FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: MEZ-HER 100 SC

Product name(s): MECORN 100 SC

Chemical active substance:

mesotrione, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Pestila Sp. z o. o.

Submission date: October 2023, March 2024

MS Finalisation date: May 2024, August 2024

Version history

When	What
03/2024	Applicant's update
05/2024	dRR assessment by zRMS PL
08/2024	The final Registration Report after 1st commenting period

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5 Analytical methods

5.1 Conclusion and summary of assessment

There are no data gaps related to analytical methods for the determination of a.s. and relevant impurities and their validations in formulation Mecorn 100 SC.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Noticed data gaps are:
• none
Note: The table *List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review* should be completed before registration

Commodity/crop	Supported/ Not supported
Maize	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Comments of zRMS:	The proposed analytical method is suitable for the determination of the active substance mesotrione in the plant protection product Mecorn 100 SC. The proposed analytical method has been fully validated in terms of interference, specificity, linearity, accuracy and precision. The proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.

Reference:	5.1.1
Report	Analytical Method Validation for Active Ingredient and impurities Content Determination of the MEZ-HER 100 SC, Digrandi S., 2023, report No. 23214-01C
Guideline(s):	Yes, 3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The content of active substance in the examined sample was determined by high performance liquid chromatography HPLC-DAD and external standard method.

Name: MEZ-HER 100 SC
Active ingredient name: Mesotrione
Active ingredient (IUPAC name): 2-(4-Mesyl-2-nitrobenzoyl)-1,3-cyclohexanedione
CAS Number: 104206-82-8
Nominal content: 100 g/L
Formulation type: Suspension concentrate (SC)
Batch number: MEZ/01/23
Manufacturing date: 02/2023
Expiry date: 02/2025

Equipment and chromatographic conditions for mesotrione analysis

LC system	HPLC Series 1100 Agilent		
Analytical column	Phenomenex, Hypersil ODS, C18 5 µm 250x4.6 mm		
Solvent A:	Acetonitrile		
Solvent C:	Water 0.1% Phosphoric acid		
Flow:	1 mL/min		
Pump Parameters	Time [min]	A (%)	C (%)
	0.00	55	45
	7.00	55	45
Oven temperature :	30 °C		
Injection volume:	2 µL		
Detector wavelength:	272 nm		
Chromatogram time:	7.00 min		
Retention times:	~4.0 min (Mesotrione)		

The preparation of standard and sample solutions

Standard solution

Taking into account the analytical standard purity, two sets of reference item stock solutions in acetonitrile were prepared as follows:

Table 1: Stock solutions preparation for Mesotrione determination

Reference item		**Purity**	**Weight**	**Final Volume**	**Conc.[1]**	**Id. Code**
Id. Code and batch	Operation n°.	[%]	[mg]	[mL]	[mg/mL]	-
SR 237 Batch BCCD6166	20	99.6	60.7	25	2.4183	SM 237-20
	21	99.6	23.1	10	2.3008	SM 237-21

[1]: Stock solution concentration is calculated as $\frac{Purity \cdot Weight}{Volume \cdot 100}$

Table 2: Reference item working solutions used in the calibration and determination of Mesotrione

Id Code	**Initial Conc.**	**Volume taken**	**Final volume**	**Final Conc.[1]**	**% w/w[2]**	**Id. Code**
	[mg/mL]	[mL]	[mL]	[mg/mL]	[%]	
SM 237-20	2.4183	0.70	5	0.3386	6.2	SL 237-20 A
SM 237-20	2.4183	0.90	5	0.4353	7.9	SL 237-20 B

SM 237-20	2.4183	1.10	5	0.5320	9.7	SL 237-20 C
SM 237-20	2.4183	1.40	5	0.6771	12.3	SL 237-20 D
SM 237-20	2.4183	1.80	5	0.8706	15.8	SL 237-20 E
SM 237-21	2.3008	1.20	5	0.5522	10.0	SL 237-21 QC

[1]: Reference item solution concentration is calculated as $\frac{Stock\ solution\ concentration \cdot Volume\ taken}{Final\ volume}$

[2]: % w/w (analyte weight/sample weight) based on nominal test item solution concentration of 5.5 mg/mL, calculated as $\frac{Working\ solution\ concentration \cdot 100}{Nominal\ test\ item\ solution\ concentration}$

Sample solution

Test item was accurately weighed into a class A volumetric flask, then made up to volume with acetonitrile and opportunely diluted to reach concentration of 5.5 mg/mL and analysed by HPLC-DAD. Five replicates were prepared for method validation and determination.

Validation - Results and discussions

Table 5.2-3: Methods suitable for the determination of active substance mesotrione in plant protection product MEZ-HER 100 SC

	mesotrione
Author(s), year	Digrandi S., 2023
Principle of method	SANCO/3030/99 rev.5
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r) n = 5	The linearity of analytical method was assessed using mesotrione standard solutions with concentration range from 0.3386 mg/mL to 0.8706 mg/mL (from 6.2% to 15.8% of nominal mesotrione content in the sample). Correlation coefficient: $R^2 = 0.9999$ Required: $R^2 \geq 0.98$.
Precision – Repeatability Mean n = 5 (%RSD)	RSD = 0.44% RSDr = 1.89% Horrat 0.23.
Accuracy (% Recovery)	Marginal recovery 97.5% (range: 98.9% - 100.5%) Required: 90-110 %.
Interference/ Specificity	No interference.
Comment	No comments.

Conclusion

The HPLC-DAD method, used to quantify mesotrione in MEZ-HER 100 SC was fully validated. Method validation included linearity, non-analyte interference, precision, accuracy and specificity. All measured parameters meet the criteria given in SANCO/3030/99 rev.5.

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

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

Comments of zRMS:	The proposed analytical methods are suitable for the determination of the relevant impurities R287431, R287432, and 1,2 dichloroethane in the plant protection product Mecorn 100 SC. The proposed analytical methods have been fully validated in terms of interference, specificity, linearity, accuracy, precision, and LOQs. The proposed methods fulfil the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical methods has been accepted.

Reference:	5.1.1/02
Report:	Analytical Method Validation for Active Ingredient and impurities Content Determination of the MEZ-HER 100 SC, Digrandi S., 2023, report no. BF – 23214-01C
Guideline(s):	Yes, SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods for R287432 impurity content
Determination of R287432 in MEZ-HER 100 SC was performed with HPLC-MS/MS instrument for specificity analysis. Spectra of analyte is the same in standard, samples and recovery samples.

Equipment and chromatographic conditions for R287432 impurity analysis

Chromatographic System:	HPLC Series 1290 AGILENT		
	TRIPLE TOF 4600 AB SCIEX detector		
Analytical Column:	Acilent, ZORBAX Eclipse Plus C18, C18 5 µm 50x2.1 mm		
Solvent A:	Water 1% Formic Acid		
Solvent B:	ACN		
Flow:	0.1 mL/min		
Pump parameters	Time [min]	Phase A (%)	Phase B (%)
	0.00	90	10
	20	90	10
	21	70	30
	23	70	30
	23.01	90	10
	25	90	10
Temp	35 °C		
Injection volume:	3 µL		
Mass Detector:	Ionisation mode: APCI Positive product ion		
	Temperature (TEM): 650 °C		
	Curtain gas (CUR): 30 psi		
	Collision gas (CAD): 5 psi		
	Ion Spray Voltage (IS): 4500 V		
	Gas 1: 45 psi		
	Gas 2: 40 psi		
	DP: 50		
	CE: 20		
Retention times:	~21.75 min (R287432)		

Test Item Solutions Preparation
Test item was weighted in a volumetric class A flask and made up to volume with Methanol to reach a concentration of 35.0 mg/mL. Samples were accurately homogenised by vigorous hand shaking, sonicated for 10 minutes and filtered with PTFE filters.

Reference Item Solutions Preparation
Taking into account the analytical standard purity, one set of reference item stock solution in methanol was prepared as follows.

Table 3: Stock solutions for R287432 determination

Stock solution	Purity	Weight	Volume	Conc.	Id. Code
Id. code and batch	[%]	[mg]	[mL]	[mg/mL]	
SR 904 Batch EPPJOW 34.1	99.80	13	20	0.6487	SM 904-3

From the above stock solution were prepared the following reference working solutions for linearity.

Table 4: Reference item working solutions for R287432 determination

Id Code	Initial Conc.	Volume taken	Final volume	Final Conc.[1]	g/Kg referred to technical material[2]	g/L referred to test Item[3]	Id. Code
	[mg/mL]	[mL]	[mL]	[mg/mL]	[g/kg]	[g/L]	
SM 904-3	0.6487	1.00	20	0.0324	8.5331	0.9573	SL 904-3 INT
SL 904-3 INT	0.0324	0.65	25	0.0008	0.2219	0.0249	SL 904-3 A
SL 904-3 INT	0.0324	0.55	10	0.0018	0.4693	0.0527	SL 904-3 B
SL 904-3 INT	0.0324	0.85	10	0.0028	0.7253	0.0814	SL 904-3 C
SL 904-3 INT	0.0324	0.65	5	0.0042	1.1093	0.1244	SL 904-3 D
SL 904-3 INT	0.0324	0.85	5	0.0055	1.4506	0.1627	SL 904-3 E
SL 904-3 INT	0.0324	0.90	3	0.0097	2.5599	0.2872	SL 904-3 F

[1]: Reference item solution concentration is calculated as $\frac{Stock\ solution\ concentration \cdot Volume\ taken}{Final\ volume}$

[2]: **g/Kg referred to technical material** based on nominal test item solution concentration of 35.0 mg/mL, %purity of technical material of 93%, Content of AI % of 10.1 %; calculated as $\frac{\text{Final Conc} * \%\text{purity of technical material} * 1000}{\text{Nominal test item solution concentration} * \text{Content of AI \%}}$

[3]: **g/L referred to the test item** based on nominal test item solution concentration of 35.0 mg/mL, Density of test item of 1.033 g/mL; calculated as $\frac{\text{Final Conc} * \text{density} * 1000}{\text{Nominal test item solution concentration}}$

Materials and methods for R287431 impurity content

The analysis was performed by the highly specific HPLC-TOF detection system; the molecular ion 344 for quantification and two fragmentation ions: 314 and 281 were detected simultaneously for confirmatory purposes.
The retention time of the analyte in the reference item matched the retention time of the analyte in the samples and recoveries solution.
The specificity of the method is confirmed by the ratio of qualifier transition to target transition: for all analytical samples with analyte the ratio was between ± 30 % of the averaged ratio calculated for the analytical standards.

Equipment and chromatographic conditions for R287431 impurity analysis

LC System:	HPLC Series 1290 AGILENT		
MS/MS detector System	TRIPLE TOF 4600 AB SCIEX deterctor		
Analytical Column:	Agilent ZORBAX Eclipse Plus Column C18 1.8 µm 50x2.1mm		
Solvent A:	Water		
Solvent B:	MeOH		
Pump parameters	Time [min]	Phase A (%)	Phase B (%)
	0.00 min	80	20
	3.00 min	20	80
	4.00 min	20	80
	4.10 min	5	95
	5.00 min	5	95
	5.10 min	80	20
	7.00 min	80	20

Flow rate:	0.3 mL/min
Column Temperature:	35 °C
Injection volume:	20 µL
Retention time:	R287431: 2.75 min
Mass Detector:	Ionisation mode: APCI negative product ion
	Temperature (TEM): 650 °C
	Curtain gas (CUR): 30 psi
	Collision gas (CAD): 5 psi
	Ion Spray Voltage (IS): -4500 V
	Gas 1: 45 psi
	Gas 2: 40 psi
	DP: -60
	CE: -22
Transitions:	R287431-1 (344/314) R287431-2 (344/281)

Test Item Solutions Preparation

The sample was accurately homogenised by vigorous hand shaking then (0.65 ± 0.05) g of the were accurately weighed into a 15 mL plastic tube. Fortification into the spike recoveries was made at this point. Then the sample was made up to the 10 mL mark with methanol solvent, the tube was tightly sealed and vigorously shaken for 1 minute, then centrifuged at 4000 rpm for 5 minutes.
Taking care not to move the test item residue on the bottom of the tube, 5 mL of the supernatant limpid layer were transferred into a second 15 mL plastic tube, made up to the 10 mL mark with pure water (1:1 dilution) and mixed thoroughly after sealing the tube.
The white emulsion in the tube was then centrifuged at 4000 rpm for 5 minutes: a yellow precipitate separetes at the bottom of the tube. An aliquot of 0.75 mL of the supernatant limpid layer was transferred into a 2-mL Eppendorf microcentrifuge tube and 0.75 mL of methanol/water 40:60 solvent mixture was added (final sample volume after dilutions = 40 mL). The tube was sealed, mixed thoroughly and ultracentrifuged at 13500 rpm for 5 minutes.
The final extract was finally sampled from the upper layer of the tube and gently filtered by 0.22 µm, 12 mm diameter PTFE filter. The filter was previously conditioned with methanol solvent (around 10 mL) and then dried with air (around 2 x 10 mL).

Reference Item Solutions Preparation

Taking into account the analytical standard purity, one set of reference item stock solution in matrix (placebo) with solvent mixture methanol/water was prepared as follows.

Table4: Stock solutions for R287431 determination

Stock solution	Purity	Weight	Volume	Conc.	Id. Code
Id. code and batch	[%]	[mg]	[mL]	[µg/mL]	
SR 893 Batch 13-GUY-162-1	96.3	5.4	50	104.05	SM 893-4

From the above stock solution were prepared the following reference working solutions for linearity.

Table 5: Reference item working solutions for R287431 determination

Id Code	Initial Conc.	Volume taken	Final volume	Final Conc.[1]	mg/Kg referred to technical material[2]	mg/L referred to test Item[3]	Id. Code
	[µg/mL]	[µL]	[mL]	[ng/mL]	[mg/kg]	[mg/L]	

SM 893-4	104.047	950	10	9884.4650	-	-	SL INT 1
SL INT 1	9.8845	400	20	197.6893	-	-	SL INT 2
SL INT 2	0.1977	5.0	1	0.9884	0.5601	0.0628	Stm 1.0 ppb
SL INT 2	0.1977	7.0	1	1.3838	0.7841	0.0880	Stm 1.4 ppb
SL INT 2	0.1977	10	1	1.9769	1.1202	0.1257	Stm 2.0 ppb
SL INT 2	0.1977	15	1	2.9653	1.6803	0.1885	Stm 3.0 ppb
SL INT 2	0.1977	20	1	3.9538	2.2404	0.2513	Stm 4.0 ppb
SL INT 2	0.1977	25	1	4.9422	2.8005	0.3142	Stm 5.0 ppb

[1]: Reference item solution concentration is calculated as $\frac{Stock\ solution\ concentration \cdot Volume\ taken}{Final\ volume}$

[2]: **mg/Kg referred to technical material** based on nominal test item solution concentration of 65.0 mg/mL, % purity of technical material of 93%, Content of AI % of 10.1%; calculated as $\frac{\text{Final Conc} * \%\text{purity of technical material} \cdot * 1000}{\text{Nominal test item solution concentration} * \text{Content of AI \%}}$

[3]: **mg/L referred to the test item** based on nominal test item solution concentration of 65.0 mg/mL, Density of test item of 1.033 g/mL; calculated as $\frac{\text{Final Conc} * \text{density} \cdot * 1000}{\text{Nominal test item solution concentration}}$

Materials and methods for 1,2 dichloroethane content

Solutions for standard and test item were analysed with GC-MS instrument for specificity analysis. Coherently with precision quantification analysis, no peak is seen in test item samples.
Solutions were prepared by diluting the sample in Methanol.

Equipment and chromatographic conditions for 1,2 dichloroethane analysis

Instrumental system:	Agilent 7890B GC with Mass Selective Detector 7000B
Analytical Column:	Agilent J&W, DB-624 UI, Chromatographic column 30m *0.25mm*1,40µm
Oven:	100°C hold 1.0 min 15°C/min to 280°C hold 1.0 min
Injector temperature:	240 °C
Injection volume	1 µL
Split ratio:	4:1
Carrier gas:	Helium
Flow(s):	1.2 mL/min
Transfer line temperature:	280°C
Mass Detector:	Quadrupole temperature 150 °C
	Source temperature 230 °C
	Electron ionization
	Positive mode
Run time:	14.0 min
Retention time:	3.85 min (1,2-Dichloroethane)

Test Item Solutions Preparation

Test item was weighted in a volumetric class A flask and made up to volume with Methanol to reach a concentration of 35.0 mg/mL. Samples were accurately homogenised by vigorous hand shaking, sonicated for 10 minutes and filtered with PTFE filters.

Reference Item Solutions Preparation

Taking into account the analytical standard purity, one set of reference item stock solution in methanol was prepared as follows.

Table 7: Stock solutions for 1,2 dichloroethane determination

Stock solution	Purity	Weight	Volume	Conc.	Id. Code
Id. code and batch	[%]	[mg]	[mL]	[mg/mL]	
SR 534 Batch LRAC7143	99.00	33.5	50	0.6633	SM 534-9
	99.00	10.3	50	0.2039	SM 534-10

From the above stock solution were prepared the following reference working solutions for linearity.

Table 8: Reference item working solutions for 1,2 dichloroethane determination

Id Code	**Initial Conc.**	**Volume taken**	**Final volume**	**Final Conc.[1]**	**g/Kg referred to technical material[2]**	**g/L referred to test Item[3]**	**Id. Code**
	[mg/mL]	[mL]	[mL]	[mg/mL]	[g/kg]	[g/L]	
SM 534-9	0.6633	0.50	20	0.0166	4.3626	0.4894	SL 534-9 INT
SL 534-9 INT	0.0166	1.00	10	0.0017	0.4363	0.0489	SL 534-9 A
SL 534-9 INT	0.0166	1.30	10	0.0022	0.5671	0.0636	SL 534-9 B
SL 534-9 INT	0.0166	1.50	10	0.0025	0.6544	0.0734	SL 534-9 C
SL 534-9 INT	0.0166	0.90	5	0.0030	0.7853	0.0881	SL 534-9 D
SL 534-9 INT	0.0166	1.20	5	0.0040	1.0470	0.1175	SL 534-9 E
SM 534-10	0.2039	0.30	25	0.0024	0.6438	0.0722	SL 534-10 QC

[1]: Reference item solution concentration is calculated as $\frac{Stock\ solution\ concentration \cdot Volume\ taken}{Final\ volume}$

[2]: **g/Kg referred to technical material** based on nominal test item solution concentration of 35.0 mg/mL, %purity of technical material of 93%, Content of AI % of 10.1%; calculated as $\frac{\text{Final Conc} * \%\text{purity of technical material} \cdot * 1000}{\text{Nominal test item solution concentration} * \text{Content of AI \%}}$

[3]: **g/L referred to the test item** based on nominal test item solution concentration of 35.0 mg/mL, Density of test item of 1.033 g/mL; calculated as $\frac{\text{Final Conc} * \text{density} \cdot * 1000}{\text{Nominal test item solution concentration}}$

Table 5.2-6: Methods suitable for the determination of the relevant impurities: R287432, R287431 and 1,2-Dichloroethane in plant protection product (PPP) MEZ-HER 100 SC

	R287432	R287431	1,2-Dichloroethane
Author(s), year	Digrandi S., 2023		
Principle of method	SANCO/3030/99 rev.5		
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the analytical method was assessed using standards solutions of R287432 in the concentration range from 0.0008 mg/mL to 0.0097 mg/mL (corresponding to R287432 conc. in test item of 0.0249 - 0.2872 g/L). n = 6 Correlation coefficient: R^2 = 0.9899 Required: $R^2 \geq 0.99$.	The linearity of the analytical method was assessed using standards solutions of R287431 in the concentration range from 0.9884 ng/mL to 4.9422 ng/mL. (corresponding to R287431 conc. in test item of 0.0628 - 0.3142 mg/L). n = 6 Correlation coefficient: R^2 = 0.9988 Required: $R^2 \geq 0.99$.	The linearity of the analytical method was assessed using standards solutions of 1,2-Dichloroethane in the concentration range from 0.0017 mg/mL to 0.0040 mg/mL. (corresponding to 1,2-dichloroethane conc. in test item of 0.0489 - 0.1175 g/L). n = 5 Correlation coefficient: R^2 = 0. 0.9994 Required: $R^2 \geq 0.99$.
Precision – Repeatability (%RSD)	n = 5 Hr = 0.26 Required: Hr ≤ 1 RSD = 1.63% RSDr = 6.14%	n = 5 Hr = 0.08 Required: Hr ≤ 1 RSD = 1.24% RSDr = 15.47%	n = 5 Hr = 0.27 Required: Hr ≤ 1 RSD = 1.55% RSDr = 5.77%
Accuracy (% Recovery)	Marginal recovery 99.1% (range: 93.7% - 102.4%) for LOQ level (n = 5) 103.6% (range: 100.6% - 106.6%) for higher level (n = 2) Required: 75% - 125%.	Marginal recovery 83.7% (range: 80.7% - 86.3%) for LOQ level (n = 5) 86.2% (range: 85.0% - 87.5%) for higher level (n = 2) Required: 75% - 125%.	Marginal recovery 118.1% (range: 115.6% - 119.9%) for LOQ level (n = 5) 114.5% (range: 113.2% - 115.9%) for higher level (n = 2) Required: 75% - 125%.
Interference/ Specificity	Fulfilled.	Fulfilled.	Fulfilled.
LOQ	0.0026% (0.0009 mg/mL) of the preparation~~)~~, 0.0268 g/L.	0.0000085% (1.3838 ng/mL) of the preparation, 0.0880 mg/L.	0.0062% (0.0022 mg/mL) of the preparation~~)~~, 0.0636 g/L.
Comment	No comments.	No comments.	No comments.

Conclusion

Determination ~~of residues~~ of relevant impurities: R287432, R287431 and 1,2-Dichloroethane was fully validated. The methods for determination are specific. The validation parameters for linearity, instrument precision, limit of quantification, repeatability and accuracy are within the acceptance range. There are not any interferences between relevant impurities and other ingredients of the samples. The methods had good precision, accuracy and the linearity and fulfil requirements of SANCO/3030/99 rev.5.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

There are no relevant formulants in product MEZ-HER 100 SC therefore no methods are required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

There is no CIPAC method available for the determination of mesotrione in SC formulations.

Information from Renewal RR for Callisto 100 SC that are also relevant for evaluation of MEZ-HER 100 SC is provided below. Since data protection for this information expired, summary of relevant information was copied from Renewal RR for Callisto 100 SC. Information copied form Renewal RR for Callisto 100 SC were evaluated and accepted in renewal process of Callisto 100 SC and are deemed to be acceptable for MEZ-HER 100 SC.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to the methods described in Renewal RR for Callisto 100 SC, which are, in applicant's opinion, fulfil requirements of "Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes" (SANTE/2020/12830, Rev.2, 14. February 2023).

An overview on the acceptable methods and possible data gaps for analysis of residues of mesotrione for the generation of pre-authorization data is given in the following tables.

Table 5.2-7: Validated methods for the generation of pre-authorization data

Component of residue definition: Mesotrione (and MNBA)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry) *Maize forage, maize grain* High water content *Maize fodder*	TMR0643B	0.01 mg/kg	HPLC-FL	Method: *Alferness, 1996 Report: TMR0643B Validation: *Bolygo, 1996 Report: RJ0689B EU agreed (UK, 2015, 2015a)
High protein/high starch content (dry) *Maize grain, maize silage, maize stover* High water content *Maize whole plant*	RAM 366/01	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: Crook, 2002 Report: RAM 366/01 Validation: Hill, 2004 Report: RJ3253B Bruns et al. 2001

Component of residue definition: Mesotrione (and MNBA)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				Report: ZA1296/0656 EU agreed (UK, 2015, 2015a)
High water content *Oilseed rape whole plant*		0.01 mg/kg		Validation: Malet & Allard, 2010 Report: RXCO00307
High oil content *Oilseed rape seed*				EU agreed (UK, 2015, 2015a)
High oil content *Linseed seed*		0.01 mg/kg		Validation: Simon, 2004 Report: gli058003 EU agreed (UK, 2015, 2015a)
High oil content *Poppyseed seed*		0.01 mg/kg		Validation: Simon, 2004a Report: gpp067003 EU agreed (UK, 2015, 2015a)
High protein/high starch content (dry) *Maize grain whole*	GRM007.11A (update to RAM 366/01)	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: Watson & Crook, 2013 Report: GRM007.11A
High water content *Maize forage*				Validation: Watson, 2013 Report: S12-03629 Amic, 2013 Report: S13-02460 EU agreed (UK, 2015, 2015a)
High oil content *Oilseed rape seed*				
High acid content *Whole orange*				

* Methods have been inserted also in the post-authorization section

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of active substance mesotrione (KCP 5.2)

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
High water content *Lettuce*	Mesotrione	0.01 mg/kg	Regulation (EU) No 2017/626
High acid content *Orange*		0.01 mg/kg	Regulation (EU) No 2017/626
High oil content *Sunflower seed*		0.01 mg/kg	Regulation (EU) No 2017/626
High protein/high starch content (dry) *Maize grain, dry broad bean*		0.01 mg/kg	Regulation (EU) No 2017/626
Difficult matrices		0.05 mg/kg	Regulation (EU) No 2017/626
Muscle	(Mesotrione)[a]	0.01 mg/kg	Regulation (EU) No 2017/626
Milk		0.01 mg/kg	Regulation (EU) No 2017/626
Eggs		0.01 mg/kg	Regulation (EU) No 2017/626
Fat		0.01 mg/kg	Regulation (EU) No 2017/626
Liver, kidney		0.01 mg/kg	Regulation (EU) No 2017/626
Soil (Ecotoxicology)	Mesotrione MNBA AMBA	~~0.05 mg/kg~~ 0.002 mg/kg	~~common limit~~ EFSA Journal 2016;14(3):4419
Drinking water (Human toxicology)	Mesotrione MNBA AMBA	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Mesotrione MNBA AMBA	0.0077 mg/L (EC50 *Lemna gibba*)	EFSA Journal 2016;14(3):4419
Air	Mesotrione	1.5 µg/m³ (AOEL sys = 0.005 mg/kg bw/day (EFSA 2016))	Calculated according to SANTE/2020/12830, Rev.2 14. February 2023
Tissue (meat or liver)	Mesotrione	0.01 mg/kg	Validation: Watson, 2013b Report S12-03250 EU agreed (UK, 2015, 2015a)
Body fluids		0.01 mg/kg	

(a) No residue definition has been set for products of animal origin.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in plant matrices is given in the following tables. No new data are submitted in the framework of this application.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: mesotrione				
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 *Maize forage*	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Method and validation: Watson, 2013a Report: S12-03251 ILV: Tessier, 2013 Report: S12-04607 EU agreed (UK, 2015, 2015a)
	ILV (QuEChERS)	0.01 mg/kg		
High acid content *Whole orange*	QuEChERS	0.01 mg/kg		
High oil content *Oilseed rape seed*	QuEChERS	0.01 mg/kg		
High protein/high starch content (dry) *Maize kernel*	QuEChERS	0.01 mg/kg		
	ILV (QuEChERS)	0.01 mg/kg		
High protein/high starch content (dry) *Maize forage, maize grain*	TMR0643B	0.01 mg/kg	HPLC-FL	Method: Alferness, 1996 Report: TMR0643B Validation: Bolygo, 1996 Report: RJ2149B EU agreed (UK, 2015, 2015a)
High water content *Maize fodder*				

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Studies on the metabolism of mesotrione in maize incorporate a number of different extraction steps, one of which is extraction with acetonitrile/water in a 1:1 ratio. Residues of mesotrione in grain were extremely low and therefore no data are available to address the extraction efficiency in grain however data are available from samples of maize fodder and forage leaf as these samples contained the majority of the radioactivity. These data indicate that the majority of the total radioactive residue obtained via solvent extraction from these matrices was extracted via use of acetonitrile/water and subsequent characterisation indicated that these extracts contained the residue of mesotrione. This would therefore indicate that the use of acetonitrile/water in a 1:1 v/v ratio is effective for extraction of residues of mesotrione. Wei & Dohn, 1997 Report: RR 96-026B Tarr & van Neste, 1997

	Method for products of plant origin
	Report: RR96-007B EU agreed (UK, 2015, 2015a)
Not required, because:	-

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in animal matrices is given in the following tables. No new data are submitted in the framework of this application.

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

Component of residue definition: (mesotrione)[a]				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Muscle	QuEChERS	0.01 mg/kg	QuEChERS LC-MS/MS (multi-residue)	QuEChERS Method and validation: Watson, 2013b Report: S12-03250 EU agreed (UK, 2015, 2015a) ILV: Bernal, 2013 Report: S12-04608 EU agreed (UK, 2015, 2015a)
Eggs Fat	QuEChERS	0.01 mg/kg		
liver	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Kidney	QuEChERS	0.01 mg/kg		
Milk	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Eggs	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Whole blood	QuEChERS	0.01 mg/kg		

(a) No residue definition has been set for products of animal origin.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Taking into account that intake of mesotrione is not significant, studies on active substance metabolism and extraction efficiency in animal matrices are not required. No monitoring method for residues in animal products is necessary. No MRLs have been set for products of animal origin.

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection

period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in soil is given in the following tables. No new studies have been submitted with this application.

Table 5.3-6: Validated methods for soil

Component of residue definition: mesotrione, MNBA and AMBA			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
GRM007.10A	0.002 mg/kg	HPLC-MS/MS	Method: Jutsum & Williams, 2013 GRM007.10A Validation: Jutsum, 2013 CEMR-5657-REG EU agreed (UK, 2015, 2015a)

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in sur-face and drinking water is given in the following tables. No new studies have been submitted with this application.

Table 5.3-7: Validated methods for water

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Component of residue definition: mesotrione, MNBA and AMBA				
Drinking water	GRM007.09A	0.05 (surface water) µg/L	HPLC-MS/MS	Method: Jutsum & Chamkesam, 2013 Report GRM007.09A Validation: Jutsum, 2013a Report CEMR-5658-REG EU agreed (UK, 2015, 2015a)
	ILV (GRM007.09A)	0.05 µg/L	HPLC-MS/MS	ILV Wiesner & Breyer, 2013 Report S13-04185 EU agreed (UK, 2015, 2015a)
Ground water	GRM007.09A	0.05 µg/L	HPLC-MS/MS	Method: Jutsum & Chamkesam, 2013 Report GRM007.09A Validation: Jutsum, 2013a

				Report CEMR-5658-REG EU agreed (UK, 2015, 2015a)
Component of residue definition: Mesotrione and MNBA				
Drinking water	TMR0707B	0.05 µg/L	GC-MSD	Method: Meyers, 1997 Report TMR0707B EU Agreed (UK, 2015, 2015a)

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in air is given in the following tables. No new studies have been submitted with this application.

Table 5.3-8: Validated methods for air

Component of residue definition: mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
RR 97-031B	0.01 µg/m^3	HPLC-UV	Method: Leung, 1997 Report RR 97-031B EU agreed (UK, 2015, 2015a)
GRM007-08B	0.45 µg/m^3	HPLC-MS/MS	Method: Jutsum, 2013b GRM007.08B Validation: Jutsum, 2013c CEMR-5403-REG EU agreed (UK, 2015, 2015a)

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

Not relevant for new registration according to art. 34 of Reg. 1107/2009 based on data which protection period has expired. For the purpose of evaluation of MEZ-HER 100 SC please refer to Renewal RR for Callisto 100 SC.

The following methods can be used to determine residue levels of mesotrione in body fluids and tissues. No new data are submitted in the framework of this application.

Table 5.3-9: Methods for body fluids and tissues

Component of residue definition: mesotrione				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Whole blood	QuEChERS Method	0.01 mg/kg	HPLC-MS/MS	Validation: Watson, 2013b Report S12-03250 EU agreed (UK, 2015, 2015a)

5.3.2.8 Other studies/ information

Not relevant.

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 KCP 5.1.1/02	Digrandi S.	2023	Analytical Method Validation for Active Ingredient and impurities Content Determination of the MEZ-HER 100 SC. Report No 23214-01C Renolab S.r.l. GLP: Yes Unpublished	N	Pestila*

* Pestila spółka z ograniczoną odpowiedzialnością

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
-	-	-	-	-	-

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
-	-	-	-	-	-

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
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for the active substance

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

New analytical method for determination of active substance and its relevant metabolites in plant protection product have been submitted and summarised in points 5.2.1.1. and 5.2.1.2. Submitted methods can be used for post-authorisation monitoring.

A 2.1.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

A 2.1.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted.

A 2.1.1.3 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

No new or additional studies have been submitted.

A 2.1.1.4 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

No new or additional studies have been submitted.

A 2.1.1.5 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

No new data have been submitted in the framework of this application.

A 2.1.1.6 Description of analytical methods for the determination of residues in support of toxicological 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)

No new or additional studies have been submitted.

A 2.1.2.2 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.1.2.5 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.