



# **Draft Assessment Report**

## **Evaluation of Active Substances**

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**  
as it applies in Great Britain

### **Elemental iron**

#### **Volume 3 – B.5 (PPP) – Final Bite**

Great Britain

January 2024

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## Version History

When	What
November 2021	Initial DAR
January 2024	Updates made after comments from the applicant

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## B.5. METHODS OF ANALYSIS

### B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

#### B.5.1.1. Analysis of the plant protection product

##### *B.5.1.1.1. Methods for the determination of the active substance in formulation*

<b>Report:</b>	<b>KCP 5.1.1/01 [REDACTED] (2018)</b>
<b>Title:</b>	Determination of Iron in Final Bite using XRF-Spectroscopy
<b>Document No.:</b>	Validation Report 42MV18001/E1 (R-39460)
<b>Guidelines:</b>	SANCO/3030/99 rev.4 (2000)
<b>GLP:</b>	Yes

Elemental iron content was determined using X-ray fluorescence (XRF) spectroscopy. Samples are heated in nitric acid until the iron is dissolved. Aliquots of the solutions were decomposed and fused together with a borate flux material to form a fused bead. The bead is irradiated with x-rays in the XRF instrument, and excited to emit fluorescent radiation which is characteristic for every element present in the sample, and proportional to its concentration. After irradiation with x-ray, the wavelength and intensity of the fluorescence radiation of every element present in the sample is detected, where intensity is measured in Kilo Counts Per Second (KCPS).

Instrument conditions: X-ray fluorescence spectrometer with computer and software Super Q (Axios Advanced Panalytical). Following measuring parameters are applied :-

Element	Line	Crystal	Collimator	Detector	U (kV)	I (mA)	Angle (°)*
Fe	KA	LiF 200	300μ	Duplex	60	66	57.5444

\*The actual angle may vary slightly due to ageing of the instrument.

Test sample preparation: Approximately 5g of grinded sample (grinded using pestle and mortar) was weighed into a beaker and approximately 50mL nitric acid (30-40%) was added. The mixture reacted for about 1 hour on a steam heated plate. The solution was filtrated hot into a 100mL measuring flask. The filter was washed twice, the washing waters were added to the solution and after cooling the flask was filled to the mark with water.

4mL of the sample solution were pipetted into a platinum crucible and allowed to dry in a drying oven at approximately 80°C. The residue was mixed with 7.9g lithium tetraborate and 0.2g lithium peroxide (flux material) in a platinum crucible.

#### Linearity

The count rate of calibration standard solutions in the range 1 – 3mg of Iron for ‘Final Bite’ were determined using the conditions above.

#### Repeatability

The procedure used for the determination of the active substance was repeated on the same batch of test substance six times. This was performed twice; over two days by two different operators.

#### Accuracy

The accuracy of the analytical procedure was assessed using samples of formulation that contained known weights of the analyte. The recovery rate was determined by spiking a placebo sample with approximately 139mg and 180mg of ‘Final Bite’ to produce spiking levels of 70% and 90% respectively. Higher spiking levels were produced by spiking a placebo material with approximately 2.22mg and 2.67mg Iron powder to produce 110% and 130% spiking levels respectively. Each spiking level was produced in triplicate. These samples were prepared in line with the test sample preparation described above and analysed by XRF-spectroscopy. The recoveries were then calculated.

#### Specificity

No non-analyte interference was observed. The iron content was determined quantitatively against the global XRF-calibration in the instrument. In the 2theta region of the iron lines, no other elements are detected.

Analytical validation data for determination of Fe in 'Final Bite'

Matrix	Analyte	Recovery Fortification Level	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
'Final Bite'	Iron	70% spike of nominal concentration (nominal concentration = 1% w/w)	100.66-102.85 (101.74, 3)	1.77 (5) <sup>1</sup> 2.55 (6) <sup>2</sup> at 1.06 % w/w  Modified Horwitz: 2.66	1.00– 3.00 mg (equivalent to 100 – 300% of nominal concentration of active in product)  Y=18.2168x + 6.8999  R = 0.9995	No non-analyte interference observed.
		90% spike of nominal concentration (nominal concentration = 1% w/w)	97.45-99.83 (98.99, 3)	Horrat H <sub>r</sub> : 0.67 - 0.96		
		110% spike of nominal concentration, using 97.14% pure Iron	96.79-98.03 (97.54, 3)			
		130% spike of nominal concentration, using 97.14% pure Iron	98.10-98.76 (98.42, 3)			

1 Day one

2 Day two

The method for determining the active substance content in the plant protection product is fully validated in accordance with SANCO 3030/99/ rev.4 and rev. 5.

#### ***B.5.1.1.2. Methods for the determination of the relevant impurities in formulation***

The applicant stated that the methods used for the determination of the relevant impurities in the technical material are also suitable for the determination of these impurities in the formulation. Full details of these methods are given in Vol 3 CA B5. However, supporting validation data in relation to the formulation was not provided. Additionally, during the course of the evaluation, additional relevant impurities were identified (cadmium and nickel). Validated analytical methods for the determination of these impurities in the formulation are also required. This has been set as a data gap, listed in Vol 1, section 3.1.4.5. The lack of these data should not impact the approval of the active substance as there is sufficient evidence that this data gap is not a safety concern. There is confidence that the levels of relevant impurities found in the technical material comply with the technical specification and given their nature, are unlikely to increase upon storage.

### B.5.1.2. Methods for the determination of residues

#### *B.5.1.2.1. Methods for Ecotoxicological Assessment*

<b>Report:</b>	<b>CA 4.1.2/01 : ██████████ (2018)</b>
Title:	Determination of the toxicity of Final Bite – 0402206 against <i>Desmodesmus subspicatus</i> according to OECD 201 resp. EU C.3
Document No.:	17121401G301 (R-39458)
Guidelines:	SANCO/3029/99 rev.4
GLP:	Yes

#### Method parameters for analytical method

Instrument:	ICP-OES 720 Agilent
Plasma:	Argon, 16.5 L/min
Ancillary gas:	Argon, 1.5 L/min
Power:	1.10 kW
Nebulizer pressure:	230kPa
Plasma view:	Axial
Repetition time:	20 sec
Stabilisation time:	15 sec
Wash time:	30 sec
Pump speed:	15 cycles per min
Wavelength Fe:	238.204 nm

Test sample preparation: Test item was pulverised with a mortar until the granules were a fine powder. All used glass vessels were rinsed with HCl before use to prevent iron contamination from e.g. drinking water.

The water soluble fractions were prepared for the test by mixing the nominal loads of 1, 3.2, 10, 32 and 100 mg/L test item with the corresponding amount of algal medium (demineralised water enriched with minerals but without algae) and shaking vigorously for 23 hours and 15 minutes. Resulting solutions of 1.3, 3.8, 10.1, 32 and 100 mg/L respectively were filtered with 0.45 µm PTFE filters and used for the test.

An aliquot of the aqueous test solutions was acidified with 100 µL HNO<sub>3</sub> (65%) and measured via ICP-OES for iron content (dilution factor 1.01).

#### Linearity

The intensity of calibration standard solutions in the range 10 – 1000 µg/L Fe in algal medium were determined using the conditions above.

#### Precision Repeatability

The procedure used for the determination of Fe in algal test medium was performed at 50 and 400 µg/L (Fe in algal test medium). Five determinations were made at each concentration.

#### Accuracy

The accuracy of the analytical procedure was assessed using samples of test medium fortified with known concentrations of the analyte. The recovery rate was determined for concentrations of 50 and 400 µg/L Fe in algal test medium. Five replicates were prepared of each concentration and measured by ICP-OES. The recoveries were calculated as follows:

$$[(\text{Concentration (Matrix)} / \text{Nominal Concentration (50 or 400 µg/L Fe)}) * 100]$$

Specificity

No non-analyte interference was observed. Calibration data has been provided, and shows the measurement of Fe in a blank control of only algal test medium lays below the lowest calibration level of 10 µg/L Fe (<30% of the LOQ). This analytical method, ICP-OES, is highly specific and provides unequivocal identification and quantification of the analyte; therefore a confirmation of analyte identification is not required.

Analytical validation data for determination of Fe in algal test medium

Matrix	Analyte	LOQ	Recovery Fortification Level	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specficity
Iron in algal test medium	Iron	50 µg/L	50 µg/L	93.6-105.2 (99.2, 5)	4.8 (5)	10 – 1000 µg/L (equivalent to 10-1000 µg/L of Fe in test sample), (n=8)	No non-analyte interference observed.
			400 µg/L	98.8 – 101.95 (99.9, 5)	1.2 (5)	Y=27.72657x + 562.79557 r = 0.999	
			Overall	93.6-105.2 (99.55, 10)	3.3 (10)		

This method is satisfactorily validated in accordance with SANCO 3029/99 rev.4.

<b>Report:</b>	<b>CA 4.1.2/02 : ████████ (2018)</b>
Title:	Determination of short term toxicity of Final Bite – 0402206 against <i>Daphnia magna</i> STRAUS according to OECD 202 rep. EU C.2
Document No.:	17121401G201 (R-39459)
Guidelines:	SANCO/3029/99 rev.4
GLP:	Yes

Method parameters for analytical method

Instrument:	ICP-OES 720 Agilent
Plasma:	Argon, 16.5 L/min
Ancillary gas:	Argon, 1.5 L/min
Power:	1.10 kW
Nebulizer pressure:	230kPa
Plasma view:	Axial
Repetition time:	20 sec
Stabilisation time:	15 sec
Wash time:	30 sec
Pump speed:	15 cycles per min
Wavelength Fe:	238.204 nm

Test sample preparation: Test item was pulverised with a mortar until the granules were a fine powder. All used glass vessels were rinsed with HCl before use to prevent iron contamination from e.g. drinking water. The water accommodated fraction was prepared by weighing the nominal load of 100.2mg/L and adding the corresponding amount of dilution water and shaking vigorously for 24 hours. The resulting solutions was filtered with 0.45µm PTFE filters and used for the limit test.

An aliquot of the aqueous test solution was acidified with 100µL HNO<sub>3</sub> (65%) and measured via ICP-OES for Fe (dilution factor 1.01).

#### Linearity

The intensity of calibration standard solutions in the range 50 – 1000 µg/L of Fe in algal medium were determined using the conditions above.

#### Precision Repeatability

The procedure used for the determination of Fe in algal test medium was performed at 50 and 600µg/L (Fe in *Daphnia* test medium). Five determinations were made at each concentration.

#### Accuracy

The accuracy of the analytical procedure was assessed using samples of test medium fortified with known concentrations of the analyte. The recovery rate was determined for concentrations of 50 and 600µg/L Fe in *Daphnia* test medium. Five replicates were prepared of each concentration and measured by ICP-OES. The recoveries were calculated as follows:

$$[(\text{Concentration (Matrix)} / \text{Nominal Concentration (50 or 600µg/L Fe)}) * 100]$$

#### Specificity

No non-analyte interference was observed. Calibration data has been provided, and shows the measurement of Fe in a blank control of only *Daphnia* test medium lays below the lowest calibration level of 50µg/L Fe. The calculated concentration in the blank solution at <5µg/L (<30% of the LOQ). This analytical method, ICP-OES, is highly specific and provides unequivocal identification and quantification of the analyte; therefore a confirmation of analyte identification is not required.

Analytical validation data for determination of Fe in *Daphnia* test medium

Matrix	Analyte	LOQ	Recovery Fortification Level	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
Iron in <i>Daphnia</i> test medium	Iron	50µg/L	50µg/L	100-101.4 (100.4, 5)	0.7 (5)	50 - 1000µg/L (equivalent to 50-1000 µg/L of Fe in test sample), (n=7)  Y= 26.72825x + 16.93328  r = 0.999	No non-analyte interference observed.
			600 µg/L	98.7-100.6 (99.8, 5)	0.8 (5)		
			Overall	98.7-101.4 (100.125, 10)	0.75 (10)		

This method is satisfactorily validated in accordance with SANCO 3029/99 rev.4.



**B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES**

Please refer to DAR Vol 3 CA B5 for data to address this requirement.

**B.5.3. REFERENCES 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	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.1.1	██████	2018	Determination of Iron in Final Bite using XRF- Spectroscopy  Henkel AG&Co. KGaA Report No. Validation Report 42MV18001/E1 (R-39460) ADAMA Makhteshim Ltd GLP Unpublished	N	Y	Article 59(1) & (2) of Regulation (EC) 1107/2009 applies.	ADAMA Makhteshi m Ltd	N/A
B.5.1.2.1.	██████	2018	Determination of the toxicity of Final Bite – 0402206 against <i>Desmodemus subspicatus</i> according to OECD 201 resp. EU C.3  LAUS GmbH Report No. : 17121401G301 (R-39458) ADAMA Makhteshim Ltd GLP Unpublished	N	Y	Article 59(1) & (2) of Regulation (EC) 1107/2009 applies.	ADAMA Makhteshi m Ltd	N/A
B.5.1.2.1	██████	2018	Determination of short term toxicity of Final Bite – 0402206 against <i>Daphniamagna</i> STRAUS according to OECD 202 rep. EU C.2  LAUS GmbH Report No. 17121401G201 (R-39459) ADAMA Makhteshim Ltd GLP Unpublished	N	Y	Article 59(1) & (2) of Regulation (EC) 1107/2009 applies.	ADAMA Makhteshi m Ltd	N/A