoxicology)				Appendix 2, KCP 5.1.2/05
	Primary	0.12 µg/L	HPLC-MS	Hutchinson, K.A. and Sharpe, A.D. (2012b) report No. BR0586/B Appendix 2, KCP 5.1.2/05
	Primary	0.12 µg/L	HPLC-MS	Hutchinson, K.A. and Sharpe, A.D. (2012c) report No: BR0585/B Appendix 2, KCP 5.1.2/05
	Confirmatory (if required)	-	-	Not required
Water, buffer solutions (Properties)	Primary	-	-	Not relevant since no study was required to be provided.
	Confirmatory (if required)	-	-	Not relevant since no study was required to be provided.

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)

The methods for the determination of the active substances and relevant impurities in the plant protection product are already submitted in accordance with the requirements set out in point 5.2.1.

5.3.2 Description of analytical methods for the determination of residues of copper (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 (include its addenda) the current legal residue definition is identical.[4]

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 to 100 mg/kg	Commission Regulation (EC) No 149/2008
Plant, high acid content		5 mg/kg to 50 mg/kg	
Plant, high protein/high starch content (dry commodities)		10 mg/kg	
Plant, high oil content		20 mg/kg to 30 mg/kg	
Plant, difficult matrices (hops, spices, tea)		40 mg/kg to 100 mg/kg	
Muscle, milk, eggs, fat,	Total copper	2 mg/kg to 30 mg/kg	Commission Regulation (EC)

[4] Draft Assessment Report (DAR), 2007; Final addendum to the Draft Assessment Report, 2008

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
liver and kidney			No 149/2008
Soil (Ecotoxicology)	Total copper	5 mg/kg	NOEC (*Eisenia andrei*) = 8.4 mg Cu/kg soil
Drinking water (Human toxicology)	Dissolved copper	0.1 µg/L	Council directive 98/83/EC; general limit for drinking water
Surface water (Ecotoxicology)		2.087 µg/L	PECsw for dissolved copper
Air	Total copper	22.5 µg/m^3	$AOEL_{sys}$: 0.075 mg/kg bw/day
Body tissues (meat or liver)	Total copper	0.01 mg/kg	SANTE/2020/12830 Rev.1; general limit
Body fluids		0.01 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 in plant matrices is given in the following tables. For the detailed evaluation of additional studies, it is referred to **Błąd! Nie można odnaleźć źródła odwołania.**.

Table 5.3-2: Validated methods for food and feed of plant 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 / EU agreed**
High water content and High acid content	Primary	5.0 mg/kg in grape	AAS (wavelength 324.8 nm).	Sicbaldi, F. (2002a) report n° 00123 EU agreed RAR Annex B.5.2.1, France, 2017
	Primary	2.0 mg/kg in tomato	AAS (wavelength 324.8 nm)	Sicbaldi, F. (2002b) report n°00119 EU agreed RAR Annex B5.1.2, France, 2017
	Primary	0.2 mg/kg in cauliflower, strawberry, sugarbeet (roots), canned peaches and beer 0.4 mg/kg in strawberry jam 0.8 mg/kg in cooked peas 2.0 mg/kg in apple dry pomace	AAS (wavelength 324.8 nm)	Sicbaldi, F. and Riccelli, S. (2010) report n° RA.09.23 EU agreed RAR Annex B.5.1.2, France, 2017
	Primary	3.0 mg/kg in tomato juice	AAS (wavelength 324.8 nm)	Riccelli, S. (2016) report n° RA.16.08

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**
		1.1 mg/kg in melon pulp 0.8 mg/kg in melon peel		EU agreed RAR Annex B.5.1.2, France, 2017
	Primary (matrix effect evaluation)	Not applicable	FAAS (wavelength 324.8 nm)	Riccelli, S. (2017) report n° RA.17.02 Appendix 2, KCP 5.2/01
	Primary **EN 13805**	Not applicable	Atomic absorption (flame, electrothermal (ET), hydride, cold-vapour) techniques and ICP-MS	Anononymus (2014) report n° EN 13805 EU agreed RAR Annex B5.2.1, France, 2017
	ILV	Not required		EU agreed RAR Annex B5.2.1, France, 2017
	Confirmatory	Not required		
High oil content	Primary	1.0 mg/kg in oil seed rape	AAS (wavelength 324.8 nm)	Sicbaldi, F. and Riccelli, S. (2010) report n° RA.09.23 EU agreed RAR Annex B5.1.2, France, 2017
	Primary	5.5 mg/kg	FAAS (wavelength 324.8 nm)	Riccelli, S. (2017) report n° RA.17.02 Appendix 2, KCP 5.2/01
	Primary **EN 13805**	Not applicable	Atomic absorption (flame, electrothermal (ET), hydride, cold-vapour) techniques and ICP-MS	Anononymus (2014) report n° EN 13805 EU agreed RAR Annex B5.2.1, France, 2017
	ILV	Not required		EU agreed RAR Annex B5.2.1, France, 2017
	Confirmatory	Not required		
High protein/high starch content (dry)	Primary	7.5 mg/kg	FAAS (wavelength 324.8 nm)	Riccelli, S. (2017) report n° RA.17.02 Appendix 2, KCP 5.2/01
	Primary **EN 13805**	Not applicable	Atomic absorption (flame, electrothermal (ET), hydride, cold-vapour) techniques and ICP-MS	Anononymus (2014) report n° EN 13805 EU agreed RAR Annex B5.2.1, France, 2017
	ILV	Not required		EU agreed RAR Annex B5.2.1, France, 2017
	Confirmatory	Not required		
Difficult	Primary	0.5 mg/kg in hops	AAS (wavelength 324.8 nm)	Sicbaldi, F. and Riccelli, S. (2010) report n° RA.09.23 EU agreed RAR Annex B5.2.1, France, 2017
	ILV	Not required		

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**
	Confirmatory	Not required		

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Due to the nature of the analysis (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 methods and possible data gaps for analysis of copper in animal matrices is given in the following tables.

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	AAS	Anononymus (2003) report n° EN14082:2003 EU agreed RAR Annex B5.2.2, France, 2017
	ILV	Not required		EU agreed RAR Annex B5.2.2, France, 2017
	Confirmatory	Not required		

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Due to the nature of the analysis (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 in soil is given in the following table.

Table 5.3-6: Validated methods for soil

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 for Speyer soil type 2.2 and type 2.3	ICP-AES (emission wavelength 324.754 nm) (bio-available copper)	Kiefer, R. (2003) report n° 20031084/02-UVX EU agreed RAR Annex B5.2.3, France, 2017
	40 mg/kg	AAS (wavelength 324.8 nm)	Carey, D. O. (1989) report n° 88-003 EU agreed RAR Annex B5.2.3, France, 2017
	Soil: 15 mg/kg for Speyer soil type 2.2 55 mg/kg for Speyer soil type 2.3	ICP-AES (emission wavelength 324.754 nm)	Kiefer, R. (2004) report n° 20031084/01-UVX EU agreed RAR Annex B5.2.3, France, 2017
Confirmatory	Not required		

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

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	100 µg/L	ICP-AES (emission wavelength 324.754 nm)	Heintze, A. (2000 and 2001) report n° 99520/01-ASCr and 99507/01-ASCr RAR Annex B.5.2.4, France, 2017
	Primary	60 µg/L	AAS (wavelength 324.8 nm)	Carey, D.O. (1989) report n° 88-003 RAR Annex B.5.2.4, France, 2017
	Primary	0.3 µg/L	ICP-MS (m/z 63: quantitative, m/z 65 qualitative)	Pardo Martinez, M. (2016) report n° CH – 157/2016 RAR Annex B.5.2.4, France, 2017
	Primary **DIN 38406 Part 7**	2 µg/L	AAS	Anononymus (1991) report n° DIN 38406 Part 7 RAR Annex B.5.2.4, France, 2017

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**
	Primary **ISO 15586:2003**	Not applicable	AAS	Anononymus (2004) RAR Annex B.5.2.4, France, 2017
	ILV	Not required		RAR Annex B.5.2.4, France, 2017
	Confirmatory	Not required		

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 in air is given in the following tables.

Table 5.3-8: Validated methods for air

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	Not applicable (depend on the actual background copper levels)	AAS (wavelength 324.8 nm)	Anononymus (1999) report n° VDI 2267, Part 1 RAR Annex B.5.2.5, France, 2017
Primary	Not applicable (depend on the actual background copper levels)	ICP-OES (wavelength 324.8 nm and 327.4 nm)	Anononymus (1997) report n° VDI 2267, Part 5 RAR Annex B.5.2.5, France, 2017
Primary	0.3 µg/m^3	ICP-MS or ICP-OES	Pardo Martinez, M. (2018) report n° CH-657/2017 Appendix 2, KCP 5.2/02
Confirmatory	Not required		

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

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,	ICP-AES (emission wavelength 324.752 nm)	Himmelstein, M.W. (2003) report n° 11784 RAR Annex B.5.2.6,

Component of residue definition: Total copper			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	841 mg/kg for faeces and 46 mg/kg for carcass		France, 2017
Confirmatory	Not required		

5.3.2.8 Other studies/ information

No other studies / information are required to be provided

5.3.3 Description of analytical methods for the determination of residues of Cymoxanil (KCP 5.2)

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

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

Table 5.3-10: 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	Cymoxanil	0.03 mg/kg	~~Commission Regulation (EC) No 2018/832~~ Reg. (EU) 2022/1363[5]
Plant, high acid content		0.3 mg/kg	
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	
Plant, high oil content		0.01 mg/kg	
Plant, difficult matrices (hops, spices, tea)		0.1 mg/kg	
Muscle	No MRL is set, no enforcement methods required.		
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Cymoxanil	0.05 mg/kg soil	SANTE/2020/12830 Rev.1; common limit
Drinking water (Human toxicology)	Cymoxanil and IN-KQ960	0.1 µg/L	Directive 98/83/EC[6]
Surface water (Ecotoxicology)	Cymoxanil and IN-KQ960	0.034 mg/L	Lowest NOEC for *Anabaena flos-aquae* (EFSA Scientific Report, 2008)
Air	Cymoxanil	3 µg/m^3	$AOEL_{sys}$/$AOEL_{inhal}$: 0.01 mg/kg bw/day (EFSA Scientific Report, 2008)
Body tissues (meat or liver)	Cymoxanil	0.01 mg/kg	SANTE/2020/12830 Rev.1; general limit
Body fluids		0.01 mg/L	

5.3.3.2 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 Cymoxanil in plant matrices is given in the following tables. For the detailed evaluation of additional studies, it is referred to **Błąd! Nie można odnaleźć źródła odwołania.**.

Table 5.3-11: Validated methods for food and feed of plant origin

[5] COMMISSION REGULATION (EU) 2018/832 of 5 June 2018 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for cyantraniliprole, cymoxanil, deltamethrin, difenoconazole, fenamidone, flubendiamide, fluopicolide, folpet, fosetyl, mandestrobin, mepiquat, metazachlor, propamocarb, propargite, pyrimethanil, sulfoxaflor and trifloxystrobin in or on certain products.
[6] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.

Component of residue definition: active ingredient Cymoxanil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water, high acid, high starch and high oil matrices (Tomato, grapes, oilseed rape and wheat grain)	Primary	0.01 mg/kg	LC-MS/MS	Lakaschus, S., Gizler, A. (2013) report n° DuPont-35769 and Seck, C., Goody, T. (2019) report n° Not applicable - position paper) Appendix 2, KCP 5.2/03
	ILV	0.01 mg/kg	LC-MS/MS	Cermak, J. (2013a) report n° DuPont-35770 Appendix 2, KCP 5.2/04
Difficult (if required, depends on intended use)	Primary	Not required.		
	Confirmatory	Not required.		
	ILV	Not required.		

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

Not required as no residue definition is proposed.

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

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

Table 5.3-12: Validated methods for soil

Component of residue definition: Cymoxanil			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS	Garofani, S. (2009a) report n° CH-285/2008 Appendix 2, KCP 5.2/05
Primary (additional validation on linearity)	0.01 mg/kg	LC-MS/MS	Nichetti, S. (2017a) report n° CH-199/2016 Appendix 2, KCP 5.2/07
Confirmatory	0.01 mg/kg	LC-MS/MS	Garofani, S. (2013) report n° CH-377/2013 Appendix 2, KCP 5.2/06

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

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

Table 5.3-13: Validated methods for drinking, ground and surface water

Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	Cymoxanil (0.1 µg/L) IN-KQ960 (0.1 µg/L)	LC-MS/MS	Leak, T. (2010) report n° ABC-65072 (Dupont-27500) Appendix 2, KCP 5.2/08
ILV and confirmation	Cymoxanil (0.1 µg/L) IN-KQ960 (0.1 µg/L)	LC-MS/MS	Cermak, B. (2013b) report n° DuPont-35792 Appendix 2, KCP 5.2/09

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

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

Table 5.3-14: Validated methods for air

Component of residue definition: Cymoxanil			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary/ Confirmation	0.17 µg/m^3	HPLC-UV (primary) HPLC-DAD (confirmation)	Garofani, S. (2009b) report n° CH-287/2008 Appendix 2, KCP 5.2/10
Primary (additional validation on linearity)	0.17 µg/m^3	HPLC-UV	Nichetti, S. (2017b) report n° CH-200/2016 Appendix 2, KCP 5.2/11

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

Table 5.3-15: Validated methods for body fluids and tissues

Component of residue definition: Cymoxanil			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/L (body tissues)	LC-MS/MS	Perboni, A. (2016) report n° RAU-065-16 Appendix 2, KCP 5.2/12
ILV	0.01 mg/L (body tissues)	LC-MS/MS	Fifi, A.P. (2016) report n° BT300/16 Appendix 2, KCP 5.2/13

Component of residue definition: Cymoxanil			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L (body fluids)	LC-MS/MS	Andrews, G. (2019) report n° ZE/19/001 Appendix 2, KCP 5.2/14

5.3.3.8 Other studies/ information

No other studies available, none deemed necessary.

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 5.1.1/02~~	~~Heugens, R.~~	~~2003~~	~~VALIDATION OF DRAFT SOP DLA 251 VERSION 0 SOLUTION DISPERSS (ATOFDH01), DETERMINATION OF FAMOXADONE CYMOXANIL AND COPPER CONTENT~~ ~~United Phosphorus Ltd., DL 03-034~~ ~~Cerexagri B.V., Rotterdam, NL~~ ~~GLP: yes~~ ~~Published: no~~	~~N~~	~~UPL Europe Ltd.~~
KCP 5.1.1/02	Diepenhorst, P. C.	1999	VALIDATION OF DRAFT SOP DLA-229.2 MANCOZEB/CYMOXANIL WG DETERMINATION OF ACTIVE INGREDIENTS AND SUSPENSIBILITY UPL Europe Ltd., DL 99-024 Development Laboratory Elf Atochem Agri B.V., The Netherlands GLP: yes Published: no	N	UPL Europe Ltd.
KCP 5.1.1/03	Diepenhorst, P. C.	2010	RE-VALIDATION OF SOP DLA-249.1 VERSION 2 “COPPER-CYMOXANIL MIXED FORMULATIONS (ATOFELNN) DETERMINATION OF CONTENT OF ACTIVE INGREDIENTS”, DIEPENHORST, P. C. (2010), UPL Europe Ltd.,: DL 09-102 Development Laboratory Elf Atochem Agri B.V., The Netherlands GLP: yes Published: no	N	UPL Europe Ltd.

KCP 5.1.1/~~03~~ 04	Pardo Martinez, M.	2019	FEL02: VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF THE METALLIC IMPURITIES CONTENT (ARSENIC, CADMIUM, LEAD, NICKEL, ANTIMONY, CHROMIUM, COBALT AND MERCURY) United Phosphorus Ltd., CH - 204/2019 Chemservice S.r.l., Novate Milanese, Italy GLP/GEP: yes Published: no	N	UPL Europe Ltd.
KCP 5.1.2/01	Maas, W.J.M., Brufau Dones, G.	2016	*IN VITRO* PERCUTANEOUS ABSORPTION OF COPPER, FORMULATED AS COPPER HYDROXIDE (DPX-GFJ52) 53.8WG (35% AS METALLIC COPPER), THROUGH HUMAN SKIN E.I. du Pont de Nemours and Company, DuPont-42821 TNO Triskelion B.V., Zeist, The Netherlands GLP/GEP: yes Published: no	N	E.I. du Pont de Nemours and Company (*)
KCP 5.1.2/02	Maas, W.J.M., Bogaards, J.J.P., de Bie, A.Th.	2016	*IN VIVO* PERCUTANEOUS ABSORPTION OF COPPER, FORMULATED AS COPPER HYDROXIDE (DPX-GFJ52) 53.8WG (35% AS METALLIC COPPER) IN RATS E.I. du Pont de Nemours and Company, DuPont-42648 TNO Triskelion B.V., Zeist, The Netherlands GLP/GEP: yes Published: no	Y	E.I. du Pont de Nemours and Company
KCP 5.1.2/03	Falconer, D.	2019	METHOD VALIDATION FOR THE ANALYSIS OF COPPER IN EIGHT PLANT MATRICES UPL Europe Ltd., 41027 Charles River Laboratories GLP/GEP: no Published: no	N	UPL Europe Ltd.
KCP 5.1.2/04	Maccaferri, L.	2009	COPPER RESIDUES IN POTATOES AFTER FOUR APPLICATIONS OF COPPER OXYCHLORIDE 37.5 WG. TWO HARVEST TRIALS IN NORTHERN EUROPE (GERMANY AND POLAND) IN 2008, ANALYTICAL PHASE REPORT Isagro, RA.08.26 Isagro Ricerca Srl, Novara, Italy GLP/GEP: no Published: no Published: no	N	UPL Europe Ltd.

KCP 5.1.2/05	Brotherhood, A. and Guzman, M.H.	2013 2011	GLP VALIDATION STUDY FOR DETERMINATION OF COPPER IN FRESHWATER, ALGAE AND DAPHNIA MEDIA BY ICP-MS AstraZeneca UK limited, 1323923 Intertek ASG, Manchester, UK GLP: yes Published: no FRESHWATER, ALGAE AND DAPHNIA COPPER ANALYSIS BY ICP-MS AstraZeneca UK limited, TM1192-Issue No. 2 Intertek ASG, Manchester, UK GLP: yes Published: no	N	UPL Europe Ltd.
KCP 5.1.2/06	Ruhland, S.	2018	CHRONIC TOXICITY OF COPPER 20% + CYMOXANIL 4% WG TO THE HONEY BEE *APIS MELLIFERA* L. UNDER LABORATORY CONDITIONS: *VERIFICATION OF THE CONCENTRATION OF THE ACTIVE INGREDIENT IN THE TEST ITEM FEEDING SOLUTIONS* UPL Europe Ltd., 17 35 CRB 0157 BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	UPL Europe Ltd.

KCP 5.1.2/07	Scheller, K. Scheller, K.	2018a 2018b	COPPER 20% + CYMOXANIL 4% WG - REPEATED EXPOSURE OF HONEY BEE (*APIS MELLIFERA* L.) LARVAE UNDER LABORATORY CONDITIONS (*IN VITRO*): *VERIFICATION OF THE CONCENTRATION OF THE ACTIVE INGREDIENT IN THE TEST ITEM FEEDING SOLUTIONS* UPL Europe Ltd., 17 35 CRB 0150 BioChem Agrar, Gerichshain, Germany GLP: yes Published: no and COPPER 20% + CYMOXANIL 4% WG – REPEATED EXPOSURE OF HONEY BEE (*APIS MELLIFERA* L.) LARVAE UNDER LABORATORY CONDITIONS (*IN VITRO*): *VERIFICATION OF THE CONCENTRATION OF THE ACTIVE INGREDIENT IN THE TEST ITEM FEEDING SOLUTIONS* UPL Europe Ltd., 17 35 CRB 0149 BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	UPL Europe Ltd.
KCP 5.1.2/08	Colli, M.	2018	CHRONIC ORAL EFFECTS OF COPPER OXYCHLORIDE 50% WP TO ADULT WORKER HONEYBEES APIS MELLIFERA L., 10-DAY FEEDING LABORATORY TEST EU Copper Task Force, BT215/17 Biotecnologie BT Srl, Todi, Italy GLP: yes Published: no	N	EUCuTF (*)
KCP 5.1.2/09	Richter, S.	2009	CYMOXANIL: VALIDATION OF AN ENFORCEMENT METHOD FOR VARIOUS CROP TYPES UPL Europe Ltd., P/B 1668 G PTRL Europe, Ulm, Germany GLP: yes Published: no	N	UPL Europe Ltd.

KCP 5.1.2/10	Weber, H.	2008	VALIDATION OF THE ANALYTICAL METHODS FOR THE DETERMINATION OF RESIDUES OF CYMOXANIL, MANCOZEB AND ITS METABOLITE ETU IN POTATO (TUBER) Belchim Crop Protection and Indofil Industries Limited, GAB-0703V Eurofins GLP: yes Published: no	N	Belchim Crop Protection and Indofil Industries Limited (*)
KCP 5.1.2/11	Lakaschus, S.	2004	VALIDATION OF THE DFG METHOD S19 (EXTENDED AND REVISED VERSION) FOR THE DETERMINATION OF RESIDUES OF CYMOXANIL IN MATRICES WITH HIGH WATER CONTENT {MELON (PEEL AND PULP), GRAPES AND POTATOES] E.I. du Pont de Nemours and Company, DuPont-15026 Dr. Specht & Partner GLP: yes Published: no	N	E.I. du Pont de Nemours and Company (*)
KCP 5.1.2/12	Tetuan, B.	2011	DETERMINATION OF RESIDUES AT HARVEST IN POTATOES, FOLLOWING SIX BROADCAST APPLICATIONS OF HARPON WG, UNDER FIELD CONDITIONS. – NORTHERN EUROPE – SEASON 2010 Gowan Comercio Internacional & Servicos ltda, 10 F PT GW P/A (PROMO/ZOX CM/10.01) Promo-Vert GLP: yes Published: no	N	Gowan Comercio Internacional & Servicos ltda (*)
KCP 5.2/01	Riccelli, 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 EU Copper Task Force, RA.17.02 Isagro - Centro di Saggio BPL GLP/GEP: no Published: no	N	EUCuTF (*)
KCP 5.2/02	Pardo Martinez, M.	2018	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF COPPER RESIDUES IN AIR EU Copper Task Force, CH-657/2017 Chemservice S.r.l., Novate Milanese, Italy GLP/GEP: no Published: no	N	EUCuTF (*)

KCP 5.2/03	Lakaschus, S., Gizler, A. and Seck, C., Goody, T.	2013 2019	VALIDATION OF MULTI-RESIDUE METHOD DFG S19 (LC-MS/MS MODULE) FOR THE DETERMINATION OF RESIDUES OF CYMOXANIL IN TOMATO, GRAPES, OILSEED RAPE AND WHEAT GRAIN E.I. du Pont de Nemours and Company, DuPont-35769 Eurofins Agroscience Services Chem GmbH GLP/GEP: yes Published: no POSITION PAPER TO COVER THE EXTRACTION EFFICIENCY OF THE MULTI-RESIDUE METHOD DFG S19 REPORTED IN THE REPORT DUPONT-35769 CTF, not applicable Battelle UK Limited GLP/GEP: no Published: no	N N	E.I. du Pont de Nemours and Company (*) CTF (*)
KCP 5.2/04	Cermak, J.	2013a	INDEPENDENT LABORATORY VALIDATION OF MULTI-RESIDUE METHOD DFG S19 FOR THE DETERMINATION OF RESIDUES OF CYMOXANIL IN TOMATO, GRAPES, OILSEED RAPE AND WHEAT GRAIN USING LC-MS/MS E.I. du Pont de Nemours and Company, DuPont-35770 Výzkumný ústav organických syntéz a.s. GLP/GEP: yes Published: no	N	E.I. du Pont de Nemours and Company (*)
KCP 5.2/05	Garofani, S.	2009a	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CY-MOXANIL RESIDUES IN SOIL Belchim Crop Protection and Indofil Industries Limited, CH-285/2008 ChemService GLP/GEP: yes Published: no	N	Belchim Crop Protection and Indofil Industries Limited (*)

KCP 5.2/06	Garofani, S.	2013	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CY-MOXANIL RESIDUES IN SOIL. INTEGRATION OF THE GLP STUDY CH-285/2008 WITH LINEARITY AND RECOVERY TESTS USING PEAK AREAS OF QUALIFIER IONS Belchim Crop Protection and Indofil Industries Limited, CH-377/2013 ChemService GLP/GEP: yes Published: no	N	Belchim Crop Protection and Indofil Industries Limited (*)
KCP 5.2/07	Nichetti S.	2017a	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CY-MOXANIL RESIDUES IN SOIL. INTEGRATION OF THE GLP STUDY CH-285/2008 AND GLP STUDY CH-377/2013 WITH LINEARITY TEST IN A SUITABLE RANGE Belchim Crop Protection and Indofil Industries Limited, CH-199/2016 ChemService GLP/GEP: yes Published: no	N	Belchim Crop Protection and Indofil Industries Limited (*)
KCP 5.2/08	Leak, T.	2010	ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL AND IN-KQ960 IN WATER (POND, STREAM, WELL, AND TAP) USING LC/MS/MS E.I. du Pont de Nemours and Company, ABC-65072 (Dupont-27500) ABC Laboratories, Inc. GLP/GEP: yes Published: no	N	E.I. du Pont de Nemours and Company (*)
KCP 5.2/09	Cermak, B.	2013b	INDEPENDENT LABORATORY VALIDATION FOR THE DETERMINATION OF RESIDUES OF CYMOXANIL AND IN-KQ960 IN WATER (DRINKING AND STREAM) USING LC-MS/MS E.I. du Pont de Nemours and Company, DuPont-35792 Výzkumný ústav organických syntéz a.s. GLP/GEP: yes Published: no	N	E.I. du Pont de Nemours and Company (*)
KCP 5.2/10	Garofani, S.	2009b	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CY-MOXANIL RESIDUES IN AIR Belchim Crop Protection and Indofil Chemicals Company, CH-287/2008 ChemService GLP/GEP: yes Published: no	N	Belchim Crop Protection and Indofil Chemicals Company (*)

KCP 5.2/11	Nichetti, S.	2017b	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL RESIDUES IN AIR. INTEGRATION OF THE GLP STUDY CH-287/2008 WITH LINEARITY TEST USING A SUITABLE RANGE Protection and Indofil Chemicals Company, CH-200/2016 ChemService GLP/GEP: yes Published: no	N	Protection and Indofil Chemicals Company (*)
KCP 5.2/12	Perboni, A.	2016	VALIDATION OF THE ANALYTICAL METHOD TO DETERMINE RESIDUE OF CYMOXANIL IN DIFFERENT MATRICES OF ANIMAL ORIGIN (KIDNEY, LIVER, FAT, MUSCLE, MILK AND EGGS) CTF, RAU-065-16 Research Center BioSpheres GLP/GEP: yes Published: no	N	CTF (*)
KCP 5.2/13	Fifi, A.P.	2016	INDEPENDENT LABORATORY VALIDATION OF THE ANALYTICAL METHOD TO DETERMINE RESIDUE OF CYMOXANIL IN DIFFERENT ANIMAL ORIGIN MATRICES (KIDNEY, LIVER, FAT, MUSCLE, MILK AND EGG) CTF, BT300/16 BioTecnologie BT S.r.l. GLP/GEP: yes Published: no	N	CTF (*)
KCP 5.2/14	Andrews, G.	2019	METHOD VALIDATION - DETERMINATION OF RESIDUES OF CYMOXANIL IN BODY FLUID CTF, ZE/19/001 Battelle UK Limited GLP/GEP: yes Published: no	N	CTF (*)

UPL EU = UPL Europe Ltd.

EUCuTF = EU Copper Task Force

CTF = Cymoxanil task force (DuPont, Sipam Oxon, Belchim, Indofil, UPL)
(*) UPL is a full member of the EU Copper Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of copper compounds
UPL is a full member of the Cymoxanil AIR4 Task Force, UPL Europe Ltd has a full access to all the studies included in the AIR dossier submitted for the EU renewal of cymoxanil

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

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 5.1.1/01	Diepenhorst, P.C.	2000	VALIDATION OF DRAFT SOP DLA-060 COPPER COMPOUNDS DETERMINATION OF COPPER CONTENT IN FORMULATIONS UPL Europe Ltd., DL 99-065 Development Laboratory Elf Atochem Agri B.V., The Netherlands GLP: yes Published: no	N	UPL EU
KCP 5.1.2, KCP 5.2	Sicbaldi, F.	2002a	ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF COPPER IN/ON GRAPES AND THEIR PROCESSED FRACTIONS EU Copper Task Force, 00123 Isagro, Ricerca Srl GLP/GEP: no Published: no	N	EUCuTF
KCP 5.1.2, KCP 5.2	Sicbaldi, F.	2002b	ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF COPPER IN/ON TOMATOES, THEIR PROCESSED FRACTIONS AND LEAVES EU Copper Task Force, 00119 Isagro, Ricerca Srl GLP/GEP: no Published: no	N	EUCuTF
KCP 5.1.2, KCP 5.2	Sicbaldi, F., Riccelli, S.	2010	METHOD VALIDATION FOR THE REDUCTION OF THE LIMIT OF QUANTIFICATION FOR COPPER IN REPRESENTATIVE MATRICES OF PLANT ORIGIN European Copper Task Force, Petit-Lancy, Switzerland, RA.09.23 Isagro, Ricerca Srl GLP/GEP: no Published: no	N	EUCuTF
KCP 5.1.2	Hansford, R.J.	2008a	MAGNITUDE OF RESIDUES OF COPPER IN FIELD MELONS (CUCURBITS-INEDIBLE PEEL) FOLLOWING APPLICATIONS OF METALLIC COPPER (AS COPPEROXYCHLO-	N	EUCuTF

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
			RIDE)/CYMOXANIL (DPX-KK807) 44WP (9.5:1)-SOUTHERN EUROPE, SEASON 2007 EU Copper Task Force, DuPont-22565 Charles River Laboratories GLP: yes Published: no		
KCP 5.1.2	Hansford, R.J.	2008b	MAGNITUDE OF RESIDUES OF COPPER IN PROTECTED MELONS (CURCURBITS - INEDIBLE PEEL) FOLLOWING APPLICATIONS OF METALLIC COPPER (AS COPPER OXYCHLORIDE) / CYMOXANIL (DPX-KK807) 44WP (9.5:1) - SOUTHERN EUROPE, SEASON 2007 E.I. Du Pont de Nemours and Company, 691916, DuPont-22564 Charles River Laboratories GLP: yes Published: no	N	EUCuTF
KCP 5.1.2	Foster, A.C.	2006	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 E.I. Du Pont de Nemours and Company, 687805, DuPont-16970 Charles River Laboratories GLP: yes Published: no	N	EUCuTF
KCP 5.1.2	Foster, A.C., Kakkonen, J.E.	2006a	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 E.I. Du Pont de Nemours and Company, 685331, DuPont-14536 Inveresk Research International, Tranent, Scotland GLP: yes Published: no	N	EUCuTF

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	Foster, A.C.	2006b	MAGNITUDE OF RESIDUES OF COPPER AND CYMOXANIL IN FIELD MELONS (FRUITING VEGETABLES) FOLLOWING APPLICATIONS OF METALLIC COPPER (AS COPPER OX-YCHLORIDE)/CYMOXANIL (DPX-KK807) 44WG (9.5:1) UNDER MAXIMUM LABEL RATES - SOUTHERN EUROPE, 2004 E.I. Du Pont de Nemours and Company, 685326, DuPont-14542 Inveresk Research International, Tranent, Scotland GLP: yes Published: no	N	EUCuTF
KCP 5.1.2	Goebel, O.	2008a	RESIDUE DETERMINATION OF COPPER IN MELON APTER 6 APPLICATIONS OF ATOFAP02 (COPPER - 20% - WG) OR ATOFAP17NC (COPPER - 40% - WG) Cerexagri, B_06RFLME01 eurofins-GAB GmbH, Niefern-Öschelbronn, Germany GLP: yes Published: no	N	UPL
KCP 5.1.2	Goebel, O.	2008b	RESIDUE DETERMINATION OF COPPER IN MELON AFTER 6 APPLICATIONS OF ATOFAP02 (WG 20%) OR ATOFAP17NC (WG 40%) Cerexagri, B_05RFLME01 eurofins-GAB GmbH, Niefern-Öschelbronn, Germany GLP: yes Published: no	N	UPL
KCP 5.1.2	Klein, O.	2015	A FIELD STUDY TO EVALUATE THE EFFECTS OF COPPER ON THE EARTHWORM FAUNA IN CENTRAL EUROPE European Copper Task Force, Petit-Lancy, Switzerland, 20031343/G1-NFEw Eurofins Agroscience Services EcoChem GmbH GLP: yes Published: no	N	EUCuTF
KCP 5.1.2	Blust, R., Steven	2016	KINETICS AND SPECIATION OF COPPER IN COPPER BASED FUNGICIDE FORMULATIONS	N	EUCuTF

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
	Joosen, S.		USED IN CROP PROTECTION (UPDATE FEBRUARY 2016) European Copper Task Force, Petit-Lancy, Switzerland, F-Cu 2016-2 Department of Biology, University of Antwerp, Belgium GLP/GEP: no Published: no		
KCP 5.1.2	Schafers, C.	2000	COMMUNITY LEVEL STUDY WITH COPPER HYDROXIDE 50% WP IN AQUATIC MICRO-COSMS EU Copper Task Force, URA-001/4-50 Fraunhofer Institut für Umweltchemie und Ökotoxikologie, Schmallenberg-Grafschaft, Germany GLP/GEP: no Published: no	N	EUCuTF
KCP 5.1.2	Shouten, A.	2016	VALIDATION OF THE DETERMINATION OF 65CU 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 PLAS-MA MASS SPECTROMETER (HR-ICP-MS) EU Copper Task Force, V20801 Triskelion B.V., Zeist, The Netherlands GLP/GEP: no Published: no	N	EUCuTF
KCP 5.1.2	Maas, W.J.M.	2016	IN VITRO DERMAL ABSORPTION OF COPPER (CU) FROM 8 FORMULATIONS THROUGH HUMAN SKIN European Copper Task Force, Petit-Lancy, Switzerland, V9062 + Amendment 01 TNO GLP/GEP: no Published: no	N	EUCuTF
KCP 5.2	Riccelli, S.	2016	METHOD VALIDATION FOR THE REDUCTION OF THE LIMIT OF QUANTIFICATION FOR	N	EUCuTF

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
			COPPER IN REPRESENTATIVE MATRICES OF PLANT ORIGIN. EU Copper Task Force, RA.16.08 Isagro, Ricerca Srl GLP: yes Published: no		
KCP 5.2	Kiefer, R.	2003	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF BIOAVAILABLE COPPER IN SOIL SAMPLE. EU Copper Task Force, 20031084/02-UVX GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany GLP: yes Published: no	N	EUCuTF
KCP 5.2	Carey, D.O.	1989	METHOD VALIDATION REPORT FOR TERRESTRIAL OUTDOOR FIELD DISSIPATION STUDY WITH COPPER CONTAINING PESTICIDES EU Copper Task Force, 88-003 Biospherics Inc., Rockville, MD, USA GLP: yes Published: no	N	EUCuTF
KCP 5.2	Kiefer, R.	2004	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL COPPER IN SOIL SAMPLES EU Copper Task Force, 20031084/01-UVX GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany GLP: yes Published: no	N	EUCuTF
KCP 5.2	Heintze, A.	2001	ASSESSMENT OF SIDE EFFECTS OF URA-13900-F-0-WP ON THE LARVAE OF THE MIDGE, CHIRONOMOUS RIPARIUS WITH THE LABORATORY TEST METHOD. EU Copper Task Force, 99520/01-ASCr GAB Biotech GLP: yes	N	EUCuTF

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
			Published: no		
KCP 5.2	Heintze, A.	2000	ASSESSMENT OF SIDE EFFECTS OF URA-08740-F-O-WP ON THE LARVAE OF THE MIDGE, CHIRONOMUS RIPARIUS WITH THE LABORATORY TEST METHOD EU Copper Task Force, 99507/01-ASCr GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany GLP: yes Published: no	N	EUCuTF
KCP 5.2	Anonymous	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) EU Copper Task Force, DIN 38406 Part 7 not available GLP/GEP: no Published: no	N	EUCuTF
KCP 5.2	Pardo Martinez, M.	2016	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF COPPER RESIDUES IN SURFACE WATE EU Copper Task Force, CH - 157/2016 not available GLP: yes Published: no	N	EUCuTF
KCP 5.2	Anonymous	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. EUCuTF, VDI 2267, Part 1, Air Pollution,the Automobile and Public Health GLP/GEP: no Published: yes	N	EUCuTF

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	Anonymous	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. EUCuTF, VDI 2267, Part 5, not available GLP/GEP: no Published: no	N	EUCuTF
KCP 5.2	Himmelstein, M.W.	2003	FIVE COPPER SUBSTANCES: ABSORPTION, DISTRIBUTION, AND EXCRETION IN MALE RATS. EU Copper Task Force, 11784 E.I. du Pont de Nemours GLP: yes Published: no	N	EUCuTF
KCP 5.2	Wolf, S.	2009a	INDEPENDENT LABORATORY VALIDATION (ILV) OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL IN GRAPES (RAC BUNCHES) AND POTATOES (RAC TUBERS) UPL Europe Ltd., C34136 Harlan Laboratories Ltd. GLP: yes Published: no	N	UPL EU
KCP 5.2	Faessel, V.	2009a	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL RESIDUES IN WATER AND SOIL PART 1: WATER UPL Europe Ltd., R A9161-1 ANADIAG, France GLP: yes Published: no	N	UPL EU
KCP 5.2	Faessel, V.	2009b	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL RESIDUES IN WATER AND SOIL, PART 2: SOIL	N	UPL EU

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
			UPL Europe Ltd., R A9161-2 ANADIAG, France GLP: yes Published: no		
KCP 5.2	Senciuc, M.	2009	DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD(S) FOR THE DETERMINATION OF CYMOXANIL METABOLITES (IN-U3204, IN-KQ960, INT4226 AND IN-W3595) IN WATER UPL Europe Ltd., P/B 1683 G PTRL Europe, Ulm, Germany GLP: yes Published: no	N	UPL EU
KCP 5.2	Wolf, S.	2009b	DEVELOPMENT AND VALIDATION OF A RESIDUE ANALYTICAL METHOD FOR CYMOXANIL IN AIR UPL Europe Ltd., C34147 Harlan Laboratories Ltd. GLP: yes Published: no	N	UPL EU

UPL EU = UPL Europe Ltd.

EUCuTF = EU 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

For any new or additional studies please refer to the corresponding section.

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

A 2.1.1.1 Methods for risk assessments of physical and chemical properties tests

No new or additional studies have been submitted.

A 2.1.1.2 Methods for risk assessments of toxicological studies

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/01 → KCP 7.3/01 and KCP 7.3/02
Report	*In vitro* percutaneous absorption of copper, formulated as Copper Hydroxide (DPX-GFJ52) 53.8WG (35% as metallic copper), through human skin, Maas, W.J.M., Brufau Donés, G., 2016, Report No. DuPont-42821
Guideline(s):	Not specified
Deviations:	Not specified
GLP:	Yes
Acceptability:	Yes, the method complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1 (2021).

The described analytical method was also used in the following study:

In vitro percutaneous absorption of copper, formulated as Copper Hydroxide (DPX-GFJ52) 53.8WG (35% as metallic copper), through rat skin, Maas, W.J.M., Brufau Donés, G., 2016, Report No. DuPont-42649

Materials and methods

Principle of the method
The samples were digested with nitric acid, except the samples of the receptor fluid, and the receptor and donor compartment wash. These samples were diluted with nitric acid before measurement. The amount of ^{63}Cu and ^{65}Cu was determined at mass 63 *m/z* and 65 *m/z*, respectively, using a double-focusing high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) in medium mode (resolution, 4000).

Analytical conditions
ICP-MS
Detected element: Cu (mass 63) and Cu (mass 65)
RF power: 1250 Watt
Cool gas: 16 L/min
Auxiliary gas: 0.7 L/min
Sample gas: 1.0 L/min

Flow: 1 mL/min
Nebulizer: Micro flow nebulizer
Resolution: Medium (4000)

Results and discussions

Specificity

The ICP-MS is considered highly specific method for determination of the specific element. Althoug the absence of interference and matrix effects were not reported in the study report, the calculation by taking into account the measured amount of ^{63}Cu and the natural abundance of ^{65}Cu was performed.

Linearity

The correlation coefficient (r) calculated with at least four calibration points (excluding zero) was ≥ 0.998.

Recovery

The mean recoveries of all spiked samples used for the repeatability were within 70-120%. From the data obtained, this criterion was fulfilled the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1 (2021) and therefore accuracy of the analytical method can be considered acceptable for analysis of copper in receptor fluids, receptor wash, tape strip, cotton swap and membrane using ICP-MS method.

Recovery results

Matrix	Fortification level (µg/L)	n	Mean recovery [%]	RSD [%]
Receptor fluid	0.050	3	85	32.9*
	0.099	3	78	9.0*
	0.198	3	81	6.2*
	0.496	3	84	3.6*
	0.991	3	85	0*
Receptor wash	0.050	3	110	27.2
	0.099	3	106	23.4
	0.198	3	99	13.5
	0.496	3	96	12.0
	0.991	3	108	0.8
Tape strip	0.0198	3	99	8.1*
	0.1982	3	104	1.0*
Cotton swab	1.487	3	98	1.0*
	771.2	3	108	0.9*
Membrane	0.0173	3	90	14.4*
	0.0496	3	97	3.1*
	0.1734	3	101	3.0*

* Calculated by the applicant.

Conclusion

The ICP-MS analytical method was complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1 (2021). The method is therefore considered acceptable for the determination of copper in receptor fluids, receptor wash, tape strip, cotton swab and membrane in support of toxiocological study.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/02 → KCP 7.3/03
Report	*In vivo* percutaneous absorption of copper, formulated as Copper Hydroxide (DPX-GFJ52) 53.8WG (35% as metallic copper) in rats, Maas, W.J.M., Bogaards, J.J.P., de Bie, A. Th., 2016, Report No. DuPont-42648
Guideline(s):	Not specified
Deviations:	Not specified
GLP:	Yes
Acceptability:	Yes, the method complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1 (2021).

The described analytical method was used to determine the concentration of ^{65}Cu in dosing solutions, urine, feces, cage wash, skin wash, tape strips, skin of the application site, skin of the non-treated area, GI tract, residual carcass, cover tapes plus the 'O'-ring and blood collected at necropsy.

Materials and methods

Principle of the method
The samples were digested with nitric acid. In the digests, the amount of ^{63}Cu and ^{65}Cu was determined at mass 63 *m/z* and 65 *m/z*, respectively, using a double-focusing high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) in medium mode (resolution, 4000) with ^{59}Co as internal standard.

Analytical conditions
ICP-MS
Detected element: Cu (mass 63) and Cu (mass 65)
RF power: 1250 Watt
Cool gas: 16 L/min
Auxiliary gas: 0.7 L/min
Sample gas: 1.0 L/min
Flow: 1 mL/min
Nebulizer: Micro flow nebulizer
Resolution: Medium (4000)

Results and discussions
Specificity
The ICP-MS is considered highly specific method for determination of the specific element. Althoug the absence of interference and matrix effects were not reported in the study report, the calculation by taking into account the measured amount of ^{63}Cu and the natural abundance of ^{65}Cu was performed.

Linearity
The correlation coefficient (r) calculated with at least four calibration points (excluding zero) was ≥ 0.998.

Recovery
Blank matrix was spiked in fivefold with a ^{65}Cu containing solution. The mean recoveries of the spiked samples used for the repeatability were within 70-120%, except for the lowest spike level in feces. However, the lowest spike level in the feces was anticipated at 3×LOD but was, erroneously, selected at LOD level which explains the low recovery, especially when taking into account the high amount of endogenous ^{65}Cu present in the matrix samples. At about 3×LOD, recovery increased to about 70%. Considering the very low spike levels relative to the very high amount of endogenous ^{65}Cu present in the matrix samples, the results obtained were considered acceptable.

From the data obtained, this criterion was fulfilled the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1 (2021) and therefore accuracy of the analytical method can be considered acceptable for analysis of copper in body tissues and body fluids using ICP-MS method.

Recovery results

Matrix	Fortification level (ppb)	n	Mean recovery [%]	RSD [%]
Residual carcass	5	5	80.0	3.1
	15	5	81.8	1.9
Tape strips	1	5	89.6	0.9
	5	5	89.7	0.7
Skin	5	5	91.4	4.2
	15	5	96.9	2.8
Urine	10	5	81.3	25.7
	30	5	85.4	8.7
Blood	10	5	69.8 (77.7)*	26.4 (8.4)*
	30	5	91.7	7.7
Plasma	10	5	93.1	21.9
	30	5	94.6	6.1
Feces	50**	5	20.2	60.2
	150	5	69.6	5.8

* The values in the bracket derived from the calculation when an outlier is discarded.
** The lowest spike level was anticipated at 3×LOD but was, erroneously, selected at LOD level.

Conclusion
The ICP-MS analytical method was complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1 (2021). The method is therefore considered acceptable for the determination of copper in body tissues and body fluids samples in support of toxiocological study.

A 2.1.1.3 Methods for risk assessments of residues studies

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/03 → KCA 6.3/04
Report	Method Validation for the Analysis of Copper in Eight Plant Matrices, Falconer, D., 2019, Report No. 41027
Guideline(s):	SANCO/3029/99 rev. 4 (2000) SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with the validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

The described analytical method was used to determine the concentration of the active ingredient, total copper content, in eight plant matrices (Carrot, Plum, Sugar Beet, Pome Fruit, Kiwi Fruit, Grape, Field Strawberries and Flowering Brassica).

The analytical method was also used to determine the concentration of copper in potatoes in support of the residue study R CO233 (Schneider, E., 2021).

Materials and methods

Materials
Test substance: Copper 20% WG (Bordeaux Mixture 20% WG)
Batch/Lot Number: 0718088
Expiry: 29 March 2020
Concentration: 200 g/kg (20%)
Storage Conditions: Store in dry, cool area (40°C max)

Analytical reference item: Copper ICP Standard
Solution: 1000 µg Cu/mL
Batch/Lot No.: 216095132
Expiry Date: 04 October 2021
Storage Conditions: Ambient

Internal standard: Yttrium ICP Standard
Solution: 1000 µg Y/mL
Batch/Lot No.: 216045001 and 214035077
Expiry Date: 12 April 2021 and 26 March 2019
Storage Conditions: Ambient

Principle of the method
Plant matrix samples were analyzed (after digestion and dilution as necessary) by inductively coupled plasma - mass spectrometry (ICP-MS). The samples were quantified using a commercially supplied Cu standard and internal standardisation was employed (Yttrium).

Analytical conditions
Timing parameter
Detected element: Cu (mass 63) and Y (mass 89)
No. of sweeps: 60
No. of readings: 1
No. of replicate: 3
Scan mode: peak hopping
Dwell time; 50 ms
Mode: KED
Cell gas A: 4 mL/min
Signal processing
Detector mode: Dual
Blank subtraction: after internal standard
Measurement units: cps
Calibration
Curve type: weighted linear
Hotblock digestion
Temperature: 100°C
Time: 120 minutes
Dilutent: 2% HNO_3

Sample pre-treatment
The mixture of ca. 0.5 g matrix, 10 mL HNO_3 and 0.25 mL H_2O_2 was digested in a hotblock tube at 100ºC for 120 minutes. Following the digestion, the mixture was made to 50 mL in 2% HNO_3. An aliquot was filtered, followed by 10-fold further dilution in 2% HNO_3 (samples containing 850 ppb to 50,000 ppb Cu in plant matrices and blank plant matrices) or further dilution in 2% HNO_3 as necessary to bring the concentration within the calibration range (samples containing >50,000 ppb Cu in plant matrices). The final dilution was spiked with an internal standard solution prior making to final volume, such that the final concentration of 100 ng/mL Y is obtained.

Validation - Results and discussions

Analyte	Copper (Cu)
Matrix	Plum fruit
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 911 / 97.0 / 2.6 9011 / 94.1 / 1.9 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	911 ppb Cu
LOD	0.0444 ng/mL
Background copper level	888 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -15.3%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Whole sugar beet plant
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 744 / 109 / 14.5 8106 / 89.7 / 0.9 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	744 ppb Cu
LOD	0.0195 ng/mL
Background copper level	772 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -0.7%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Sugar beet tops
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 1100 / 91.1*(97.6#) / 14.6* (2.2#) 10100 / 90.9 / 1.6 * Calculated by the applicant using all 5 data points, without omitting the outliner data. # Calculated based on 4 data points, 1 outliner data was omitted. Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	1100 ppb Cu
LOD	0.0497 ng/mL
Background copper level	1035 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -0.7%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Sugar beet roots
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 832 / 107 / 13.8 8472 / 95.0 / 1.8 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	832 ppb Cu
LOD	0.0211 ng/mL
Background copper level	466 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix enhancement was observed (matrix effect 4.2%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Apple
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 893 / 92.0 / 3.2 8543 / 87.2 / 1.5 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	893 ppb Cu
LOD	0.0414 ng/mL
Background copper level	668 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -5.2%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Whole carrot plant
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 1547 / 83.1 / 9.1 10107 / 104 / 6.5 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	1547 ppb Cu
LOD	0.0193 ng/mL
Background copper level	956 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -7.2%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Carrot tops
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 23182 / 106 / 7.2 203182 / 95.0 / 0.8 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	23182 ppb Cu
LOD	0.250 ng/mL
Background copper level	20071 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix enhancement was observed (matrix effect 2.1%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Carrot roots
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 1111 / 99.9 / 9.3 12311 / 99.9 / 7.1 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	1111 ppb Cu
LOD	0.0404 ng/mL
Background copper level	1247 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -10.5%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Kiwi
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 1503 / 81.1 / 12.9 14740 / 93.0 / 0.8 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	1503 ppb Cu
LOD	0.0349 ng/mL (mature)
Background copper level	1466 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix enhancement was observed (matrix effect 14.2%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Grape berries
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 898 / 84.4 / 4.6 8546 / 91.0 / 2.5 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	898 ppb Cu
LOD	0.0125 ng/mL (mature) and 0.0123 ng/mL (immature)
Background copper level	260 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -3.7%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Strawberry
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 933 / 98.6 / 6.4 8583 / 97.2 / 0.9 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	933 ppb Cu
LOD	0.0266 ng/mL
Background copper level	0.00 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix enhancement was observed (matrix effect 9.2%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Broccoli whole plant
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 848 / 92.3 / 2.3 8498 / 88.9 / 4.5 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	848 ppb Cu
LOD	0.0123 ng/mL
Background copper level	408 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -5.1%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Broccoli inflorescence
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 816 / 102 / 4.7 8466 / 93.0 / 3.9 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	816 ppb Cu
LOD	0.0588 ng/mL
Background copper level	437 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix enhancement was observed (matrix effect 3.6%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Cauliflower whole plant
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 791 / 97.4 / 7.7 8451 / 89.4 / 0.8 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	791 ppb Cu
LOD	0.0203 ng/mL
Background copper level	253 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix enhancement was observed (matrix effect 0.7%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Analyte	Copper (Cu)
Matrix	Cauliflower inflorescence
Specificity	Blank diluent, copper ICP standard at the highest concentration used in the calibration curve, yttrium ICP standard at the same concentration used in the calibration curve and test item approximately diluted to the highest concentration used on the calibration curve all showed no significant interference at target m/z values. Carryover: level of carry over response is not considered to affect the accuracy of the assay significantly.
Calibration/ linearity	Linear within the concentration range of 0.250 – 50.0 ng Cu/mL (8 concentrations) Coefficient of determination (r^2): >0.99
Recovery and Repeatability	**Fortification level [ppb Cu, n=5]** / **Mean recovery [%]** / **RSD [%]** 798 / 102 / 15.3 8442 / 92.5 / 0.7 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	798 ppb Cu
LOD	0.0114 ng/mL
Background copper level	194 ppb Cu
Assessment of matrix effects presented?	Yes, insignificant matrix suppression was observed (matrix effect -6.8%). It is not necessary to prepare calibration standards in matrix-matched calibration solutions.
Stability of standard solution	0.500 mg Cu/mL: stable for 31 days when stored at ambient temperature 50.0 mg Cu/mL: stable for 45 days when stored at ambient temperature
Example chromatograms included in the report?	Not applicable for this analytical technique.

Notes:
- Copper is a monoatomic element and inherently stable and so it will not undergo degradation during storage. The analytical technique measures total copper content of samples. Therefore, residues of copper are expected to be stable in all residue trials samples and no assessment of stability was required.
- Assay specificity samples confirmed a significant response for copper in blank plant matrix samples. Due to this response being observed correction of copper amount in each matrix type was made to account for any background contribution in spiked validation samples.
- Assay specificity samples confirm that there is also increased response for copper in the blank digestion matrix sample. The copper response is higher than the response found for the blank diluent sample. However, this response does not significantly impact the assay as the net found intensity for this sample is smaller in comparison to the blank plant matrix samples analyzed in a similar manner.

Conclusion
The described analytical method has been demonstrated to be satisfactory in terms of specificity, linearity, recovery, repeatability, matrix effects, LOQ and LOD for the determination of copper in Carrot, Plum, Sugar Beet, Pome Fruit, Kiwi Fruit, Grape, Field Strawberries and Flowering Brassica plant matrix samples and in terms of stability of copper in standard solutions according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. The analytical method also complies with the requirements laid down in the current guideline SANTE/2020/12830 rev. 1.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/04 → KCA 6.3/01
Report(s):	Copper Residues in Potatoes after four applications of copper oxychloride 37.5 WG. Two Harvest Trials in Northern Europe (Germany and Poland) in 2008, Analytical phase report, Maccaferri, L., 2009, report number RA.08.26
Guideline(s):	SANCO/3029/99 rev. 4 (2000) SANCO/825/00 rev. 8.1
Deviations:	Yes, only 3 samples (instead of 5) were used for recovery and repeatability study.
GLP:	Yes
Acceptability:	Yes, the method complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1.

This analytical method MA RES 002 was used for analysis of copper residues in potato specimens collected during the field phase of the study RA.08.26 (Sicbaldi, F., Soddu, R. and Riccelli, S., 2009)

Materials and methods
Materials
Test substance: Copper oxychloride 37.5 WG

Analytical reference item: Copper standard for AAS Trace Cert
Solution: 1000 mg/L in 2% HNO_3, ex Fluka

Principle of the method
Residues of copper are determined from homogenized samples by acidic digestion and microwave heating. The solution is filtered and analyzed by reading of its absorbance at 324.8 nm, after calibration of the Atomic Absorption Spectrometer (AAS) with reference item solutions. Using AAS, the identity of copper is verified by the specific absorption wavelength.

Sample pre-treatment
The frozen specimens were chopped and homogenized with the cutter. 5.0 g of homogenized sample were weighed in a vessel for digestion (fortification of the recovery trials at this point) and 6 mL of nitric acid 65%, 1 mL of hydrogen peroxide 30% and 2 mL of de-mineralised water were added. The vessels were closed and loaded into the microwave apparatus. The heating program was started in accordance with the parameters reported below.

Ramp	Time (min)	Temperature (°C)	Power (W)
1	3	85	800
2	20	125	800
3	30	185	800
4	10	185	800

The solutions were left to cool and then were transferred into 20 mL volumetric flasks, bringing to volume with de-mineralised water and then filtered on 0.45 µm porosity PVDF filters.

Analytical conditions
Technique: Flame
Wavelength: 324.8 nm
Slit width: 0.7
Signal type: AA-BG
Read time: 5 sec
Read delay: 25 sec
Flame type: Air/C_2H_2
Oxidant flow: 10 mL/min

Fuel flow: 3 mL/min

Validation - Results and discussions

Specificity

The specificity of the method is given by using the AAS analytical technique, which is a highly specific technique. The method was shown to be specific for the determination of copper as no interference above 30% of the LOQ was observed in the analysis of blank samples. The background copper level for the potato untreated samples was ranging from 0.48 to 0.88 mg/kg.

Linearity

The calibration curves were found to be linear in the concentration range of 0.1 – 2.0 mg/L (seven concentration levels) with the following correlation coefficients: 0.9998, 0.9997.

Limit of quantification (LOQ)

2 mg/kg

Limit of detection (LOD)

0.08 mg/kg, it was calculated from the instrumental sensitivity of 0.02 mg/L, that is the copper concentration corresponding to an absorbance of 0.0044 absorbance units.

Recovery and repeatability

The SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of copper in potato specimens using AAS method.

Recovery and repeatability results

Fortification level (mg/kg)	n	Mean recovery [%]		RSD [%]	
		Without background subtraction	With background subtraction	Without background subtraction	With background subtraction
2	3	128.7	104.7	3.9	4.8
20	3	108.0	105.6	3.7	3.8

Confirmatory

AAS analytical technique for analysis of copper is considered a highly specific technique, therefore a confirmatory method is not required.

Conclusion

The AAS analytical method was successfully validated following SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 and also complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1. The method is considered acceptable for the determination of copper in potato specimens in support of the residue study.

A 2.1.1.4 Methods for risk assessments of environmental fate studies

No new or additional studies have been submitted.

A 2.1.1.5 Methods for risk assessments of ecotoxicology studies

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/05 → KCP 10.2.1/01, KCP 10.2.1/02, KCP 10.2.1/03
Report(s):	GLP Validation study for determination of copper in freshwater, algae and daphnia media by ICP-MS, Brotherhood, A., 2013, report number 1323923 and Freshwater, algae and daphnia copper analysis by ICP-MS, Guzman, M.H., 2011, report number TM1192-Issue No. 2
Guideline(s):	No guideline specified in the study report, but method used is comparable to SANTE/2020/12830 rev.1 (2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1.

The method described in these reports were used to determine the concentration of the active ingredient, copper, in freshwater, algae and daphnia media in support of the following ecotoxicological studies:

Cuprofix C Disperss (FEL02): Determination of acute toxicity to rainbow trout (*Oncorhynchus mykiss*), Hutchinson, K.A. and Sharpe, A.D., 2012a, report No. BR0587/B, study number 11-0134/D

Cuprofix C Disperss (FEL02): Determination of acute toxicity to *Daphnia magna*, Hutchinson, K.A. and Sharpe, A.D., 2012b, report No. BR0586/B, study number 11-0134/C

Cuprofix C Disperss (FEL02): Determination of toxicity to the green alga *Pseudokirchneriella subcapitata*, Hutchinson, K.A. and Sharpe, A.D., 2012c, Report No: BR0585/B, Study number 11-0134/B

Materials and methods (Copper)

Materials

Test substance: Bordeaux mixture
CAS: 8011-63-0

Analytical reference item: Copper
Solution: 1000 ppm w/v copper standard, ex Romil
Batch/Lot No.: I533449

Principle of the method

Freshwater, algae and daphnia samples were analyzed (directly or after dilution) by inductively coupled plasma mass spectroscopy (ICP-MS) for the concentration of copper. The results of the samples analysis are reported as ppb w/v of Cu in the samples.

Sample pre-treatment

The test samples have already been acidified by the sponsor and are to be analyzed as supplied. If the result exceeds, or is expected to exceed the highest calibration standard, the sample is allowed to be diluted with HNO_3 and deionized water. Diluted samples must contain ca. 2% HNO_3.

Analytical conditions

Detected element: Cu (mass 63)
Mode: Helium (He)
Integration time: 0.1 seconds
Replicate: 3
Wash time: 180 seconds

Material and methods (Cymoxanil)

The method was reported in the ecotoxicological study reports (Hutchinson, K.A. and Sharpe, A.D., 2012a, 2012b and 2012c).

Principle of the method

Aqueous samples were analyzed after dilution or after acidifying, solid phase extraction and dilution by high performance liquid chromatography using a mass spectrometry detector (HPLC-MS) for the concentration of cymoxanil.

Analytical conditions
HPLC-MS/MS
Column: Xbridge C18, 50 mm × 2.1 mm, 5 µm
Column temperature: 50°C
Injection volume: 10 µL
Flow rate: 0.5 mL/min
Mobile phase A: 0.1% ammonium acetate in LC-MS water
Mobile phase B: 0.1% ammonium acetate in LC-MS methanol
Gradient: 0.00 min: 90% A/ 10% B
3.00 min: 0% A/ 100% B
3.50 min: 0% A/ 100% B
3.51 min: 90% A/ 10% B
5.00 min: 90% A/ 10% B
Detector: Triple Quadrupole mass spectrometer
Ionization mode: HESI, positive
Ion mass transition: 199.0 → 111.2 m/z (quantification)
199.0 → 128.1 m/z (confirmation)
Retention time: approx. 1.6 minutes

Note: At the end of the ecotoxicological test the cymoxanil analysis was unsuccessful due to a loss of sensitivity in the analytical method.

Validation - Results and discussions

Analyte	Copper (Cu)
Matrix	Freshwater, Algal media water, Daphnia media water
Specificity	No significant interferences for the mass selected for copper. Possible polyatomic interference from titanium oxide was removed by using helium in the reaction cell. The criterion was met.
Calibration/ linearity	Linear within the concentration range of 1.0 – 1000 ppb w/v Cu (8 concentrations) Coefficient of determination (r^2): 0.9997 and 1.0000 Linear Regression Equation: y = (3.865E+3)x + (1.748E+4) and y = (5.810E+3)x + (7.001E+3) Linear within the concentration range of 1.0 – 20 ppb w/v Cu (4 concentrations): used for low copper concentration analysis to improve the accuracy Coefficient of determination (r^2): 0.9999 and 1.0000 Linear Regression Equation: y = (4.013E+3)x – 61.11 and y = (6.441E+3)x + (4.023E+2)
Recovery and Repeatability	**Fresh water** **Fortification level [ppb w/v Cu, n=6]** / **Mean recovery [%]** / **RSD [%]** 1 / 100 / 2 Criterion for recovery was partially met, i.e. mean in the range of 70 -120%, but validate at only one fortification level. Criterion for repeatability was partially met, i.e. coefficient of variation of ≤ 20% but validate at only one fortification level.

	Algal media water **Fortification level [ppb w/v Cu, n=6]** / **Mean recovery [%]** / **RSD [%]** 1 / 98.2 / 8 Criterion for recovery was partially met, i.e. mean in the range of 70 -120%, but validate at only one fortification level. Criterion for repeatability was partially met, i.e. coefficient of variation of ≤ 20% but validate at only one fortification level. **Daphnia media water** **Fortification level [ppb w/v Cu, n=6]** / **Mean recovery [%]** / **RSD [%]** 1 / 93.5 / 14 Criterion for recovery was partially met, i.e. mean in the range of 70 -120%, but validate at only one fortification level. Criterion for repeatability was partially met, i.e. coefficient of variation of ≤ 20% but validate at only one fortification level.
LOQ	1 ppb w/v Cu
Assessment of matrix effects presented?	No. It was mentioned that the matrix effects were assessed prior this study, it was assumed that there is no matric effect in these matrices.
Example chromatograms included in the report?	Not applicable for this analytical technique.

Conclusion
The described analytical method is acceptable to use for determination of copper in freshwater, algal media water and Daphnia media water as its performance (in term of specificity, linearity and recovery at LOQ level) complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/06 → KCP 10.3.1.2/01
Report(s):	Chronic toxicity of Copper 20% + Cymoxanil 4% WG to the honey bee *Apis mellifera* L. under laboratory conditions: *Verification of the concentration of the active ingredient in the test item feeding solutions*, Ruhland, S., 2018, Report No. 17 35 CRB 0157
Guideline(s):	SANCO/3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the validation complied with the current guideline SANTE/2020/12830 rev. 1 (2021).

The described analytical method was used to determine the concentration of the active ingredient, copper, in the test item feeding solution containing sucrose and xanthan in support of the following ecotoxicological study:

Chronic toxicity of Copper 20% + Cymoxanil 4% WG to the honey bee *Apis mellifera* L. under laboratory conditions, Ruhland, S., 2018, Report No. 17 48 BAC 0058

Materials and methods

Materials
Test item: Copper 20% + Cymoxanil 4% water-dispersible granule (WG)

Analytical reference item: Copper
Solution: Copper (Cu) concentration 1.000 g/L in 2% Nitric acid (HNO_3)
Batch/Lot No.: F47680
Purity: 996.8 ± 3.3 mg/L

Principle of the method
Feeding solution samples were analyzed (after dilution) by atomic absorption spectroscopy (AAS) for the concentration of copper.

Sample pre-treatment
The samples (0.250 g ± 0.006 g) were diluted with 0.5% HNO_3 in ultrapure water (30 mL). The samples were vortexed at 2500 rpm for 5 min. The 0.02 mL of the resulting solution were further diluted into 2 mL sample cups with 0.5% HNO_3.

Analytical conditions
Method: AAS-graphite furnace technique

Prefabricated condition for cooper determination:
Modifier: Pd (0.1%)/Mg (0.05%); 5 µL
Injection volume: 20 µL
Detection of copper: 324.7540 nm
Oven temperature program:

Step	Temperature [ºC]	Ramp [ºC]	Hold [s]	Time [s]
Drying	80	6	20	28.3
Drying	90	3	20	23.3
Drying	110	5	10	14.0
Pyrolyse	350	50	20	24.8
Pyrolyse	1100	300	10	12.5
Gas adjustment	1100	0	5	5.0
Atomisation	2000	1500	4	4.6
Bake out	2450	500	4	4.9

Validation - Results and discussions

Analyte	Copper (Cu)
Matrix	Sucrose solution containing 50% (w/v) of sucrose and 0.1% (w/v) xanthan
Specificity	Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected. The criterion (response < 30% of LOQ) was met. The specificity of the method was assured by a high-resolution double monochromator based on a prism and an Echelle grating monochromator. The main absorbance line was chosen for copper (324.754 nm).
Calibration/ linearity	Linear within the concentration range of 2.0 to 40 µg Cu/L (7 concentrations) Coefficient of determination (r^2): 0.9987 Linear Regression Equation: y = 0.0191829 + 0.0111666x
Recovery and Repeatability	**Fortification level [mg Cu/L, n=5]** / **Mean recovery [%]** / **RSD [%]** 1.314 / 109 / 4.3 262.8 / 100 / 1.7 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	1.314 mg Cu/L (corresponding to 3.552 µg Cu/L regarding dilution factor)
Assessment of matrix effects presented?	No. Matrix effects were not taken into account since the samples of the biological part and the validation samples were diluted with 0.5% HNO_3 with high dilution factors (370.0 and 9620).
Example chromatograms included in the report?	Yes

Conclusion
The analytical method presented above was fully validated according to SANCO/3029/99 rev. 4 and also complies with the requirements laid down in the current guideline SANTE/2020/12830 rev. 1. The analytical method is therefore acceptable for the determination of copper in sucrose solution containing 50%(w/v) sucrose and 0.1%(w/v) xanthan.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/07 → KCP 10.3.1.3/01 and KCP 10.3.1.3/02
Report(s):	Copper 20% + Cymoxanil 4% WG - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro*): *Verification of the concentration of the active ingredient in the test item feeding solutions*, Scheller, K., 2018a, Report no. 17 35 CRB 0150 and Copper 20% + Cymoxanil 4% WG – Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro*): *Verification of the concentration of the active ingredient in the test item feeding solutions,* Scheller, K., 2018b, Report no. 17 35 CRB 0149
Guideline(s):	SANCO/3029/99 rev. 4 (2000)

Deviations:	No
GLP:	Yes
Acceptability:	Yes, the validation complied with the current guideline SANTE/2020/12830 rev. 1 (2021).

The described analytical method was used to determine the concentration of the active ingredient, copper, in the test item feeding solutions (diet C) in support of the following ecotoxicological studies:

Copper 20% + Cymoxanil 4% WG - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (in vitro), Scheller, K., 2018a, Report no. 17 48 BLA 0003

Copper 20% + Cymoxanil 4% WG – Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions (*in vitro*), Scheller, K., 2018b, Report no. 17 48 BLC 0092

Materials and methods

Materials

Test item: Copper 20% + Cymoxanil 4% water-dispersible granule (WG)

Analytical reference item: Copper
Solution: Copper (Cu) concentration 1.000 g/L in 2% Nitric acid (HNO_3)
Batch/Lot No.: F47680
Purity: 996.8 ± 3.3 mg/L

Principle of the method

Feeding solution samples were analyzed (after dilution) by atomic absorption spectroscopy (AAS) for the concentration of copper.

Sample pre-treatment

The samples (0.250 g ± 0.006 g) were diluted with 0.5% HNO_3 in ultrapure water (30 mL). The samples were vortexed at 2500 rpm for 5 min. The 0.02 mL of the resulting solution were further diluted into 2 mL sample cups with 0.5% HNO_3.

Analytical conditions

Method: AAS-graphite furnace technique

Prefabricated condition for cooper determination:
Modifier: Pd (0.1%)/Mg (0.05%); 5 µL
Injection volume: 20 µL
Detection of copper: 324.7540 nm
Oven temperature program:

Step	Temperature [°C]	Ramp [°C]	Hold [s]	Time [s]
Drying	80	6	20	28.3
Drying	90	3	20	23.3
Drying	110	5	10	14.0
Pyrolyse	350	50	20	24.8
Pyrolyse	1100	300	10	12.5
Gas adjustment	1100	0	5	5.0
Atomisation	2000	1500	4	4.6
Bake out	2450	500	4	4.9

Validation - Results and discussions

Analyte	Copper (Cu)
Matrix	Diet C containing 18% (w/v) fructose, 18% (w/v) glucose, 4% (w/v) yeast
Specificity	Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected. The criterion (response < 30% of LOQ) was met. The specificity of the method was assured by a high-resolution double monochromator based on a prism and an Echelle grating monochromator. The main absorbance line was chosen for copper (324.754 nm).
Calibration/ linearity	Linear within the concentration range of 2.0 to 40 µg Cu/L (7 concentrations) Coefficient of determination (r^2): 0.9987 Linear Regression Equation: y = 0.0191829 + 0.0111666x
Recovery and Repeatability	**Fortification level [mg Cu/L, n=5]** / **Mean recovery [%]** / **RSD [%]** 140.2 / 107 / 2.1 350.5 / 95 / 4.2 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	140.2 mg Cu/L (corresponding to 10.9 µg Cu/L regarding dilution factor)
Assessment of matrix effects presented?	No. Matrix effects were not taken into account since the samples of the biological part and the validation samples were diluted with 0.5% HNO_3 with a high dilution factor of 13900.
Example chromatograms included in the report?	Yes

Conclusion
The analytical method presented above was fully validated according to SANCO/3029/99 rev. 4 and also complies with the requirements laid down in the current guideline SANTE/2020/12830 rev. 1. Therefore, the analytical method was acceptable for the determination of copper in diet C containing 18% (w/v) fructose, 18% (w/v) glucose, 4% (w/v) yeast.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/08 → KCP 10.3.1.2/02
Report(s):	Chronic oral effects of copper oxychloride 50% WP to adult worker honeybees Apis mellifera L., 10-day feeding laboratory test, Colli, M., 2018, Report No: BT215/17
Guideline(s):	SANCO/3029/99 rev. 4 (2000)
Deviations:	Yes, (1) temperature and humidity occasionally deviated from the guideline norm values. As this occurred for < 2 hours/day this deviation is not considered to adversely affect the results of the study and (2) only 3 samples were used for recovery and repeatability study.
GLP:	Yes

Acceptability: Yes, the validation complied with the minimum validation requirement for the assessment of existing methods for risk assessment stated in the current guideline SANTE/2020/12830 rev.1.

Validation - Results and discussions

Analyte	Copper (Cu)
Matrix	Water / sugar feeding solution
Specificity	The absence of interference >30% of LOQ in blank sample was demonstrated.
Calibration/ linearity	**Water stock solution** Linear within the concentration range of 0.1-100 µg/L Coefficient of determination (r^2): 1.0000 Linear Regression Equation: y = 15002.0417 x + 6790.3067 **Sugar feeding solution** Linear within the concentration range of 10-100 µg/L Coefficient of determination (r^2): 0.9996 Linear Regression Equation: y = 14782.7641 x + 3770.5233
Recovery and Repeatability (water stock solution)	**Fortification level [mg/L, n=3]** / **Mean recovery [%]** / **RSD [%]** 70 / 104.13 / 3.45 700 / 96.61 / 5.27 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
Recovery and Repeatability (sugar feeding solution)	**Fortification level [mg/kg, n=3]** / **Mean recovery [%]** / **RSD [%]** 1.8 / 105.06 / 1.28 18 / 103.04 / 2.02 Criterion for recovery (mean in the range of 70 -120%) was met. Criterion for repeatability (coefficient of variation of ≤ 20%) was met.
LOQ	70 mg/mL in water stock solution 1.8 mg/kg in sugar feeding solution
Assessment of matrix effects presented?	Yes
Example chromatograms included in the report?	No

Conclusion
The ICP-MS method for determination of copper content in water stock solution and sugar feeding solution was successfully validated. The method is to be considered validated and fitting its purpose.

A 2.1.1.6 Methods for risk assessments of efficacy studies

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)

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/01
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, Riccelli, S., 2017, report No. RA.17.02
Guideline(s):	SANCO/3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the validation complied with the validation requirement stated in the current guideline SANTE/2020/12830 rev.1.

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 analyzed 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

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%.

Validation - Results and discussions
Matrix effect
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 SANCO/3029/99 rev. 4 guideline. Method validation data are summarised in the tables below.

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%.

Recovery and repeatability result

Crop group	**Matrix**	**Fortification level [mg/kg]**	**n**	**Mean recovery** [%]	**RSD** [%]
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	106.3	3.4

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

Parameter	**Total copper**
Specificity	The specificity of the method is given by using the FAAS analytical technique at the copper specific wavelength of 324.8 nm. No 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 in the concentration range of 0.01 – 10.0 mg/L, corresponding to the following concentration: 0.2 – 200 mg/kg in wheat grain, y = 0.000509 + 0.110782 x, r^2 = 0.9999 0.4 – 400 mg/kg in oilseed rape, y = -0.000391 + 0.101070 x, r^2 = 1.0000 0.04 – 40 mg/kg in lettuce and grape
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	Tested LOQ Oilseed rape seed: 5.5 mg/kg Wheat grain: 7.5 mg/kg Calculated LOD Oilseed rape seed: 1.6 mg/kg Wheat grain: 1.0 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, please refer to the RAR (2017) for information.

A 2.1.2.3 Description of analytical methods for the determination of residues in soil (KCP 5.2)

No new or additional studies have been submitted, please refer to the RAR (2017) for information.

A 2.1.2.4 Description of analytical methods for the determination of residues in water (KCP 5.2)

No new or additional studies have been submitted, please refer to the RAR (2017) for information.

A 2.1.2.5 Description of analytical methods for the determination of residues in air (KCP 5.2)

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/02
Report	Validation of the Analytical Method for the determination of Copper residues in Air, Pardo Martinez, M., 2018, report No. CH-657/2017
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	Yes, although the matrix effect test was not required in the study plan and in the SANCO/825/00 rev. 8.1, it was conducted.
GLP:	Yes
Acceptability:	Yes, the method complied with the validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

Materials and methods
Principle of the method
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 µm Metricel® 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 m^3 volume of air and therefore to reach the required LOQ of 0.30 $\mu g/m^3$.

2) For retention capacity: The air sampling was performed at temperature of 35°C and relative humidity of 80% on a 0.30 $\mu g/m^3$ 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.

Validation - Results and discussions
Matrix effect
The matrix effects for copper in air were found not significant (lower than 20%) for both stable isotopes, the quantification of the air samples was performed using copper standard solutions prepared in dilution medium.

Specificity and confirmatory
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 $\mu g/m^3$ to 8.33 $\mu g/m^3$ in the air sample and was found to be linear for both ^{63}Cu quantifier and ^{65}Cu 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 and limit of detection
The limit of quantification (LOQ) was the low fortification level at 0.30 $\mu g/m^3$ for copper in air samples, corresponding to a final injected solution of 1.80 µg/L.

The limit of detection (LOD), defined as half of the lowest calibration level, was 0.50 µg/L, corresponding to 0.08 $\mu g/m^3$ for copper in air samples.

Recovery and repeatability
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 ^{63}Cu quantifier and ^{65}Cu qualifier isotopes.

Recovery and repeatability results

Matrix	Qualifier isotopes	Fortification level	n	Mean recovery [%]	RSD [%]
Air	^{63}Cu	0.30 $\mu g/m^3$	6	83.7	2.69
		2.99 $\mu g/m^3$	6	83.0	1.90
	^{65}Cu	0.30 $\mu g/m^3$	6	84.2	3.32

Matrix	Qualifier isotopes	Fortification level	n	Mean recovery [%]	RSD [%]
		2.99 µg/m^3	6	84.2	1.50

Retention capacity
The air sampling was performed at temperature of 35°C and relative humidity of 80% on a 0.30 µg/m^3 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. 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 according to SANCO/825/00 rev 8.1 and also complied with the requirements laid down in the current guideline SANTE/2020/12830 rev. 1. The method is therefore suitable for the determination of copper residues in air.

A 2.1.2.6 Description of analytical methods for the determination of residues in body fluids and tissues (KCP 5.2)

No new or additional studies have been submitted, please refer to the RAR (2017) for information.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for Cymoxanil

For any new or additional studies please refer to the corresponding section.

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.2.1.1 Methods for risk assessments of physical and chemical properties tests

No new or additional studies have been submitted.

A 2.2.1.2 Methods for risk assessments of toxicological studies

No new or additional studies have been submitted.

A 2.2.1.3 Methods for risk assessments of residues studies

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/09 → KCA 6.3/05
Report	Cymoxanil: Validation of an enforcement method for various crop types, Richter, S., 2009, report No. P/B 1668 G
Guideline(s):	SANCO/3029/99, rev. 4 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

The objective of this study was to adopt and to validate the QuEChERS multi-residue analytical method (EN 15662:2008) for determination of cymoxanil in/on various crop types, exemplified by lettuce (high water content), grape berries (high acid content), hazelnuts (high oil content), and potato tuber (high starch content), all with a target limit of quantification (LOQ) of 0.02 mg/kg.

Materials and methods

Principle of the method

The homogenized sample is extracted with acetonitrile. After addition of $MgSO_4$, NaCl, and buffering citrate salts (pH 5-5.5), the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is cleaned-up by freezing out oil (hazelnut only), followed by dispersive SPE on PSA, $MgSO_4$ and for hazelnut additionally with $C1_8$ SPE material. Extracts are acidified with formic acid to stabilize base-sensitive analytes for subsequent LC-MS/MS analysis, monitoring two mass transitions for quantitation and confirmation.

Analytical conditions

HPLC-MS/MS

Column:	Aquasil C18, 3 mm × 150 mm, 3 µm
Column temperature:	40°C
Injection volume:	10 µL
Flow rate:	0.3 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	0.00 min: 80% A/ 20% B 1.00 min: 80% A/ 20% B 3.00 min: 5% A/ 95% B 10.00 min: 5% A/ 95% B 10.10 min: 80% A/ 20% B 13.00 min: 80% A/ 20% B
Detector:	Triple Quadrupole mass spectrometer
Ionization mode:	ESI, positive
Ion mass transition:	199 → 128 m/z (quantification) 199 → 111 m/z (confirmation)

Validation - Results and discussions

Matrix effect

Matrix matched standard calibration solutions were used by default.

Specificity

No interfering peaks were observed at the retention time of the analyte when matrix blank control extracts were injected.

Linearity

Calibration curves of cymoxanil were linear using five matrix-matched standards in the range of 1.0 ng/mL to 100 ng/mL of cymoxanil (equivalent to 0.005 – 0.5 mg/kg in the sample). This range covers the concentration range from 25% of the LOQ to at least 20% above the highest fortification level.

Transition mass: 199 → 128, Intercept: 22600, Slope of the line: 1210, R^2: 1.0000
Transition mass: 199 → 111, Intercept: 12200, Slope of the line: 4170, R^2: 0.9999

Limit of quantification (LOQ)
0.02 mg/kg (corresponding to 4 ng/mL in the extract)

Recovery and repeatability
The SANCO/3029/99, rev. 4 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 analysis of cymoxanil in grape, lettuce, potato and hazelnut using HPLC-MS/MS method.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Grape	199 → 128	0.02	5	87	3
		0.20	5	104	6
	199 → 111	0.02	5	91	3
		0.20	5	105	7
Lettuce	199 → 128	0.02	5	110	1
		0.20	5	110	0
	199 → 111	0.02	5	106	1
		0.20	5	109	0
Potato	199 → 128	0.02	5	107	2
		0.20	5	101	1
	199 → 111	0.02	5	105	2
		0.20	5	100	1
Hazelnut	199 → 128	0.02	5	109	1
		0.20	5	106	2
	199 → 111	0.02	5	104	1
		0.20	5	106	1

Confirmatory
Simultaneous confirmation of the analytical method was achieved by monitoring one additional SRM transition. The method was considered highly specific, thus the use of an alternative method was not necessary.

Stability
Cymoxanil was stable when stored in the dark as solution in acetonitrile/water (containing 0.1% (v/v) formic acid) in the refrigerator during the duration of analysis (1 week).

Conclusion
The QuEChERS method was assessed and validated at the 0.02 mg/kg LOQ level and at the 0.20 mg/kg (10xLOQ) level for determination of cymoxanil in lettuce, grape berries, hazelnut, and potato tuber.

The method was successfully validated following SANCO/3029/99, rev. 4 and complied with the current guideline SANTE/2020/12830 rev.1. The method demonstrated to be applicable to used in support of the residue studies.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/10 → KCA 6.3/07
Report	Validation of the analytical Methods for the Determination of Residues of cymoxanil, mancozeb and its Metabolite ETU in Potato (Tuber), Weber, H., 2008, report No. GAB-0703V
Guideline(s):	SANCO/3029/99, rev. 4 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

The study objective was to validate the analytical method as described in multi method L 00.00-34 opf the Official Collection of Test methods according to § 64 LFGB (food, Commodity and feed code) (formerly DFG S19) for the determination of cymoxanil in/on potato (tuber). Only the validation for cymoxanil is reported below.

Materials and methods

Principle of the method

The method used was the extended and revised version of DFG method S19. This method describes the analytical procedures for the determination of residues of organochlorine and organophosphorus compounds, nitrogen-containing and other pesticides in food. This method includes all former version of DFG method S19, but provides and extended range of application.

The sample was extracted with acetone. Warm water is added beforehand in an amount that takes full account of the natural water content of the specimen so that during extraction the acetone/water ratio remains constant at 2/1 (v/v). for liquid-liquid partition ethyl acetate/cyclohexane and sodium chloride are added and after repeated mixing the phases are separated. The evaporated residue of an aliquot of the organic phase is cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane as eluant. An aliquot of the purified extract is dissolved in methanol/acetic acid and analyzed by LC-MS/MS for residues of cymoxanil.

Analytical conditions

UPLC-MS/MS	
Column:	Macherey-Nagel MN® C18 Gravity, 50 x 2.0 mm
Column temperature:	40°C
Flow rate:	0.4 mL/min
Mobile phase A:	Methanol/ 5mM ammonium acetate/ 0.1% formic acid
Mobile phase B:	Water/ 5mM ammonium acetate/ 0.1% formic acid
Gradient:	0.0 min: 20% A/ 80% B 0.1 min: 20% A/ 80% B 1.5 min: 90% A/ 10% B 2.5 min: 90% A/ 10% B 2.51 min: 20% A/ 80% B 4.0 min: 20% A/ 80% B
Detector:	Mass detector
Ionization mode:	ESI, positive
Ion mass transition:	199 → 128 m/z (quantification) 199 → 111 m/z (confirmation)

Validation - Results and discussions

Matrix effect

No data provided.

Specificity

No interfering peaks above 30% of the LOQ were observed at the retention time of the analyte when blank control extracts were injected.

Linearity

Calibration curves of cymoxanil were linear using seven calibration solutions of cymoxanil in the range of 0.4 to 80

ng/mL, (corresponding to 0.002 - 0.34 in mg/kg). This range covers the concentration range from 20% of the LOQ to at least 20% above the highest fortification level.

Transition mass: 199 → 128, Intercept: 146.48, Slope of the line: 363.07, R^2: 0.9991
Transition mass: 199 → 111, Intercept: 29.912, Slope of the line: 225.85, R^2: 0.9998

Limit of quantification (LOQ)
0.01 mg/kg (corresponding to 2.34 ng/mL in the extract)

Recovery and repeatability
The SANCO/3029/99, rev. 4 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 analysis of cymoxanil in grape, lettuce, potato and hazelnut using HPLC-MS/MS method.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Potato	199 → 128	0.01	5	99	2.7
		0.1	5	103	2.7
	199 → 111	0.01	5	97	4.2
		0.1	5	98	3.9

Confirmatory
Simultaneous confirmation of the analytical method was achieved by monitoring one additional SRM transition. The method was considered highly specific, thus the use of an alternative method was not necessary.

Conclusion
This analytical method based on the extended and revised version of DFG method S19 is suitable to determine cymoxanil in potato. The method was successfully validated according SANCO/3029/99, rev. 4 and complied with the current guideline SANTE/2020/12830 rev.1.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/11 → KCA 6.3/08 and KCA 6.3/09
Report	Validation of the DFG Method S 19 (Extended and Revised Version) for the determination of residues of cymoxanil in matrices with high water content [Melon (peel and pulp), grapes and potatoes], Lakaschus, S., 2004, report No. DuPont-15026
Guideline(s):	SANCO/825/00 rev. 7 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

Materials and methods
Principle of the method
DFG method S 19 (extended and revised version) published as "Modular Multiple Method for the Determination of Pesticides Residues in Foodstuffs, L 00.00-34", Collection of Official Methods under Article 35 of the German Foods and Commodities Act (LMBG) with extraction module A 1 for matrix with high water content [melon (peel and pulp), grapes and potatoes], gel permeation chromatography (module GPC), silica gel mini column chromatography (module C 1) and nitrogen phosphorous detection (NPD, module D 3), confirmatory analysis with LC-MS/MS.

Analytical conditions

GC-NDP

Column:	15 m fused silica capillary column DB-1, internal diameter 0.53 mm
Column temperature:	Initial 100°C, hold for 1 min., heat rate 10°C/min to 250°C, hold for 12 min
Injector Temperature:	250°C
Detector Temperature:	270°C
Detector:	Nitrogen phosphorous detection (NPD)

HPLC-MS/MS

Column:	LUNA C18(2), 2.0 x 150 mm
Column temperature:	25°C
Flow rate:	0.4 mL/min
Mobile phase A:	Methanol/ 0.05% acetic acid
Mobile phase B:	Water/ 0.05% acetic acid
Gradient:	3.0 min: 10% A/ 90% B (equilibration time) 0.0 min: 10% A/ 90% B 2.0 min: 90% A/ 10% B 7.0 min: 90% A/ 10% B
Detector:	QTrap tandem spectrometer
Ionization mode:	MRM, positive
Ion mass transition:	199 → 128 m/z (quantification) 199 → 111 m/z (confirmation)

Validation - Results and discussions

Matrix effect

No data provided.

Specificity

No significant interference from the specimen matrix were detected in any of the control specimens by GC-NPD and LC-MS/MS.

Linearity

GC-NPD: Calibration curves of cymoxanil were linear using seven calibration solutions of cymoxanil in the range of 0.02- 2.0 µg/mL (corresponding to 0.003 - 0.25 in mg/kg). This range covers the concentration range from 23% of the LOQ to at least 20% above the highest fortification level. Intercept: -1763, Slope of the line: 62147, R^2: 0.9997

LC-MS/MS: Calibration curves of cymoxanil were linear using six calibration solutions of cymoxanil in the range of 0.001 – 0.100 µg/mL. Intercept: 2090.2, Slope of the line: 13166, R^2: 0.9998

Limit of quantification (LOQ)

0.01 mg/kg (corresponding to 0.08 µg/mL in the extract) for melon pulp, potatoes, grapes
0.02 mg/kg (corresponding to 0.15 µg/mL in the extract) for melon peel

Recovery and repeatability

The SANCO/3029/99, rev. 4 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 analysis of cymoxanil in melon pulp, melon peel, potatoes and grapes.

Recovery and repeatability results

Matrix	Detection method	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Melon peel	GC-NPD	0.02	5	95	5.3
		0.2	5	104	8.0
	LC-MS/MS	0.02	5	88	9.7
Melon pulp	GC-NPD	0.01	3	105	18

Matrix	Detection method	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
		0.1	5	107	9.8
	LC-MS/MS	0.01	3	90	3.4
Potatoes	GC-NPD	0.01	5	93	4.2
		0.1	5	89	7.9
	LC-MS/MS	0.01	3	83	13
Grapes	GC-NPD	0.01	5	98	10
		0.1	5	91	5.9
	LC-MS/MS	0.01	3	84	13

Conclusion
This analytical method based on the extended and revised version of DFG method S19 is suitable to determine cymoxanil in melon (peel and pulp), potatoes and grapes. The method was successfully validated according SANCO/3029/99, rev. 4 and complied with the current guideline SANTE/2020/12830 rev.1.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.1.2/12 → KCA 6.3/06
Report	Determination of residues at harvest in potatoes, following six broadcast applications of HARPON WG, under field conditions. – Northern Europe – Season 2010, Tetuan, B., 2011, report No. 10 F PT GW P/A (PROMO/ZOX-CM/10.01)
Guideline(s):	SANCO/3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

Materials and methods
Principle of the method
Residues of cymoxanil and zoxamide are extracted from potatoes with an acetonitrile/2% potassium bicarbonate aqueous solution mixture. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (QuEChERS), the mixture was shaken intensively and centrifuged for phase separation. An aliquot of the organic phase was cleaned-up by dispersive solid phase extraction (D-SPE). The determination was performed by liquid chromatography with detection by mass spectrometric in tandem (LC-MS/MS).

Analytical conditions
LC-MS/MS

Column:	PHENOMENEX C18, 4.6 mm × 150 mm
Column temperature:	35°C
Injection volume:	1.0 µL
Mobile phase A:	Ultra pure water/glacial acetic acid (100/0.1, v/v) and 5mM ammonium acetate
Mobile phase B:	Methanol/glacial acetic acid (100/0.1, v/v) and 5mM ammonium acetate.
Gradient:	0 min: 50% A/ 50% B 5 min: 50% A/ 50% B 9 min: 0% A/ 100% B 13 min: 0% A/ 100% B 14 min: 50% A/ 50% B 20 min: 50% A/ 50% B
Detector:	Quadrupole mass detection
Ionization mode:	ESI, positive

Ion mass transition: 199 → 128 m/z (quantification)
199 → 111 m/z (confirmation)

Validation - Results and discussions
Validation data presented below are data of cymoxanil only.

Matrix effect
No data provided.

Specificity
No interfering peaks above 30% of the LOQ were observed at the retention time of the analyte when blank control extracts were injected.

Linearity
Calibration curves of cymoxanil were linear using five calibration solutions of cymoxanil in the range of 0.004 to 0.030 mg/L, (corresponding to 0.004 at 0.03 in mg/kg). This range covers the concentration range from 40% of the LOQ to at least 20% above the highest fortification level. Intercept: 1325.7, Slope of the line: 10452, R^2: 0.9999

Limit of quantification (LOQ)
0.01 mg/kg (corresponding to 0.01 mg/L in the extract)

Recovery and repeatability
The SANCO/3029/99, rev. 4 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 analysis of cymoxanil in grape, lettuce, potato and hazelnut using HPLC-MS/MS method.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Potato	199 → 128	0.01	5	101	4
		0.1	5	110	5

Confirmatory
The analyses were carried out by LC-MS/MS, monitoring two transitions. The method was considered highly specific, thus the use of an alternative method was not necessary.

Stability
No data provided.

Conclusion
This QuEChERS method is suitable to determine cymoxanil in potato with LC-MS/MS. This validation is fully validated according SANCO/3029/99, rev. 4 and complied with the current guideline SANTE/2020/12830 rev.1.

A 2.2.1.4 Methods for risk assessments of environmental fate studies

No new or additional studies have been submitted.

A 2.2.1.5 Methods for risk assessments of ecotoxicology studies

Please refer to point A 2.1.1.5 for the respective summaries.

A 2.2.1.6 Methods for risk assessments of efficacy studies

No new or additional studies have been submitted.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/03
Report	Validation of Multi-Residue Method DFG S19 (LC-MS/MS Module) for the Determination of Residues of Cymoxanil in Tomato, Grapes, Oilseed Rape and Wheat Grain, Lakaschus S., Gizler A., 2013, report No. DuPont-35769 and Position paper to cover the extraction efficiency of the Multi-Residue Method DFG S19 reported in the report DuPont-35769, Seck C, Goody T., 2019
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	The method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

Materials and methods

Principle of the method

Tomato, wheat grain and grapes: Specimens are extracted with acetone using a homogeniser. Water is added beforehand in an amount that takes into account the natural water content of the specimen so that during extraction the acetone/water ratio remains constant (for wheat grain the water is heated to 40°C and samples are allowed to soak). For grapes only: A small amount of sodium bicarbonate is added to adjust the pH value to pH 7.
After addition of sodium chloride and ethyl acetate/cyclohexane and repeated homogenisation, the organic layer containing cymoxanil is allowed to separate from the aqueous layer. The evaporated residue of an aliquot of the organic phase is cleaned up by gel permeation chromatography (GPC) on Bio Beads S-X3 (polystyrene gel) using a mixture of ethyl acetate and cyclohexane as eluent and an automated gel permeation chromatograph. The residue-containing GPC fraction is concentrated, re-dissolved in the HPLC solvent and analyzed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

Oilseed rape: Specimens are extracted with acetone and acetonitrile in the presence of Calflo E and Celite. The suspension is blended intensively and filtered with suction through a paper filter in a Buchner porcelain funnel. Then the filtrate is filtered through a dry fluted filter covered with Calflo E into a graduated cylinder. The volume of the filtrate is measured, and transferred, rinsing with acetone, into a round-bottomed flask. Isooctane is added, and the solution is reduced using rotary-evaporation. Last traces of solvent are removed with a gentle stream of air at room temperature. The weight of the residue is determined. The evaporated residue of the organic phase is dissolved in ethyl acetate/cyclohexane and cleaned up by gel permeation chromatography on Bio Beads S-X3. The residue-containing GPC fraction is concentrated, re-dissolved in the HPLC solvent and analyzed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

Analytical conditions

HPLC-MS/MS for tomato, grapes and wheat grain stability test; oilseed rape validation and stability test

Column:	Luna C18, 150 × 2.0 mm, 5.0 µm and pre-column
Column temperature:	40 °C
Injection volume:	15 µL
Flow rate:	0.4 mL/min

Mobile phase A:	0.05% acetic acid in methanol
Mobile phase B:	0.05% acetic acid in water
Gradient:	0.0 min: 10% A/ 90% B
	0.0 → 5.0 min: 10→95% A/ 90→5% B
	5.0 → 7.0 min: 95% A/ 5% B
	7.0 → 7.1 min: 95→10% A/ 5→90% B
	7.1 → 9.0 min: 10% A/ 90% B
Detector:	Tandem mass detector
Ion source:	ESI, positive mode
Ion mass transitions:	199 → 128 m/z (quantification)
	199 → 111 m/z (confirmation)

HPLC-MS/MS for tomato, grapes and wheat grain validation

Column:	Ascentis Express, C18 2.1 x 50 mm, 2.7 µm
Column temperature:	40 °C
Injection volume:	7 µL
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% formic acid / methanol / 5 mM ammonium formate
Mobile phase B:	0.1% formic acid / 5 mM ammonium formate
Gradient:	0.0 → 2.5 min: 10→95% A/ 90→5% B
	2.5 → 3.0 min: 95% A/ 5% B
	3.0 → 3.1 min: 95→10% A/ 5→90% B
	3.1 → 4.0 min: 10% A/ 90% B
Detector:	Tandem mass detector
Ion source:	ESI, positive mode
Ion mass transitions:	199 → 128 m/z (quantification)
	199 → 111 m/z (confirmation)

Validation - Results and discussions

Matrix effect

Matrix effects of <20% were measured for tomato and grapes and considered not to be significant. Therefore, solvent standards were used for calibration and quantification.

For wheat grain and oilseed rape, minor matrix effects were detected, so matrix matched standards were used for calibration and quantification.

Specificity

The concentration of cymoxanil in the final extracts was determined by HPLC-MS/MS. In order to ensure unambiguous identification two mass transitions were monitored (MRM 199→128 and MRM 199→111). No significant interferences from the specimen matrix were detected at the retention time corresponding to cymoxanil in any of the control specimens. For cymoxanil, the control specimens of tomato, grapes, oilseed rape and wheat grain and the blank samples indicated no detectable residues.

Linearity

Linearity in the concentration range of 0.250 to 25.0 ng/mL (corresponding to 0.002 to 0.214 mg/kg in samples) covering a range from less than 30% of the LOQ to 20% above the highest fortification level.

Matrix	Transition mass (m/z)	Intercept	Slope of the line	R^2
Solvent standard (tomato, grapes)	199 → 128	17.94	119.01	0.9994
	199 → 111	10.616	75.095	0.9998
Wheat grain	199 → 128	14.585	144.04	0.9999
	199 → 111	4.1339	92.699	0.9999
Oilseed rape	199 → 128	-1829	19074	0.9996
	199 → 111	-700.28	7113.3	0.9997

Limit of quantification (LOQ)

0.01 mg/kg (corresponding to 1.17 ng/mL in the extract) of cymoxanil in tomato, grapes, oilseed rape and wheat grain

Recovery and repeatability

The SANCO/825/00 rev. 7 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in tomato, grapes, oilseed rape and wheat grain.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Tomato	199 → 128	0.01	5	71	12
		0.10	5	83	9.0
	199 → 111	0.01	5	77	10
		0.10	5	86	8.3
Grape	199 → 128	0.01	5	87	7.4
		0.10	5	74	4.3
	199 → 111	0.01	5	87	4.4
		0.10	5	75	3.9
Oilseed rape	199 → 128	0.01	5	87	5.9
		0.10	5	88	7.6
	199 → 111	0.01	5	94	6.5
		0.10	5	91	8.9
Wheat grain	199 → 128	0.01	5	91	6.4
		0.10	5	102	7.3
	199 → 111	0.01	5	91	6.9
		0.10	5	100	4.4

Confirmatory
Simultaneous confirmation of the analytical method was achieved by monitoring one additional SRM transition. The method was considered highly specific, thus the use of an alternative method was not necessary.

Extraction efficiency
A position paper has been written to cover this point. A summary is presented below.

By comparison with the extraction system of the metabolism studies, the extraction efficiency has been confirmed:
In the metabolism studies, the extraction solvent was acetonitrile with a small fraction of a buffer solution (1.0 M, pH 4.0). This solvent was confirmed as an adequate extraction solvent. The solvent of the proposed monitoring method, acetone, is considered as a similar solvent than acetonitrile due to the similar solubility for cymoxanil (about 62 g/L for acetone and 58 g/L for acetonitrile). The addition of a buffer solution or water in the extraction solvent is irrelevant as cymoxanil is pH independent. So, the extraction efficiency is not affected if the samples are extracted only with acetonitrile or acetone.

Also, as cymoxanil is non-systemic, it is recognised that cymoxanil will stay on the surface of the plant after application and therefore no incurred residue is expected. On this basis, the extraction efficiency has also been addressed by looking at the recoveries on stored samples. As recoveries from fresh fortified samples and stored frozen fortified samples, stored for a minimum of 1 year, were in line, it can be concluded that the extraction efficiency has been confirmed too.

Conclusion
The HPLC-MS/MS method was successfully validated following SANCO/825/00 rev. 8.1 and complied with the current guideline SANTE/2020/12830 rev.1. The method is therefore suitable for the determination of residues of cymoxanil in tomato, grapes, oilseed rape and wheat grain.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/04
Report	Independent Laboratory Validation of Multi-Residue Method DFG S19 for the Determination of Residues of Cymoxanil in Tomato, Grapes, Oilseed Rape and Wheat Grain Using LC-MS/MS, Cermak J., 2013, report No. DuPont-35770
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	The method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

Materials and methods

Principle of the method

Tomato, wheat grain and grapes: Specimens are extracted with acetone using a homogeniser. Water is added beforehand in an amount that takes into account the natural water content of the specimen so that during extraction the acetone/water ratio remains constant (for wheat grain the water is heated to 40°C and samples are allowed to soak).

After addition of sodium chloride and ethyl acetate/cyclohexane and repeated homogenisation, the organic layer containing cymoxanil is allowed to separate from the aqueous layer. The evaporated residue of an aliquot of the organic phase is cleaned up by gel permeation chromatography (GPC) on Bio Beads S-X3 (polystyrene gel) using a mixture of ethyl acetate and cyclohexane as eluent and an automated gel permeation chromatograph. The residue-containing GPC fraction is concentrated, re-dissolved in the HPLC solvent and analyzed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

Oilseed rape: Specimens are extracted with acetone and acetonitrile in the presence of Calflo E and Celite. The suspension is blended intensively and filtered with suction through a paper filter in a Buchner porcelain funnel. Then the filtrate is filtered through a dry fluted filter covered with Calflo E into a graduated cylinder. The volume of the filtrate is measured, and transferred, rinsing with acetone, into a round-bottomed flask. Isooctane is added. Last traces of solvent are removed with a gentle stream of nitrogen at room temperature. The evaporated residue of the organic phase is dissolved in ethyl acetate/cyclohexane and cleaned up by gel permeation chromatography on Bio Beads S-X3. The residue-containing GPC fraction is concentrated, re-dissolved in the HPLC solvent and analyzed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

Analytical conditions

HPLC-MS/MS

Column:	Ascentis Express C18, 50 x 2.1 mm, 2.7 µm and precolumn C18, 20 x 2.1 mm, 3 µm
Column temperature:	40 °C
Injection volume:	25 µL
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	0.0 min: 90% A/ 10% B 0.0 → 6.0 min: 5% A/ 95% B 6.0 → 8.0 min: 5% A/ 95% B 8.0 → 8.1 min: 90% A/ 10% B 8.1 → 10.0 min: 90% A/10% B
Detector:	Triple Quadrupole mass spectrometer
Ion source:	ESI, positive mode
Ion mass transitions:	199.0 → 128.0 ± 0.1 m/z (quantification) 199.0 → 111.0 ± 0.1 m/z (confirmation)

Validation - Results and discussions

Matrix effect

Matrix matched standards were used for calibration and quantification because matrix effects were detected for all

matrices.

Specificity
The concentrations of cymoxanil in the final solutions were determined by HPLC-MS/MS. In order to ensure unambiguous identification two mass transitions were monitored (MRM 199→128 and MRM 199→111). No significant interferences from the specimen matrix were detected at the retention time corresponding to cymoxanil in any of the control specimens.

Linearity
Linearity in the concentration range of 0.250 to 25.0 ng/mL (corresponding to 0.002 to 0.214 mg/kg in samples) covering a range from less than 30% of the LOQ to 20% above the highest fortification level.

Matrix	Transition mass (m/z)	Intercept	Slope of the line	R^2
Tomato	199 → 128	137.57	1461.9	1
	199 → 111	72.34	1994.4	0.9999
Wine grape	199 → 128	121.85	892.44	0.9997
	199 → 111	165.41	1223.4	0.9999
Oilseed rape	199 → 128	189.95	1253.3	0.9995
	199 → 111	81.678	1739.3	0.9999
Wheat grain	199 → 128	7.782	686.37	0.9999
	199 → 111	-47.087	961.51	1

Limit of quantification (LOQ)
0.01 mg/kg (corresponding to 1.17 ng/mL in the extract) of cymoxanil in tomato, grapes, oilseed rape and wheat grain

Recovery and repeatability
The SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in tomato, wine grapes, oilseed rape and wheat grain.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Tomato	199 → 128	0.01	5	77	5.3
		0.10	5	81	2.0
	199 → 111	0.01	5	77	7.0
		0.10	5	84	2.2
Wine grapes	199 → 128	0.01	5	104	4.1
		0.10	5	99	3.3
	199 → 111	0.01	5	103	7.98
		0.10	5	96	6.7
Oilseed rape	199 → 128	0.01	5	75	1.7
		0.10	5	74	3.5
	199 → 111	0.01	5	72	2.3
		0.10	5	75	1.8
Wheat grain	199 → 128	0.01	5	91	11
		0.10	5	105	3.8
	199 → 111	0.01	5	91	14
		0.10	5	108	1.5

Confirmatory
Simultaneous confirmation of the analytical method was achieved by monitoring one additional SRM transition. The method was considered highly specific, thus the use of an alternative method was not necessary.

Conclusion
The HPLC-MS/MS method was successfully validated following SANCO/825/00 rev. 8.1 and complied with the current guideline SANTE/2020/12830 rev.1. The method is therefore suitable for the determination of residues of cymoxanil in tomato, wine grapes, oilseed rape and wheat grain.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.3 Description of analytical methods for the determination of residues in soil (KCP 5.2)

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/05
Report	Validation of the Analytical Method for the Determination of Cymoxanil Residues in Soil, Garofani S., 2009a, report No. CH-285/2008
Guideline(s):	SANCO/825/00 rev. 7 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	The method does not comply with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021). Additional validation on the linearity and confirmatory method were conducted and reported in KCP 5.2/07 and KCP 5.2/06, respectively.

Materials and methods
Principle of the method
An aliquot of acetonitrile is added to a dry soil sample and the mixture is shaken. An aliquot is filtered on a filter paper and the filtrate is injected into a HPLC coupled with a triple quadrupole mass detector.

Soil type used: Sandy loam.
Analytical conditions
HPLC-MS/MS

Column:	Supelco Ascentis RP-Amide, 2.1 mm × 150 mm, 5-µm
Column temperature:	Room temperature
Injection volume:	10 µL
Flow rate:	0.2 mL/min
Mobile phase:	Water / acetonitrile / acetic acid (40/60/0.05, v/v/v)
Detector:	Triple quadrupole mass detector
Ion source:	ESI, positive mode
Ion mass transitions:	199 → 128 m/z (quantification) 199 → 111 m/z (confirmation) 199 → 83 m/z (confirmation)

Validation - Results and discussions
Matrix effect

No matrix effect data present.

Specificity
A comparison of the chromatograms of the cymoxanil reference material, solvent wash, control soil sample and fortified soil sample showed no interference at or above 30% of the LOQ.

Linearity
Nominally 1.0 to 20 ng/mL of cymoxanil (equivalent to 4 – 80 ng/g in soil) covering a range from 40% of the LOQ to at least 20% above the highest fortification level. Mass transition: 199 → 128, Intercept: 1981, Slope of the line: 4519, R^2: 0.99354

Deviation from SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 (2021), where the concentration range shall be covered from 30% of the LOQ to 20% above the highest level. The calibration is re-validated in the study CH-199/2016 (KCP 5.2/07).

Limit of quantification
The limit of quantification (LOQ) was the low fortification level at 10.1 ng/g (0.01 mg/kg) in soil samples (corresponding to 2.5 ng/mL), below the minimum required LOQ of 0.05 mg/kg.

Recovery and repeatability
The SANCO/825/00 rev. 7 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in soil samples with mass transition of 199 → 128 m/z.

Recovery and repeatability results

Matrix	Mass transition (m/z)	Fortification level (ng/g)	n	Mean recovery [%]	RSD [%]
Sandy loam soil	199 → 128	10.1	6	90.7	4.1
		101	6	90.7	6.7

Confirmatory
Quantification was conducted using the total ion chromatogram and multiple reaction monitoring (TIC MRM). The confirmatory method was conducted in the separate study KCP 5.2/06.

Stability
No significant degradation of cymoxanil was observed during the validation of the analytical method. The organic extracts in acetonitrile were stored at room temperature until analysis which was performed on the same day.

Conclusion
The analytical method was shown to be specific for cymoxanil residues in soil samples. In order to fully comply to SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1, the confirmatory method was conducted in a separate study KCP 5.2/06 and the calibration was re-validated in the separate study KCP 5.2/07.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/06
Report	Validation of the Analytical Method for the Determination of Cymoxanil Residues in Soil. Integration of the GLP Study CH-285/2008 with Linearity and Recovery Tests Using Peak Areas of Qualifier Ions, Garofani S., 2013, report No. CH-377/2013
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	The method does not comply with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021). Additional validation on the linearity was conducted and reported in KCP 5.2/07.

This analytical method was conducted to additionally validate the confirmation parameter for a corresponding method CH-285/2008 (KCP 5.2/05).

Materials and methods

Principle of the method

An aliquot of the soil sample is extracted with acetonitrile. The organic extract is filtered and injected into a HPLC coupled with a triple quadrupole mass detector.

Analytical conditions

HPLC-MS/MS

Column:	Supelco Ascentis RP-Amide, 2.1 mm × 150 mm, 5-µm
Column temperature:	Room temperature
Injection volume:	10 µL
Flow rate:	0.2 mL/min
Mobile phase:	Water / acetonitrile / acetic acid (40/60/0.05, v/v/v)
Detector:	Triple quadrupole mass detector
Ion source:	ESI, positive mode
Ion mass transitions:	199 → 128 m/z (quantification) 199 → 111 m/z (confirmation) 199 → 83 m/z (confirmation)

Validation - Results and discussions

Matrix effect

No matrix effect data present.

Specificity

From corresponding study CH-285/2008 (KCP 5.2/05) - a comparison of the chromatograms of the cymoxanil reference material, solvent wash, control soil sample and fortified soil sample showed no interference at or above 30% of the LOQ.

Linearity

Nominally 1.0 to 20 ng/mL of cymoxanil (equivalent to 4 – 80 ng/g in soil) covering a range from 40% of the LOQ to at least 20% above the highest fortification level.
Mass transition: 199 → 128, Intercept: 1981, Slope of the line: 4519, R^2: 0.99354
Mass transition: 199 → 111, Intercept: 567, Slope of the line: 1447, R^2: 0.99306
Mass transition: 199 → 83, Intercept: 509, Slope of the line: 1494, R^2: 0.99365

Deviation from SANTE/2020/12830 rev.1, where the concentration range shall be covered from 30% of the LOQ to 20% above the highest level. The calibration is re-validated in the study CH-199/2016 (KCP5.2/07).

Limit of quantification

The limit of quantification (LOQ) was the low fortification level at 10.12 ng/g (0.01 mg/kg) in soil samples (corresponding to 2.5 ng/mL), below the minimum required LOQ of 0.05 mg/kg.

Recovery and repeatability

The SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in soil samples with mass transitions of 199 → 128 m/z, 199 → 111 m/z and 199 → 83 m/z.

Recovery and repeatability results

Matrix	Mass transition (m/z)	Fortification level (ng/g)	n	Mean recovery [%]	RSD [%]
Soil	199 → 128	10.12	6	90.5	4.1
		101.2	6	89.6	6.8
	199 → 111	10.12	6	96.1	3.9
		101.2	6	92.7	6.5
	199 → 83	10.12	6	87.8	7.4
		101.2	6	88.9	7.1

Conclusion
The analytical method was shown to be specific for cymoxanil residues in soil samples. In order to fully comply to SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1, the calibration was re-validated in the separate study KCP 5.2/07.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/07
Report	Validation of the analytical method for the determination of Cymoxanil residues in soil. Integration of the GLP Study CH – 285/2008 and GLP Study CH – 377/2013 with Linearity Test in a suitable range, Nichetti S., 2017, report No. CH-199/2016
Guideline(s):	(2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

This analytical method was conducted to additionally validate the linearity parameter for a corresponding method CH-285/2008 (KCP 5.2/05).

Materials and methods
Principle of the method
An aliquot of the soil sample is extracted with acetonitrile. The organic extract is filtered and injected into a HPLC coupled with a triple quadrupole mass detector.

Analytical conditions
HPLC-MS/MS

Column:	Supelco Ascentis RP-Amide, 2.1 mm × 150 mm, 5-µm
Column temperature:	Room temperature
Injection volume:	10 µL
Flow rate:	0.2 mL/min
Mobile phase:	Water / acetonitrile / acetic acid (40/60/0.05, v/v/v)
Detector:	Triple quadrupole mass detector
Ion source:	ESI, positive mode
Ion mass transitions:	199 → 128 m/z (quantification)

199 → 111 m/z (confirmation)
199 → 83 m/z (confirmation)

Validation - Results and discussions
Matrix effect
No matrix effect data present.

Linearity
Nominally 0.52 to 20.62 ng/mL of cymoxanil (equivalent to 2 – 80 ng/g in soil) covering a range from 20% of the LOQ to at least 20% above the highest fortification level.

Mass transition: 199 → 128, Intercept: -156, Slope of the line: 2341, R^2: 0.99969
Mass transition: 199 → 111, Intercept: -344, Slope of the line: 963, R^2: 0.99687
Mass transition: 199 → 83, Intercept: -160, Slope of the line: 755, R^2: 0.99946

Limit of quantification
The limit of quantification (LOQ) was determined to be 10 ng/g in soil samples.

Conclusion
In this study, a new linearity test was performed. The described analytical method is acceptable to use for determination of cymoxanil in soil samples as its performance complied with the validation requirements stated in the current guideline SANTE/2020/12830 rev.1.

A 2.2.2.4 Description of analytical methods for the determination of residues in water (KCP 5.2)

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/08
Report	Analytical Method for the Determination of Cymoxanil and IN-KQ960 in Water (Pond, Stream, Well, and Tap) Using LC/MS/MS, Leak T., 2010, report No. ABC-65072 (Dupont-27500)
Guideline(s):	SANCO/825/00 rev. 7 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	The method does not comply with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021). Additional validation on confirmation was conducted and reported in KCP 5.2/09.

Materials and methods
Principle of the method
Water samples were prepared by placing 10 mL of test sample into a culture tube, removing an aliquot of water equal to the volume of the intended fortification, and fortifying with appropriate spiking solution. Analyses were accomplished by HPLC-MS/MS. Detection of the analytes was by turbo ion spray (TIS) in the positive ion mode. One parent-to-daughter ion transition per analyte was monitored during analysis.

Analytical conditions
HPLC-MS/MS

Column:	Phenomenex Luna C8, 2 mm × 150 mm, 5-µm
Column temperature:	30°C
Injection volume:	40 µL
Flow rate:	0.5 mL/min
Mobile phase A:	0.1% formic acid in water

Mobile phase B:	HPLC-grade methanol
Gradient:	0.00 min: 98% A/ 2% B
	4.00 min: 50% A/ 50% B
	5.00 min: 50% A/ 50% B
	5.10 min: 98% A/ 2% B
	10.00 min: 98% A/ 2% B
Detector:	Triple quadrupole mass detector
Ion source:	Turbo Ion Spray (TIS), positive mode
Ion mass transitions:	199.1 → 128.1 m/z (cymoxanil, quantification)
	217.1 → 146.0 m/z (IN-KQ960, quantification)

Validation - Results and discussions

Matrix effect

No matrix effect data present.

Specificity

Samples are analyzed by HPLC-MS/MS, which is highly specific. Interferences were not observed for cymoxanil and IN-KQ960 at their respective chromatographic retention times in the water matrices tested.

Linearity

Linearity in the concentration range of 0.03 - 2.0 ng/mL of cymoxanil and IN-KQ960 (equivalent to 0.03 – 2.0 ppb) covering a range from 30% of the LOQ to at least 20% above the highest fortification level.

Cymoxanil, Intercept: 1.65E+03, Slope of the line: 3.56E+05, r: 0.9991
IN-KQ960, Intercept: 415, Slope of the line: 9.42E+04, r: 0.9987

Limit of quantification (LOQ)

0.1 µg/L (corresponding to 0.10 ng/mL in the extracted sample) for both cymoxanil and IN-KQ960

Recovery and repeatability

The SANCO/825/00 rev. 7 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in water matrices.

Recovery and repeatability results

Matrix	Residue	Fortification level (ng/mL)	n	Mean recovery [%]	RSD [%]
Pond water	Cymoxanil	0.1	5	81	5.30
		1.0	5	79	3.32
	IN-KQ960	0.1	5	93	4.69
		1.0	5	97	2.22
Stream water	Cymoxanil	0.1	5	84	3.77
		1.0	5	85	1.04
	IN-KQ960	0.1	5	98	3.84
		1.0	5	99	1.41
Well water	Cymoxanil	0.1	5	96	2.79
		1.0	5	93	3.20
	IN-KQ960	0.1	5	99	6.42
		1.0	5	104	2.11
Tap water	Cymoxanil	0.1	5	92	2.74
		1.0	5	94	0.811

Matrix	Residue	Fortification level (ng/mL)	n	Mean recovery [%]	RSD [%]
	IN-KQ960	0.1	5	98	6.10
		1.0	5	103	2.06

Confirmatory
The confirmatory method was conducted in the separate study DuPont-35792 (KCP 5.2/09).

Conclusion
Although the described HPLC-MS/MS analytical method is suitable for the quantitation of cymoxanil and IN-KQ960 in water matrices at an LOQ of approximately 0.1 µg/L, it does not fully comply to SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 guidelines. The confirmatory method was conducted in a separate study DuPont-35792 (KCP 5.2/09).

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/09
Report	Independent Laboratory Validation for the Determination of Residues of Cymoxanil and IN-KQ960 in Water (Drinking and Stream) Using LC-MS/MS, Cermak J., 2013b, report No. DuPont-35792
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

This analytical method was conducted in order to additionally validate the confirmation parameter for the corresponding analytical method ABC-65072 (Dupont-27500) (KCP 5.2/08). An independent Laboratory Validation (ILV) have been conducted as well.

Materials and methods
Principle of the method
Water samples were prepared by placing an aliquot of test sample into a culture tube and fortifying with appropriate spiking solution. Analyses were accomplished by LC-MS/MS.

Analytical conditions
HPLC-MS/MS

Column:	Nucleodur C8 Gravity EC 150/2, 150 x 2 mm, 5-µm
Column temperature:	30°C
Injection volume:	90 µL
Flow rate:	0.5 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% ammonium hydroxide in methanol
Gradient:	0.0 min: 98% A/ 2% B 0.0 → 4.0 min: 50% A/ 50% B 4.0 → 5.0 min: 50% A/ 50% B 5.0 → 5.1 min: 98% A/ 2% B 5.1 → 10.0 min: 98% A/ 2% B
Detector:	Triple quadrupole mass detector
Ion source:	ESI, positive mode
Ion mass transitions:	199.0 → 128.0 ± 0.1 m/z (cymoxanil, quantification) 199.0 → 111.0 ± 0.1 m/z (cymoxanil, confirmation) 217.0 → 146.0 ± 0.1 m/z (N-KQ960, quantification)

217.0 → 71.0 ± 0.1 m/z (N-KQ960, confirmation)

Validation - Results and discussions

Matrix effect

No matrix effect data present.

Specificity

The concentrations of cymoxanil and IN-KQ960 in the final solutions were determined by HPLC-MS/MS. In order to ensure unambiguous identification two mass transitions were monitored (Cymoxanil MRM 199 →128 and MRM 199 →111, IN-KQ960 MRM 217 →146 and MRM 217 →71). No significant interferences from the specimen matrix were detected at the retention time corresponding to cymoxanil and IN-KQ960 in any of the control specimens.

Linearity

Linearity in the concentration range of 0.03 - 2.0 ng/mL of cymoxanil and IN-KQ960 (corresponding to 0.03 – 2.0 ppb in samples), covering a range from 30% of the LOQ to at least 20% above the highest fortification level.

Cymoxanil, mass transition: 199 → 128, Intercept: 1863.9, Slope of the line: 243385, R^2: 0.9999
Cymoxanil, mass transition: 199 → 111, Intercept: 958.54, Slope of the line: 105553, R^2: 0.9999
IN-KQ960, mass transition: 217 → 146, Intercept: 83.982, Slope of the line: 100971, R^2: 0.9999
IN-KQ960, mass transition: 217 → 71, Intercept: 141.54, Slope of the line: 40090, R^2: 1

Limit of quantification (LOQ)

0.100 ppb (ng/mL) for both cymoxanil and IN-KQ960

Recovery and repeatability

The SANCO/825/00 rev. 7 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in water matrices.

Recovery and repeatability results

Matrix	Residue	Mass transition (m/z)	Fortification level (ng/mL)	n	Mean recovery [%]	RSD [%]
Drinking water	Cymoxanil	199 → 128	0.1	5	100	2.8
			1.0	5	101	5.7
		199 → 111	0.1	5	95	4.2
			1.0	5	99	5.5
	IN-KQ960	217 → 146	0.1	5	99	3.3
			1.0	5	96	6.4
		217 → 71	0.1	5	98	3.6
			1.0	5	95	6.1
Stream water	Cymoxanil	199 → 128	0.1	5	99	3.8
			1.0	5	96	1.6
		199 → 111	0.1	5	97	2.5
			1.0	5	94	1.8
	IN-KQ960	217 → 146	0.1	5	110	3.4
			1.0	5	108	1.4
		217 → 71	0.1	5	108	3.6
			1.0	5	108	1.7

Conclusion

The method is suitable for the determination of residues of cymoxanil and IN-KQ960 in water samples. The method has been successfully independently validated according SANCO/825/00, rev 8.1 and complies with the current guideline SANTE/2020/12830 rev.1.

A 2.2.2.5 Description of analytical methods for the determination of residues in air (KCP 5.2)

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/10
Report	Validation of the Analytical Method for the Determination of Cymoxanil Residues in Air, Garofani S., 2009b, report No. CH-287/2008
Guideline(s):	SANCO/825/00 rev. 7 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	The method does not comply with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021). Additional validation on the linearity was conducted and reported in KCP 5.2/11.

Materials and methods

Principle of the method

Residues of cymoxanil in air are adsorbed with Tenax glass tubes. The tubes are extracted with acetone and the determination of cymoxanil is performed by HPLC using an external standard and UV detector. For confirmatory purposes, HPLC/DAD was used.

Analytical conditions

HPLC-UV (primary method)

Column:	Zorbax Eclipse XDB-C8, 4.6 mm × 150 mm, 5-µm (Agilent)
Column temperature:	Room temperature
Injection volume:	10 µL
Flow rate:	1.0 mL/min
Mobile phase:	Water / acetonitrile / phosphoric acid (75/25/0.2, v/v/v)
Detector:	UV (245 nm)

HPLC-DAD (confirmation method)

Column:	Zorbax Eclipse XDB-C8, 4.6 mm × 150 mm, 5-µm (Agilent)
Column temperature:	Room temperature
Injection volume:	10 µL
Flow rate:	1.0 mL/min
Mobile phase:	Water / acetonitrile / phosphoric acid (75/25/0.2, v/v/v)
Detector:	Diode Array

Validation - Results and discussions

Matrix effect

No matrix effect data present.

Specificity

A comparison of the chromatograms of the cymoxanil analytical standard, solvent wash, unfortified control tube and fortified Tenax tube spiked at 0.12 µg, did not show any interference.

Linearity

Linearity in the concentration range of 101 to 303 ng/mL of cymoxanil (equivalent to 0.10 to 0.30 µg/tube or 0.14 to 0.42 µg/m^3 based on an air flow rate of 2 L/min and a sampling time of 6 hours). Primary method; Intercept: -4721,

Slope of the line: 247, r: 0.99839.

This concentration range covers a range from 84% of the LOQ to at least 20% above the highest fortification level. Deviation from SANTE/2020/12830 rev.1, where the concentration range shall be covered from 30% of the LOQ to 20% above the highest level. The calibration is re-validated in the study CH-200/2016 (KCP5.2/11).

Limit of quantification
The LOQ was determined as 0.17 µg/m^3 (equivalent to 0.12 µg/tube) based on an air flow rate of 2 L/min and a sampling time of 6 hours. The value falls below MRL of 3 µg/m^3 as specified in EFSA Scientific Report, 2008.

Recovery and repeatability
The SANCO/825/00 rev. 7 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in air using HPLC-UV method.

Recovery and repeatability results

Matrix	Method	Fortification level (µg/tube)	n	Mean recovery [%]	RSD [%]
Air	HPLC-UV	0.12	6	95.2	1.7
		1.20	6	95.7	1.1

Retention capacity
The obtained recovery values (see table below) after 6 hours of 2 L/min air flow at 35˚C and 80% relative humidity were in the acceptable range which demonstrates that the retention capacity was considered sufficient and no significant breakthrough occurred into the secondary sorbent section of the air sampling tube.

Retention capacity results

Matrix	Method	Fortification level (µg/tube)	n	Mean recovery [%]	RSD [%]
Air	HPLC-UV	0.12	5	94.1	2.4
		1.20	5	94.4	1.2

Confirmatory
The presence of cymoxanil in control and fortified samples was confirmed using HPLC with diode array detector (DAD) by comparison with the UV/Vis spectrum of cymoxanil standard solutions at the particular retention time. However, no confirmatory methods are required for the determination of residues in air if sufficient confirmatory methods are available for the determination in soil or water, according SANTE/2020/12830 rev.1. This is the case for cymoxanil.

Stability
The stability of fortified Tenax tubes stored at room temperature, at 4˚C and at below -15˚C for 8 days was analyzed. No significant degradation was observed over time.

Conclusion
The analytical method for determination of cymoxanil in air was successfully validated. However, in order to fully comply to the current guideline SANTE/2020/12830 rev.1, the calibration range was re-validated in the separate study CH-200/2016 (KCP 5.2/11).

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/11
Report	Validation of the analytical method for the determination of Cymoxanil residues in air. Integration of the GLP Study CH – 287/2008 with Linearity Test in a suitable range, Nichetti S., 2017b, report No. CH-200/2016
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

This supplementary validation for linearity over a lower concentration range was conducted in support of study CH – 287/2008 (KCP 5.2/10). All the other conditions and experimental details of the new test were identical to study CH-287/2008.

Materials and methods

Analytical conditions

HPLC-UV

Column:	Zorbax Eclipse XDB-C8, 4.6 mm × 150 mm, 5-µm (Agilent)
Column temperature:	Room temperature
Injection volume:	10 µL
Flow rate:	1.0 mL/min
Mobile phase:	Water / acetonitrile / phosphoric acid (75/25/0.2, v/v/v)
Detector:	UV (245 nm)

Validation - Results and discussions

Linearity

Linearity in the concentration range of 32.97 to 329.67 ng/mL of cymoxanil (equivalent to 0.033 to 0.33 µg/tube or 0.046 to 0.46 µg/m^3). Intercept: 6366, Slope of the line: 169, R^2: 0.99933

This concentration range, covering a range from 27% of the LOQ to at least 20% above the highest fortification level, complied with the requirement stated in SANTE/2020/12830 rev.1.

Conclusion

The HPLC-UV analytical method for quantitative analysis of cymoxanil residues in air was successfully validated for linearity according to the current guideline SANTE/2020/12830 rev.1.

A 2.2.2.6 Description of analytical methods for the determination of residues in body fluids and tissues (KCP 5.2)

The following studies have not been previously evaluated during EU approval process; therefore, study summaries are provided.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/12
Report	Validation of the analytical method to determine residue of cymoxanil in different matrices of animal origin (kidney, liver, fat, muscle, milk and eggs), Perboni A., 2016, report No. RAU-065-16
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

A method for the determination of residues of cymoxanil in different animal matrices (kidney, liver, fat, muscle, milk and eggs) at a target LOQ of 0.01 mg/kg has been validated to fulfil the requirements according to guidance document SANCO/3029/99, rev. 4 and SANCO/825/00 rev. 8.1. The validation in muscle is suitable to cover the validation for body tissues. Therefore, only the validation in muscle is summarised below.

Materials and methods

Principle of the method

The method consists in an extraction using acetonitrile and liquid/liquid partition after addition of NaCl, $MgSO_4$ and citrate buffer. The extract was further purified with hexane for fat and kidney matrices. The samples were centrifuged twice and analyzed by using a HPLC-MS.

Analytical conditions

HPLC-MS/MS

Column:	MAX-RP 100 Å, 30 x 2.0 mm, 2.5 µm (Synergi)
Column temperature:	35°C
Injection volume:	5 µL
Flow rate:	0.3 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	0 min: 90% A/ 10% B 1 min: 90% A/ 10% B 2 min: 20% A/ 80% B 6 min: 20% A/ 80% B 7 min: 90% A/ 10% B 10 min: 90% A/ 10% B
Detector:	Triple Quadrupole mass spectrometer
Ionization mode:	ESI, positive
Ion mass transition:	199 → 128 m/z (quantification) 199 → 111 m/z (confirmation)

Validation - Results and discussions

Matrix effect

No matrix effect data present. Matrix-matched standards were used by default as recommended in SANCO/825/00 rev. 8.1 guideline.

Specificity

Interfering peaks in control samples that eluted at the same retention time of cymoxanil were less than 30% of the LOQ demonstrating acceptable specificity (for both quantitation and confirmatory transitions). The specificity of the method has been confirmed by the quantification of two LC-MS/MS transitions for cymoxanil (m/z 199 → 128 and m/z 199 → 111).

Linearity

Calibration curves of cymoxanil were linear using seven matrix-matched standards in the range of 0.003 – 0.2 µg/mL (corresponding to 0.003 – 0.2 mg/kg in the sample), covering the concentration range from 30% to 20% above the highest measured concentration.

Transition mass: 199 → 128, Intercept: -0.0001, Slope of the line: 0.000000817, R^2: 0.9994
Transition mass: 199 → 111, Intercept: 0.0004, Slope of the line: 0.000001738, R^2: 0.999

Limit of quantification (LOQ)
0.01 mg/kg

Limit of detection (LOD)
0.003 mg/kg

Recovery and repeatability
The SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in muscle using HPLC-MS/MS method.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Muscle (body tissue)	199 → 128	0.01	5	97.14	2.86
		0.1	6	83.92	2.83
	199 → 111	0.01	5	91.89	4.58
		0.1	5	82.76	2.82

Confirmatory
Simultaneous confirmation of the analytical method was achieved by monitoring one additional SRM transition. The method was considered highly specific, thus the use of an alternative method was not necessary.

Conclusion
The HPLC-MS/MS analytical method was successfully validated following SANCO/825/00 rev. 8.1 and also complied with the current guideline SANTE/2020/12830 rev.1. The method is suitable for the determination of residues of cymoxanil in body tissues of animal origin. Satisfactory validation data was also achieved for the second transition demonstrating that any mass transition can be used for quantification and/or confirmation.

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/13
Report	Independent Laboratory Validation of the analytical method to determine residue of cymoxanil in different animal origin matrices (kidney, liver, fat, muscle, milk and egg), Fifi A. P., 2016, report No. BT300/16
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

An Independent Laboratory Validation (ILV) of the analytical method for the determination of residues of cymoxanil in the different animal matrices (kidney, liver, fat, muscle, milk and eggs) at a target LOQ of 0.01 mg/kg has been validated to fulfil the requirements according to guidance document SANCO/825/00 rev. 8.1 . The ILV in muscle is suitable to cover the ILV for body tissues. Therefore, only the validation in muscle is summarised below.

Materials and methods
Principle of the method
The method consists in an extraction using acetonitrile and liquid/liquid partition after addition of NaCl, $MgSO_4$ and citrate buffer. The extract was further purified with hexane for fat and kidney matrices. The samples were centrifuged twice and analyzed by using a HPLC system coupled with a triple quadropole mass analyser (HPLC-

MS/MS).

Analytical conditions

HPLC-MS/MS

Column:	Zorbax Eclipse Plus C18, Narrow Bore RR, 100 x 2.1 mm, 3.5 µm (this HPLC column considered as equivalent to the HPLC column from the original analytical method)
Column temperature:	35°C
Injection volume:	5 µL
Flow rate:	0.3 mL/min
Mobile phase A:	0.1% formic acid in ultrapure water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	0 min: 90% A/ 10% B 1 min: 90% A/ 10% B 2 min: 20% A/ 80% B 6 min: 20% A/ 80% B 7 min: 90% A/ 10% B 10 min: 90% A/ 10% B
Detector:	Triple Quadrupole mass spectrometer
Ionization mode:	ESI, positive
Ion mass transition:	199 → 128 m/z (quantification) 199 → 111 m/z (confirmation)

Validation - Results and discussions

Matrix effect

No matrix effect data present. Matrix-matched standards were used by default as recommended in SANCO/825/00 rev. 8.1 guideline.

Specificity

Interfering peaks in control samples that eluted at the same retention time of cymoxanil were less than 30% of the LOQ demonstrating acceptable specificity (for both quantitation and confirmatory transitions). The specificity of the method has been confirmed by the quantification of two LC-MS/MS transitions for cymoxanil (m/z 199 → 128 and m/z 199 → 111).

Linearity

Calibration curves of cymoxanil were linear using seven matrix-matched standards in the range of 0.003 – 0.2 µg/mL (corresponding to 0.003 – 0.2 mg/kg in the sample), covering the concentration range from 30% to 20% above the highest measured concentration.

Transition mass: 199 → 128, Intercept: 4.3089, Slope of the line: 721.1, R^2: 0.9993
Transition mass: 199 → 111, Intercept: 195.28, Slope of the line: 281.19, R^2: 0.9992

Limit of quantification (LOQ)

0.01 mg/kg

Limit of detection (LOD)

0.003 mg/kg

Recovery and repeatability

The SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in muscle using HPLC-MS/MS method.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
Muscle	199 → 128	0.01	5	88.9	4.9

Matrix	Transition mass (m/z)	Fortification level (mg/kg)	n	Mean recovery [%]	RSD [%]
(body tissue)		0.1	6	89.2	3.1
	199 → 111	0.01	5	88.5	9.5
		0.1	5	90.1	2.0

Confirmatory
Simultaneous confirmation of the analytical method was achieved by monitoring one additional SRM transition. The method was considered highly specific, thus the use of an alternative method was not necessary.

Conclusion
The method was successfully independently validated following SANCO/825/00 rev. 8.1 for body tissues. The method also complied with the current guideline SANTE/2020/12830 rev.1. Therefore, the analytical method was suitable for determination the residues of cymoxanil in body tissues (muscle).

Comments of zRMS:	Method is accepted

Reference:	KCP 5.2/14
Report	Method Validation - Determination of Residues of Cymoxanil in Body Fluid, Andrews G., 2019, report No. ZE/19/001
Guideline(s):	SANCO/825/00 rev. 8.1 (2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes, the method complied with all validation requirements stated in the current guideline SANTE/2020/12830 rev.1 (2021).

The aim of the study is to validate the method for determination of cymoxanil residues in body fluid (plasma) at a target LOQ of 0.05 mg/L.

Materials and methods
Principle of the method
The extraction of cymoxanil consisted of the addition of 0.1% formic acid in acetonitrile to human plasma, shaking of the samples followed by centrifugation. Final determination was by HPLC-MS/MS.

Analytical conditions
HPLC-MS/MS

Column:	Aquasil C18 150 x 3 mm, 3µm (BAF 583)
Column temperature:	40°C
Injection volume:	20 µL
Flow rate:	0.3 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	0 min: 80% A/ 20% B 1 min: 80% A/ 20% B 3 min: 5% A/ 95% B 10 min: 5% A/ 95% B 10.1 min: 80% A/ 20% B 13 min: 80% A/ 20% B
Detector:	Triple Quadrupole mass spectrometer
Ionization mode:	ESI, positive
Ion mass transition:	199 → 128 m/z (quantification) 199 → 83 m/z (confirmation)

Validation - Results and discussions

Matrix effect
Matrix effects of <20 % were measured for body fluids and considered not to be significant. Therefore, solvent standards were used for calibration and quantification.

Specificity
Interfering peaks in control samples that eluted at the same retention time of cymoxanil were less than 30% of the LOQ demonstrating acceptable specificity (for both quantitation and confirmatory transitions). The specificity of the method has been confirmed by the quantification of two LC-MS/MS transitions for cymoxanil (m/z 199 → 128 and m/z 199 → 111).

Linearity
Calibration curves of cymoxanil were linear using eight solvent standards in the range of 2 – 120 ng/mL (corresponding to 0.01 – 0.6 mg/L in the sample), covering the concentration range from 30% to 20% above the highest measured concentration.

Transition mass: 199 → 128, Intercept: 106000, Slope of the line: 217000, R^2: 0.9992
Transition mass: 199 → 83, Intercept: 24900, Slope of the line: 49800, R^2: 0.9990

Limit of quantification (LOQ)
0.05 mg/L

Limit of detection (LOD)
0.00003 mg/L for transition mass of 199 → 128 m/z
0.0004 mg/L for transition mass of 199 → 83 m/z

Recovery and repeatability
The SANCO/825/00 rev. 8.1 and SANTE/2020/12830 rev.1 guidelines require mean recoveries for each level in the range from 70 to 120% 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 analysis of cymoxanil in human plasma using HPLC-MS/MS method.

Recovery and repeatability results

Matrix	Transition mass (m/z)	Fortification level (mg/L)	n	Mean recovery [%]	RSD [%]
Muscle (body tissue)	199 → 128	0.05	5	77.1	2.8
		0.5	5	78.7	1.8
	199 → 83	0.05	5	78.7	2.9
		0.5	5	78.5	2.2

Confirmatory
Simultaneous confirmation of the analytical method was achieved by monitoring one additional SRM transition. The method was considered highly specific, thus the use of an alternative method was not necessary.

Conclusion
The HPLC-MS/MS analytical method was successfully validated following SANCO/825/00 rev. 8.1 for body fluid and also complied with the current guideline SANTE/2020/12830 rev.1. All the requirements concerning matrix effect, specificity, linearity, LOQ, confirmation, recovery and repeatability were satisfied. The LOQ of the method was 0.05 mg/L. The analytical method was suitable for determination of the residues of cymoxanil in body fluid (human plasma).

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted.