

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB2106cF

Product name: Revus Pro

Chemical active substances:

Propamocarb-HCl, 450 g/L

Mandipropamid, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem NV

Submission date: March 2023

MS Finalisation date: 06/03/2024

Version history

When	What
March 2023	Initial dossier submission by applicant for approval of new product
July 2023	Dossier sent for evaluation
November 2023	zRMS evaluation of dRR
March 2024	Final version prepared by zRMS after Commenting period

Table of Contents

5	Analytical methods.....	5
5.1	Conclusion and summary of assessment.....	5
5.2	Methods used for the generation of pre-authorization data (KCP 5.1).....	6
5.2.1	Analysis of the plant protection product (KCP 5.1.1)	6
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	6
5.2.1.2	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	8
5.2.1.3	Description of analytical methods for the determination of formulants (KCP 5.1.1)	10
5.2.1.4	Applicability of existing CIPAC methods (KCP 5.1.1).....	10
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	11
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2)	13
5.3.1	Analysis of the plant protection product (KCP 5.2)	13
5.3.2	Description of analytical methods for the determination of residues of Propamocarb-HCl (KCP 5.2).....	13
5.3.2.1	Overview of residue definitions and levels for which compliance is required	13
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	15
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2).....	15
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2)	16
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	17
5.3.2.6	Description of methods for the analysis of air (KCP 5.2).....	17
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	18
5.3.2.8	Other studies/ information	18
5.3.3	Description of analytical methods for the determination of residues of Mandipropamid (KCP 5.2)	18
5.3.3.1	Overview of residue definitions and levels for which compliance is required	18
5.3.3.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	19
5.3.3.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2).....	20
5.3.3.4	Description of methods for the analysis of soil (KCP 5.2)	20
5.3.3.5	Description of methods for the analysis of water (KCP 5.2).....	21
5.3.3.6	Description of methods for the analysis of air (KCP 5.2).....	21
5.3.3.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	21
5.3.3.8	Other studies/ information	22
Appendix 1	Lists of data considered in support of the evaluation.....	23
Appendix 2	Detailed evaluation of submitted analytical methods.....	25

A 2.1	Analytical methods for Propamocarb-HCl	25
A 2.1.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	25
A 2.1.2	Methods for post-authorization control and monitoring purposes (KCP 5.2)	35
A 2.2	Analytical methods for Mandipropamid.....	36
A 2.2.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	36
A 2.2.2	Methods for post-authorization control and monitoring purposes (KCP 5.2)	36

zRMS comments:

This report has been completed by the Applicant.

The text highlighted in grey was provided by the zRMS. The text highlighted in yellow was added by zRMS after the commenting process.

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:

- no data gap

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

Noticed data gaps are:

for propamocarb:

- a primary, confirmatory and ILV methods for the determination of propamocarb in drinking water
- a primary method for the determination of propamocarb in surface water
- a primary method and confirmation for the analysis of propamocarb in body tissues and body fluids

In the opinion of the zRMS, the Applicant should complete them post-registration after the renewal of approval for propamocarb. However, the final decision should be taken by risk managers.

for mandipropamide:

- an ILV method for the determination of mandipropamid in drinking water is missing
- a primary method and confirmation is required for the analysis of mandipropamid in body tissues and body fluids
- the extraction efficiency of method for products of plant origin

In the opinion of the zRMS, the Applicant should complete them post-registration after the renewal of approval for mandipropamid. However, the final decision should be taken by risk managers.

Commodity/crop	Supported/ Not supported
Seed, ware and starch potato	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 Propamocarb-HCl and Mandipropamid in plant protection product is provided as follows:

Comments of zRMS:	The method of determination of propamocarb HCl and Mandipropamid in a formulation GLOB2106cF was validated in accordance SANCO/3030/99 rev. 5 in compliance with GLP. The method is acceptable for determination of active substances in PPP.
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Reference:	KCP 5.1.1
Report	Validation of the methods of determination of propamocarb HCl and Mandipropamid and a specified impurity in a formulation GLOB2106cF, in compliance with good laboratory practice, Sowle J., 2022, DNA6689
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Propamocarb-HCl

The assay of was performed using approximately 0.1 g of sample. The mass of the formulation was accurately recorded, transferred to a 100 mL volumetric flask and partially made to volume with Acetonitrile. The sample was sonicated for 5 minutes, allowed to cool to room temperature, and made up to volume with Acetonitrile. The sample was further diluted 1:1000 by taking 100µL into a 100mL volumetric flask and made to volume with Acetonitrile. The samples were then assayed by injecting each solution once into the LC-QQQ under the following conditions:

LC-QQQ Conditions – Propamocarb HCl:

Instrument:	Agilent 6470 QQQ Mass Spectrometer
Mode:	Isocratic Reverse Phase
Column:	Agilent Zorbax Eclipse (50mm x 4.6mm)
Packing:	XDB-C18, 1.8µm
Eluent A (60%):	5mM Ammonium Formate with 0.1% (v/v) Formic Acid in 95% (v/v) Methanol and 5% (v/v) Deionised Water
Eluent B (40%):	5mM Ammonium Formate with 0.1% (v/v) Formic Acid in 95% (v/v) Deionised Water and 5% (v/v) Methanol
Flow Rate:	0.3mL/minute
Injection Volume:	2µL
Column Temperature:	25°C
Retention Time:	Propamocarb HCl approximately 1.6 to 1.8 minutes

Data Acquisition: MassHunter

Ionisation:	Positive	Sheath Gas Temperature:	400°C
Gas Temperature:	230°C	Sheath Gas Flow:	12L/minute
Gas Flow:	4L/min ute	Capillary Voltage:	4000V
Nebulizer:	40psi	Nozzle Voltage:	1500V

Propamocarb HCl MRM Precursor Ion: 189.16m/z

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)	Accelerator Voltage (V)
189.16	74.1	200	45	28	5
189.16	58.2	200	45	32	5
189.16	41.1	200	45	48	5

The reference standard of Propamocarb-HCl was prepared in acetonitrile.

Mandipropamid

The assay of Mandipropamid was performed using approximately 0.1g of sample. The mass of the Formulation was accurately recorded, transferred to a 100mL volumetric flask and partially made to volume with Acetonitrile. The sample was sonicated for 5 minutes, allowed to cool to room temperature, and made up to volume with Acetonitrile. The samples were then assayed by injecting each solution once into the HPLC-PDA under the following conditions:

HPLC-PDA Conditions – Mandipropamid Validation

Instrument:	Shimadzu HPLC-PDA
Mode:	Isocratic Reverse Phase
Column:	Grace Genesis (250mm x 4.6mm)
Packing:	C8, 3µm
Eluent A (50%):	Acetonitrile
Eluent B (50%):	Deionised Water adjusted to pH3 with Formic Acid
Wavelength:	225nm
Flow Rate:	1.0 mL/minute
Injection Volume:	10µL
Column Temperature:	25°C
Data Collection:	LabSolutions
Retention Time:	Approximately 15.5 to 15.8 minutes

The reference standard of Mandipropamid was prepared in acetonitrile.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of Propamocarb-HCl and Mandipropamid in plant protection product GLOB2106cF

	Propamocarb-HCl	Mandipropamid
Author(s), year	Sowle J., 2022	
Principle of method	LC-QQQ	HPLC-PDA
Linearity (linear between)	0.02 – 1 mg/mL R ² = 0.9965	0.005 – 0.2 mg/mL R ² = 0.9999

	Propamocarb-HCl	Mandipropamid
Author(s), year	Sowle J., 2022	
mg/L / % range of the declared content) (correlation coefficient, expressed as r)	y=0.00000001420x-0.0239602476 (equivalent concentration (g/l) in formulation: 20-1000	y=0.0000000420x-0.0004991477 (equivalent concentration (g/l) in formulation: 5-200
Precision – Repeatability Mean (%RSD) n = 6	Mean = 447.8 ± 1.070 g/L %RSD = 0.239 Hr = 0.158	Mean = 76.00 ± 0.262 g/L %RSD = 0.345 Hr = 0.174
Accuracy (% Recovery) n = 6	Mean: 101.4 ± 1.512% % RSD = 1.490 Hr = 0.988 (Total Recovery at 450 g/L)	Mean Recovery = 97.98 ± 0.764% %RSD = 0.780 Hr = 0.393 (Total Recovery at 75 g/L)
Interference/ Specificity	Propamocarb HCl eluted at 1.6 to 1.8 minutes, and there were no other peaks present above the LOQ at the same elution time as Propamocarb HCl.	Mandipropamid eluted at 15.7 to 15.8 minutes and there were no other peaks present at the same elution time as Mandipropamid HCl.
Comment	-	-

Conclusion

The validation parameters for these methodologies have been met for this study under the SANCO/3030/99 rev. 5 guidelines.

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 method of determination of relevant impurity in a formulation GLOB2106cF was validated in accordance SANCO/3030/99 rev. 5 in compliance with GLP. The method is acceptable for determination of relevant impurity in PPP.
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Reference:	KCP 5.1.1
Report	Validation of the methods of determination of propamocarb HCl and Mandipropamid and a specified impurity in a formulation GLOB2106cF, in compliance with good laboratory practice, Sowle J., 2022, DNA6689
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Impurity 1 (N-{2-[4-(2-chloro-allyloxy)-3-methoxy-phenyl]-ethyl}-2-(4-chloro-phenyl)-2-prop-2-ynyloxy-acetamide)

The assay of Impurity 1 was performed using approximately 0.5g of sample. The mass of the Formulation was accurately recorded, transferred to a 20mL volumetric flask and partially made to volume with Acetonitrile. The sample was sonicated for 5 minutes, allowed to cool to room temperature, and made up to volume with Acetonitrile. The samples were centrifuged at 5000 rpm for 5 minutes and then assayed by injecting each solution once into the LC-QTOF under the following conditions:

LC-QTOF Conditions – Impurity 1:

LC Conditions:

Instrument:	Agilent 1200 Series HPLC-DAD
Mode:	Isocratic Reverse Phase
Column:	Grace Alltima C18, (250 mm x 4.6 mm)
Packing:	C18, 5µm
Eluent A (60%):	Acetonitrile
Eluent B (40%):	Deionised Water adjusted to pH3 with Formic Acid
Flow Rate:	1.0mL/minute
Injection Volume:	5µL
Column Temperature:	25°C
Wavelength:	Not Applicable

MS Conditions:

Instrument:	Agilent 6500 Series Q-ToF Mass Spectrometer		
Mode:	Agilent Jetstream ESI		
Ionisation:	Positive		
MS Scan Range:	50-1000m/z		
MS/MS Scan Range:	50-500m/z (MS Spectral Analysis Only)		
Extracted Ions:	Quantitation by Molecular Formula Accurate Mass Extraction of Impurity 1 (C ₂₃ H ₂₃ Cl ₂ NO ₄) equating to 448.1077 m/z [M+H] ⁺		
Acquisition Rate:	1 Spectra/Second		
Acquisition Time:	1000 ms/Spectrum		
Retention Time:	Approximately 12.9 to 13.0 minutes		
Gas Temperature:	250°C	VCap:	3000 V
Drying Gas Flow:	6 L/minute	Nozzle Voltage:	2000 V
Nebulizer:	30 psig	Fragmentor:	150 V
Sheath Gas:	250°C	Skimmer:	65 V
Sheath Gas Flow:	6 L/minute	OCT 1 RF Vpp:	750 V
Collision Energy:	0 V		
Data Acquisition:	MassHunter		

The reference standard of Impurity 1 was prepared in acetonitrile.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) GLOB2106cF

	Impurity 1 max. content in PPP: 0.0075 g/L
Author(s), year	Sowle J., 2022
Principle of method	LC-QTOF
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0.1 – 15 mg/mL $R^2 = 0.9999$ $y = 0.00000002366x - 0.0524144606$ equivalent concentration (g/Kg) in formulation: 0.004-0.6
Precision – Repeatability Mean n = 6 (%RSD)	No detectable Impurity 1 above the LOQ Level of 0.004 g/Kg.
Accuracy n = 6 (% Recovery)	Mean = 97.54 ± 1.237% %RSD = 1.268 Hr = 0.205
LOQ Recovery (Total Recovery) at 0.004 g/kg	Mean Recovery = 98.86% %RSD=1.634 Hr=0.187
Interference/ Specificity	Impurity 1 eluted at 13.0 minutes and there were no other peaks present at the same elution time as Impurity 1
Spectral analysis	The MS spectra for Impurity 1 confirmed the species identification
LOQ	0.004 g/kg (0.1 mg/L)
Comment	-

Conclusion

The validation parameters for these methodologies have been met for this study under the SANCO/3030/99 rev. 5 guidelines.

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

Not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method (399) for determination of Propamocarb-HCl exists for the determination of Propamocarb hydrochloride in an SL formulation.

For Mandipropamid, a CIPAC method (783) exists but it does not provide a method for Mandipropamid analysis in the presence of other active ingredients.

In conclusion: there are no CIPAC methods available for the determination of Propamocarb-HCl and

Mandipropamid in GLOB2106cF.

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 Propamocarb-HCl and Mandipropamid for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: Propamocarb-HCl				
Food/feed of plant origin (Residues)	Primary	0.01 mg/kg (Wheat and Barley grain, Rape seed, Tomato, Orange fruit) 0.05 mg/kg (Wheat, Barley forage and straw)	HPLC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
Food/feed animal origin (Residues)	-	Not required	-	EFSA Scientific Report (2006) 78, 1-80
Soil (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.02 mg/kg	HPLC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
Surface water and drinking water (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.05 mg/kg	HPLC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
Feed, body fluids,... (Toxicology)	-	Not required Required	-	EFSA Scientific Report (2006) 78, 1-80 Commission Regulation (EU) No 283/2013
Air (Exposure)	Primary	9 µg/m ³	LC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
	Primary	0.4 µg/m ³	GC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
Water (Ecotoxicology)	Primary	9.9 µg/L	HPLC-MS/MS	Lührs U., 2023 (KCP 10.2.1)
	Primary	6.6 µg/L	HPLC-MS/MS	Ganßmann M., 2023a (KCP 10.2.1)
	Primary	13 µg/L	HPLC-MS/MS	Ganßmann M., 2023b (KCP 10.2.1)
	Primary	2558 mg/L	HPLC-UV	Stead A., 2023a; Stead A., 2023b (KCP 10.6)

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: Propamocarb-HCl				
	Confirmatory	Not required		
Other (Ecotoxicology)	Primary	3.1 µg/L; 3.1 µg/L	HPLC-MS/MS	Chwiesko D., 2023 (KCP 10.3.1.1)
	Primary	0.31 g/kg	LC-MS/MS	Venturi S., 2023 (KCP 10.3.1.2)
	Primary	0.21 g/L	LC-MS/MS	Colli M., 2023 (KCP 10.3.1.3)
	Confirmatory	Not required		

An overview on the acceptable methods and possible data gaps for analysis of residues of Mandipropamid for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: Mandipropamid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Food/feed of plant origin (Residues)	Primary	Mandipropamide:0.01 mg/kg SYN500003: 0.005 mg/kg	DFG-S19: LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935 Draft Assessment Report (DAR)/Monograph prepared in the context of inclusion of the active substance mandipropamid in Annex I of the Council Directive 01/414/EEC, Revision1, July 2011;
Food/feed of animal origin (Residues)	-	Not required 0.01 mg/kg	LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935 EFSA Journal 2018;16(5):5284
Soil (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.0005 mg/kg	LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935
Surface water and drinking water (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.05 µg/L	LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935
Feed, body fluids,... (Toxicology)	-	Not required Required	-	EFSA Scientific Report (2012) 10(11), 2935 Commission Regulation (EU) No

Component of residue definition: Mandipropamid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				283/2013
Air (Exposure)	Primary	0.56 µg/m ³	LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935
New studies				
Water (Ecotoxicology)	Primary	1.6 µg/L	HPLC-MS/MS	Lührs U., 2023 (KCP 10.2.1)
	Primary	1.1 µg/L	HPLC-MS/MS	Ganßmann M., 2023a (KCP 10.2.1)
	Primary	2.3 µg/L	HPLC-MS/MS	Ganßmann M., 2023b (KCP 10.2.1)
	Primary	435.1 mg/L	HPLC-UV	Stead A., 2023a; Stead A., 2023b (KCP 10.6)
	Confirmatory	Not required		
Other (Ecotoxicology)	Primary	2.6 µg/L; 2.1 µg/L	HPLC-MS/MS	Chwiesko D., 2023 (KCP 10.3.1.1)
	Primary	0.05 g/kg	LC-MS/MS	Venturi S., 2023 (KCP 10.3.1.2)
	Primary	0.036 g/L	LC-MS/MS	Colli M., 2023 (KCP 10.3.1.3)
	Confirmatory	Not required		

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of Propamocarb-HCl (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Sum of propamocarb and its salts, expressed as propamocarb	0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Plant, high acid content		0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Plant, high oil content		0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Muscle	N-oxide propamocarb in ruminant and pig matrices and N-desmethyl propamocarb in poultry matrices	Not required 0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Milk		Not required 0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Eggs		Not required 0.05 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Fat		Not required 0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Liver, kidney		Not required 0.01 mg/kg	EFSA Scientific Report (2006) 78, 1-80 Reg. (EU) 2020/856
Soil (Ecotoxicology)	Sum of propamocarb and its salts, expressed as propamocarb	0.05 mg/kg	General limit for soil SANCO/825/00 rev. 8.1
Drinking water (Human toxicology)	Sum of propamocarb and its salts, expressed as propamocarb	0.1 µg/L	General limit for drinking water SANCO/825/00 rev. 8.1
Surface water (Ecotoxicology)	Sum of propamocarb and its salts, expressed as propamocarb	6.3 µg/L	EFSA Scientific Report (2006) 78, 1-80 NOEC (<i>Lepomis macrochirus</i>)
Air	Sum of propamocarb and its salts, expressed as propamocarb	0.4 µg/m ³	EFSA Scientific Report (2006) 78, 1-80
Tissue (meat or liver)	-	Not required Required 0.01 mg/kg	- Commission Regulation (EU) No 283/2013
Body fluids		Not required Required 0.01 mg/L	SANTE/2020/12380 rev. 2.

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 Propamocarb-HCl in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: Sum of propamocarb and its salts, expressed as propamocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	0.01 mg/kg	HPLC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
High acid content				
High oil content				
High protein/high starch content (dry)				

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Demonstration of the extraction efficiency should be provided at the renewal of the active substance.

zRMS comments:

The Applicant provided additional information:

According to the RAR (June 2017) of Propamocarb (Vol. 3 – CA- B5, page 17): *“The extraction efficiency has been checked and judged acceptable without the conduct of studies. This expert statement has been written based on the analytical procedures in place, the solubility of Propamocarb in common solvents and on the extraction procedures used within the metabolism studies. The residue definition in plants is either defined as Propamocarb (free base) or as Propamocarb hydrochloride, depending on the country. During the EU review of Propamocarb hydrochloride under Council Directive 91/414/EEC, the European Food Safety Authority proposed the residue definition in plants as “Propamocarb and its salts expressed as Propamocarb”. The proposed residue definitions in animals in EFSA Journal 2013; 11 (4): 3214 was defined as N-oxide Propamocarb in milk, pig and ruminants tissues and as N-desmethyl Propamocarb in poultry products for enforcement. For risk assessment, the residue is defined in milk, pig and ruminant tissues as the sum of Propamocarb, N-oxide Propamocarb, Oxazolidine-2-one Propamocarb and 2-hydroxy-Propamocarb expressed as Propamocarb; for poultry tissues, the residue is defined as the sum of Propamocarb and N-desmethyl Propamocarb, expressed as Propamocarb. The determination of Propamocarb residues in plants and animals involves the extraction of Propamocarb residues from the sample*

material with dilute acetic acid. An aliquot of the extract is concentrated by solid-phase-extraction (SPE) on C18 phase. Propamocarb is then eluted with acetonitrile/water/acetic acid. Final determination is performed by HPLC-MS/MS. By this procedure, the Propamocarb residues including all Propamocarb salts are extracted from the matrix and the different salts are hydrolysed into the free Propamocarb base. Other analytical methods use either acidified methanol or hydrochloric acid for hydrolysis and extraction of the Propamocarb residues. For the determination of Propamocarb and metabolites residues in animal commodities the extraction step is similar.

The extraction procedure used in all plant and animal metabolism studies usually involves hydrolysis and maceration into an organic solvent (Methanol/1M HCl (99:1 v/v)) followed by a further soxhlet extraction with the same solvent. Due to the high solubility of Propamocarb and its metabolites in highly polar solvents (water, methanol, acetone, dichloromethane, acetonitrile), and due to the fact that in metabolism studies the total radioactive residues in all matrices were at least 90% of the total residues extracted (except for muscle cow with 83 %TRR), it can be concluded that the analytical procedures in place within the analytical methods for the determination of Propamocarb and its metabolites allow a satisfactory extraction of Propamocarb and its metabolites residues in the different matrices (plants and animals).”

This statement, presented in the RAR, is not based on new studies, is non-GLP and does therefore not deserve data protection. “Expert statement (KCA 4.1.2/15; Theurig, M.; 2006; M-267054-01-1, non-GLP, unprotected).

In the opinion of zRMS, the explanation presented is sufficient.

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

An analytical method is not required due to fact that no MRL is proposed.

zRMS comments:

zRMS does not agree with the Applicant’s explanation. MRLs for propamocarb in products of animal origin were proposed by the Reg. (EU) No 289/2014. They are still valid.

According to the EFSA Journal 2013;11(4):3214: During the peer review under Directive 91/414/EEC, an analytical method using HPLC-MS/MS and its ILV was reported for the determination of propamocarb in food of animal origin with an LOQ of 0.01mg/kg in milk, meat, liver, kidney and eggs (Ireland, 2004; FAO, 2006b). In addition, after Annex I inclusion, the RMS also reported an HPLC-MS/MS method for the determination of propamocarb with an LOQ of 0.01 mg/kg in meat, fat, liver, kidney, milk and eggs (Ireland, 2012). Nevertheless, as the residue for enforcement is defined as N-oxide propamocarb in ruminant and pig matrices and N-desmethyl propamocarb in poultry matrices, a fully validated analytical method, with its ILV and a confirmatory method for the determination of each analyte are required.

Taking into account that no residues are expected in potatoes or products of animal origin after use in accordance with the proposed GAP, the above mentioned lack of data is not considered critical for this dossier.

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 Propamocarb-HCl in soil is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-4: Validated methods for soil (if appropriate)

Component of residue definition: Sum of propamocarb and its salts, expressed as propamocarb			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.02 mg/kg	HPLC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
Confirmatory	0.02 mg/kg	HPLC-MS/MS	EFSA Scientific Report (2006) 78, 1-80

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 Propamocarb in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

Table 5.3-5: Validated methods for water (if appropriate)

Component of residue definition: Sum of propamocarb and its salts, expressed as propamocarb				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	HPLC-MS/MS	EFSA Scientific Report (2006) 78, 1-80
Surface water				

zRMS comments:

The methods used in the DAR and in the EFSA Scientific Report (2006) 78, 1-80 of propamocarb, include dichloromethane, both for surface and drinking water, , which does not meet SANTE 2020/12830 rev. 2 requirements. Therefore, a primary, confirmatory and ILV methods in drinking water is missing - data gap. In addition, a primary method is missing for the determination of propamocarb in surface water – data gap.

In the opinion of the zRMS, the Applicant should complete them post-registration after the renewal of approval for propamocarb. However, the final decision should be taken by risk managers.

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 Propamocarb in air is given in the following tables. For the detailed evaluation of new/ additional studies please refer to Appendix 2.

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

Component of residue definition: Sum of propamocarb and its salts, expressed as propamocarb			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	9 µg/m ³	LC-MS/MS	EFSA Scientific Report

Component of residue definition: Sum of propamocarb and its salts, expressed as propamocarb			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
			(2006) 78, 1-80
Primary	0.4 µg/m ³	GC-MS/MS	EFSA Scientific Report (2006) 78, 1-80

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

A method for the determination of Propamocarb in body fluids is only required at the renewal of the active substance.

zRMS comments:

According to Regulation No. 283/2013 a primary method and confirmation is required for the analysis of propamocarb in body tissues and body fluids - data gap. Data should be completed after renewal of propamocarb.

5.3.2.8 Other studies/ information

In several ecotoxicological studies summarized in section B9 of the dRR, analytical methods were used for the detection of Propamocarb-HCl in the different test mediums. The analytical part of these studies is summarized in Appendix 2.

5.3.3 Description of analytical methods for the determination of residues of Mandipropamid (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 (incl. its addenda) the current legal residue definition is identical.

Table 5.3-7: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Food/feed of plant origin	Mandipropamid (any ratio of constituent isomers)	0.01 mg/kg	EFSA Scientific Report (2012) 10(11), 2935 Reg. (EU) 2023/1069
Food/feed of animal origin	Mandipropamid (any ratio of constituent isomers)	Not required 0.01 mg/kg	EFSA Scientific Report (2012) 10(11), 2935 Reg. (EU) 2023/1069
Soil (Ecotoxicology)	Mandipropamid (sum of isomers)	0.05 mg/kg 0.0005 mg/kg	General limit for soil SANCO/825/00 rev. 8.1 EFSA Scientific Report

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
			(2012) 10(11), 2935
Drinking water (Human toxicology)	Mandipropamid (sum of isomers)	0.1 µg/L 0.05 µg/L	General limit for drinking water SANCO/825/00 rev. 8.1 EFSA Scientific Report (2012) 10(11), 2935
Surface water (Ecotoxicology)	Mandipropamid (sum of isomers)	76 µg/L	EFSA Scientific Report (2012) 10(11), 2935 NOEC (<i>Daphnia magna</i>)
Air	Mandipropamid (sum of isomers)	0.56 µg/cm ³	EFSA Scientific Report (2012) 10(11), 2935
Tissue (meat or liver)	Mandipropamid	Not required Required 0.01 mg/kg	not classified as T / T+ EFSA Scientific Report (2012) 10(11), 2935 Commission Regulation (EU) No 283/2013 SANTE/2020/12380 rev. 2.
Body fluids		Not required Required 0.01 mg/L	not classified as T / T+ EFSA Scientific Report (2012) 10(11), 2935 Commission Regulation (EU) No 283/2013 SANTE/2020/12380 rev. 2.

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 Mandipropamid in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-8: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Mandipropamid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	0.01 mg/kg	DFG-S19: LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935
High acid content				
High oil content				
High protein/high starch content (dry)				

Table 5.3-9: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Demonstration of the extraction efficiency should be provided at the renewal of the active substance.

zRMS comments:

The residue dossier is based on studies evaluated at EU level. The Applicant did not provided new studies. According to the EFSA Journal 2018;16(5):5284: During the peer review, a multi-residue analytical method using liquid chromatography with tandem mass spectrometry (LC-MS/MS) was validated for the determination of mandipropamid in high water, high acid, high oil and dry content commodities with a LOQ of 0.01 mg/kg (EFSA, 2012). Furthermore, the EURL reported a multi-residue analytical method using LC-MS/MS for the four main plant matrices with a LOQ of 0.01 mg/kg (EURL, 2017). A multi-residue analytical method using LC-MS/MS for the four main plant matrices with a LOQ of 0.01 mg/kg was also reported by Greece (2017). Hence, it is concluded that mandipropamid can be enforced with a LOQ of 0.01 mg/kg in high water content, high acid content, high oil content and dry commodities.

zRMS agrees with Applicant that demonstration of the extraction efficiency should be provided at the renewal of the active substance. For authorization of GLOB2106cF no change of the MRL is needed and for a product authorization a no different analytical methodology is used, compared to that of the approval procedure of the active substance. However, it was clearly indicated in point 5.1 that this is a data gap and must be completed post-registration.

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

An analytical method is not required due to fact that no MRL is proposed.

zRMS comments:

zRMS does not agree with the Applicant's explanation. MRLs for mandipropamid in fat (products of animal origin) were proposed for the first time by the Reg. (EU) No 2023/1069. They are still valid. It should be noted, however, that the introduction of these values is related to the adoption of the CXL values to EU legislation. According to the EFSA Journal 2018;16(5):5284: no feeding studies were available or required for this MRL review. Two validated analytical methods for animal matrices each with a LOQ of 0.01 mg/kg were reported by the RMS (Austria, 2013) and Greece (2017).

Taking into account that no residues are expected in potatoes or products of animal origin after use in accordance with the proposed GAP, the above mentioned lack of data is not considered critical for this dossier.

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 Mandipropamid in soil is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-10: Validated methods for soil (if appropriate)

Component of residue definition: Mandipropamid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.0005 mg/kg	LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935

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 Mandipropamid in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

Table 5.3-11: Validated methods for water (if appropriate)

Component of residue definition: Mandipropamid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935
Surface water				

zRMS comments:

An ILV method for the determination of mandipropamid in drinking water is missing. Data gap.

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 Mandipropamid in air is given in the following tables. For the detailed evaluation of new/ additional studies please refer to Appendix 2.

Table 5.3-12: Validated methods for air (if appropriate)

Component of residue definition: Mandipropamid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.56 µg/m ³	LC-MS/MS	EFSA Scientific Report (2012) 10(11), 2935

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

A method for the determination of Mandipropamid in body fluids is only required at the renewal of the active substance.

zRMS comments:

According to Regulation No. 283/2013 a primary method and confirmation is required for the analysis of

mandipropamid in body tissues and body fluids. Data gap.

5.3.3.8 Other studies/ information

In several ecotoxicological studies summarized in section B9 of the dRR, analytical methods were used for the detection of Mandipropamid in the different test mediums. The analytical part of these studies is summarized in Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

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	Sowle J.	2022	Validation of the methods of determination of propamocarb HCl and Mandipropamid and a specified impurity in a formulation GLOB2106cF, in compliance with good laboratory practice DNA6689 David Norris Analytical Laboratories Ltd GLP Unpublished	N	Globachem NV
KCP 5.2.1 5.1.2 Submitted as KCP 10.2.1	Lührs U.	2023	GLOB2106cF: acute toxicity to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in a 96-hour static test 169461230 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.2.1 5.1.2 Submitted as KCP 10.2.1	Ganßmann M.	2023a	GLOB2106cF: Acute toxicity to <i>Daphnia magna</i> in a static 48-hour immobilisation test 169461220 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.2.1 5.1.2 Submitted as KCP 10.2.1	Ganßmann M.	2023b	GLOB2106cF: Toxicity to <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test 169461210 Ibacon GmbH GLP Unpublished	N	Globachem NV
KCP 5.2.1 5.1.2 Submitted as KCP	Chwiesko D.	2023	GLOB2106cF: acute contact and oral toxicity to bumblebees (<i>Bombus terrestris</i> L.) in the laboratory 169461105 Ibacon GmbH GLP	N	Globachem NV

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
10.3.1.1			Unpublished		
KCP 5.2.1 5.1.2 Submitted as KCP 10.3.1.2	Venturi S.	2023	Chronic oral effects of GLOB2106cF to adult worker honeybees (<i>Apis mellifera</i> L.) in a 10-day feeding laboratory test BT262/22 BioTecnologie BT S.r.l. GLP Unpublished	N	Globachem NV
KCP 5.2.1 5.1.2 Submitted as KCP 10.3.1.3	Colli M.	2023	Effects of GLOB2106cF on honeybees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure. BT126/22 BioTecnologie BT S.r.l. GLP Unpublished	N	Globachem NV
KCP 5.2.1 5.1.2 Submitted as KCP 10.6	Stead, A.	2023a	GLOB2106cF: OECD Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test STC/22/E1576 Stockbridge Technology Centre Ltd GLP Unpublished	N	Globachem NV
KCP 5.2.1 5.1.2 Submitted as KCP 10.6	Stead, A.	2023b	GLOB2106cF: OECD Terrestrial Plant Test - Vegetative Vigour Test STC/22/E1575 Stockbridge Technology Centre Ltd GLP Unpublished	N	Globachem NV

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Propamocarb-HCl

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

Reference is made to 5.2.1 for a summary of the method used to determine the active substance in the formulated product.

A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

No new or additional studies have been submitted.

A 2.1.1.1.1 Analytical methods in water used in aquatic toxicity studies

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	Accepted. The method was sufficiently validated according to SANTE/2020/12830 rev. 2.
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Reference:	KCP 10.2.1
Report	GLOB2106cF: Acute toxicity to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in a 96-hour static test, Lührs U., 2023, 169461230
Guideline(s):	Yes, OECD 203 and SANTE/2020/12830 rev. 1 guideline
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The purpose of the analytical part was to perform the analysis of the concentrations of Mandipropamid and Propamocarb-HCl, based on its parent Propamocarb, of the test item GLOB2106cF in the test samples.

Method for Determination:

Direct dilution of samples followed by analysis via LC-MS/MS

All standards were prepared in acetonitrile / HPLC-water (1/1, v/v).

Results and discussions

Table A 1: Recovery results from method validation of Propamocarb-HCl and Mandipropamid using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Reconstituted water	Propamocarb-HCl	30 (5)	99	4
		120000 (5)	100	1
	Mandipropamid	30 (5)	76	6
		120000 (5)	93	2

Table A 2: Characteristics for the analytical method used for validation of Propamocarb-HCl and Mandipropamid residues in reconstituted water

	Propamocarb-HCl	Mandipropamid
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y=15214 * x - 3564 R ² = 0.9966 number of data points: 7	Linear y= 34856 * x + 2205 R ² = 0.9996 number of data points: 7
Calibration range	3.53 – 35.3 µg/L	0.5-5 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	3.53 µg/L / 30 µg test item/L	0.5 µg/L / 30 µg test item/L
Extract and standard stability	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between end of sample preparation and beginning of analysis. Standard stability was not investigated since freshly prepared standard solutions were used for all analyses.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 1 and can be used for analytical determination of Propamocarb-HCl and Mandipropamid in reconstituted water.

Comments of zRMS:	Accepted. The method was sufficiently validated according to SANTE/2020/12830 rev. 2.
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Reference:	KCP 10.2.1
Report	GLOB2106cF: Acute toxicity to <i>Daphnia magna</i> in a static 48-hour immobilisation test, Ganßmann M., 2023a, 169461220
Guideline(s):	Yes, OECD 202 and SANTE/2020/12830 rev. 1 guideline
Deviations:	No
GLP:	Yes

Acceptability: **Yes**

Materials and methods

The purpose of the analytical part was to perform the analysis of the concentrations of Mandipropamid and Propamocarb-HCl, based on its parent Propamocarb, of the test item GLOB2106cF in the test samples.

Method for Determination:

Direct dilution of samples followed by analysis via LC-MS/MS

All standards were prepared in acetonitrile / HPLC-water (1/1, v/v).

Results and discussions

Table A 3: Recovery results from method validation of Propamocarb-HCl and Mandipropamid using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Reconstituted water	Propamocarb-HCl	20 (5)	103	6
		120000 (5)	108	6
	Mandipropamid	20 (5)	103	5
		120000 (5)	118	5

Table A 4: Characteristics for the analytical method used for validation of Propamocarb-HCl and Mandipropamid residues in reconstituted water

	Propamocarb-HCl	Mandipropamid
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y=9969 * x - 793 R ² = 0.9998 number of data points: 7	Linear y=42478 * x + 2019 R ² = 0.9997 number of data points: 7
Calibration range	3.5-35 µg/L	0.5-5 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, > 20%, matrix matched solution used.
Limit of detection/quantification	3.6 µg/L / 20 µg test item/L	0.5 µg/L / 20 µg test item/L
Extract and standard stability	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between end of sample preparation and beginning of analysis. Standard stability was not investigated since freshly prepared standard solutions were used for all analyses.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 1 and can be used for analytical determination of Propamocarb-HCl and Mandipropamid in reconstituted water.

Comments of zRMS:	Accepted. The method was sufficiently validated according to SANTE/2020/12830 rev. 2.
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Reference:	KCP 10.2.1
Report	GLOB2106cF: toxicity to <i>Pseudokirchneriella subcapitata</i> in an algal growth inhibition test, Ganßmann M., 2023b, 169461210
Guideline(s):	Yes, OECD 201 and SANTE/2020/12830 rev. 1 guideline
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The purpose of the analytical part was to perform the analysis of the concentrations of Mandipropamid and Propamocarb-HCl, based on its parent Propamocarb, of the test item GLOB2106cF in the test samples.

Method for Determination:

Direct dilution of samples followed by analysis via LC-MS/MS

All standards were prepared in acetonitrile / HPLC-water (1/1, v/v).

Results and discussions

Table A 5: Recovery results from method validation of Propamocarb-HCl and Mandipropamid using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Reconstituted water	Propamocarb-HCl	800 (5)	114	3
		120000 (5)	112	2
	Mandipropamid	800 (5)	96	7
		120000 (5)	75	17

Table A 6: Characteristics for the analytical method used for validation of Propamocarb-HCl and Mandipropamid residues in reconstituted water

	Propamocarb-HCl	Mandipropamid
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y=16854 * x - 4421 R ² = 0.9992 number of data points: 7	Linear y=5035 * x + 1252 R ² = 0.9983 number of data points: 7
Calibration range	3.6 - 36 µg/L	0.5 - 5 µg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched	Yes, > 20%, matrix matched

	Propamocarb-HCl	Mandipropamid
	solution used.	solution used.
Limit of detection/quantification	3.6 µg/L / 800 µg test item/L	0.5 µg/L / 800 µg test item/L
Extract and standard stability	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between end of sample preparation and beginning of analysis. Standard stability was not investigated since freshly prepared standard solutions were used for all analyses.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 1 and can be used for analytical determination of Propamocarb-HCl and Mandipropamid in reconstituted water.

Comments of zRMS:	Accepted. The method was sufficiently validated according to SANTE/2020/12830 rev. 2.
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Reference: KCP 10.6
 Report GLOB2106cF: OECD Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test, Stead A., 2023a, STC/22/E1576
 Guideline(s): Yes, OECD 208 (2006)
 Deviations: No
 GLP: Yes
 Acceptability: **Yes**

Reference: KCP 10.6
 Report GLOB2106cF: OECD Terrestrial Plant Test - Vegetative Vigour Test, Stead A., 2023b, STC/22/E1575
 Guideline(s): Yes, OECD 227 (2006)
 Deviations: No
 GLP: Yes
 Acceptability: **Yes**

Materials and methods

Samples were analysed for Propamocarb-HCl and Mandipropamid using HLPC.

All standards were prepared in methanol.

Results and discussions

Table A 7: Recovery results from method validation of Propamocarb-HCl and Mandipropamid using the analytical method

Matrix	Analyte	Fortification level (mg/L) ($n = x$)	Mean recovery (%)	RSD (%)
Aqueous solution	Propamocarb-HCl	2558 (5)	99	1.4
		13826 (5)	98	0.5
	Mandipropamid	435.1 (5)	103	0.5
		2352 (5)	102	0.3

Table A 8: Characteristics for the analytical method used for validation of Propamocarb-HCl and Mandipropamid residues in aqueous solution

	Propamocarb-HCl	Mandipropamid
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear $y = 0.07143x + 0.1294$ $R^2 = 1.0000$ number of data points: 9	Linear $y = 0.4123x + 0.2622$ $R^2 = 0.9999$ number of data points: 9
Calibration range	66.90-1673 mg/L	11.84-296.1 mg/L
Residuals analysis	The regression residuals are randomly distributed and no trends are visible.	
Assessment of matrix effects is presented	Yes, < 20%	Yes, < 20%
Limit of detection/quantification	79.88 mg/L / 2558 mg/L	11.84 mg/L / 435.1 mg/L
Extract and standard stability	The stock solutions of the reference items were prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 1 and can be used for analytical determination of Propamocarb-HCl and Mandipropamid in aqueous solution.

A 2.1.1.1.2 Analytical methods used in other ecotoxicological studies

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	Accepted. The method was sufficiently validated according to SANTE/2020/12830 rev. 2.
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Reference:	KCP 10.3.1.1 (Submitted as KCP 10.3.1.1)
Report	GLOB2106cF: acute contact and oral toxicity to bumblebees (<i>Bombis terrestris</i> L.) in the laboratory, Chwiesko D., 2023, 169461105
Guideline(s):	Yes, OECD 246 and 247
Deviations:	No

GLP: Yes
 Acceptability: Yes

Materials and methods

The purpose of the analytical part was to perform the analysis of the concentrations of Mandipropamid and Propamocarb-HCl, based on its parent Propamocarb, of the test item GLOB2106cF in the test samples.

Method for Determination:

Direct dilution of samples followed by analysis via LC-MS/MS

All standards were prepared in acetonitrile / pure water (50/50, v/v).

Results and discussions

Table A 9: Recovery results from method validation of Propamocarb-HCl and Mandipropamid using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Application solution (0.1% Triton X-100)	Propamocarb-HCl	3.1 (11)	91	8
		6.1 (11)	92	11
	Mandipropamid	2.6 (8)	96	11
		5.3 (8)	91	5
Feeding solution (50 % w/v sucrose solution)	Propamocarb-HCl	3.1 (5)	90	8
		5.1 (5)	88	2
	Mandipropamid	2.1 (5)	100	9
		7.0 (5)	89	4

Table A 10: Characteristics for the analytical method used for validation of Propamocarb-HCl and Mandipropamid residues in application solution (contact test)

	Propamocarb-HCl	Mandipropamid
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear y = 95289 x + 30236 R ² = 0.9988 number of data points: 6	Linear y = 27870 x + 1354 R ² = 1.0000 number of data points: 6
Calibration range	0.6 – 11.9 µg/L	0.5 – 10 µg/L
Residuals analysis	Regression residuals of linear regression are randomly distributed and no trends are visible.	Regression residuals of linear regression are randomly distributed and no trends are visible.
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	0.6 µg/L / 3.1 µg/L	0.5 µg/L / 2.6 µg/L

	Propamocarb-HCl	Mandipropamid
Extract and standard stability	The stock solutions of the reference items were prepared on each day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.	

Table A 11: Characteristics for the analytical method used for validation of Propamocarb-HCl and Mandipropamid residues in feeding solution (oral test)

	Propamocarb-HCl	Mandipropamid
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Linear $y = 221781 x + 26083$ $R^2 = 0.9999$ number of data points: 6	Linear $y = 45907 x - 458$ $R^2 = 0.9996$ number of data points: 6
Calibration range	0.6 – 11.9 µg/L	0.5 – 10 µg/L
Residuals analysis	Regression residuals of linear regression are randomly distributed and no trends are visible.	Regression residuals of linear regression are randomly distributed and no trends are visible.
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, < 20%, but matrix matched solution used.
Limit of detection/quantification	0.6 µg/L / 3.1 µg/L	0.5 µg/L / 2.1 µg/L
Extract and standard stability	The stock solutions of the reference items were prepared on each day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 1 and can be used for analytical determination of Propamocarb-HCl and Mandipropamid in the application solution and feeding solution used in the contact and oral test, respectively.

Comments of zRMS:	Accepted. The method was sufficiently validated according to SANTE/2020/12830 rev. 2.
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Reference:	KCP 10.3.1.2 (Submitted as KCP 10.3.1.2)
Report	Chronic oral effects of GLOB2106cF to adult worker honeybees (<i>Apis mellifera</i> L.) in a 10-day feeding laboratory test, Venturi S., 2023, BT262/22
Guideline(s):	Yes, OECD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

All samples were analysed by LC-MS/MS.

All standards were prepared in acetonitrile.

Results and discussions

Table A 12: Recovery results from method validation of Mandipropamid and Propamocarb-HCl using the analytical method

Matrix	Analyte	Fortification level (g a.s./kg) (n = x)	Mean recovery (%)	RSD (%)
50 % w/v sucrose solution	Mandipropamid	0.05 (5)	88.02	1.24
		2.12 (5)	96.75	0.72
	Propamocarb-HCl	0.31 (5)	106.92	1.05
		12.44 (5)	86.31	0.75

Table A 13: Characteristics for the analytical method used for validation of Mandipropamid and Propamocarb-HCl residues in sucrose solution

	Mandipropamid	Propamocarb-HCl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic $y = 1.250058x^2 + 1311.106645x - 787.079602$ $R^2 = 0.99950673$ number of data points: 5	Quadratic $y = -0.196113x^2 + 867.33965x - 5480.10478$ $R^2 = 0.99881326$ number of data points: 5
Calibration range	2.9636–148.1801 µg/L	8.9758–897.5761 µg/L
Residuals analysis	Regression residuals are randomly distributed and no trends are visible.	Regression residuals are randomly distributed and no trends are visible.
Assessment of matrix effects is presented	Yes, < 20%, but matrix matched solution used.	Yes, > 20%, matrix matched solution used.
Limit of detection/quantification	2.9636 µg/L / 0.05 g/kg	8.9758 µg/L / 0.31 g/kg
Extract and standard stability	The standard solutions and the diluted samples were prepared once and used in the same day of preparation, so no stability control was carried out.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 1 and can be used for analytical determination of Propamocarb-HCl and Mandipropamid in sucrose solution.

Comments of zRMS:	Accepted. The method was sufficiently validated according to SANTE/2020/12830 rev. 2.
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Reference: KCP 5.2.1 (Submitted as KCP 10.3.1.3)

Report Effects of GLOB2106cF on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Colli M., 2023, BT126/22

Guideline(s): Yes, OECD 239 (2016)

Deviations: Deviation No. 1 issued on 09th September 2022
 Description:
 - 03rd August 2022 (day 3 of the test - during the treatments) the temperature was lower than 34°C for about 4 hours (minimum value 32.7°C), and the humidity was lower than 90% for about 2 hours (minimum value 76.8%) this was due to the operations carried out for the selection of the larvae for each treatment plate.
 - 04 th August 2022, (day 4 of the test) the temperature was lower than 34°C for about 2 hours (minimum value 33.0°C), this occurred during the assessment and treatment operations.
 - 05 th August 2022, (day 5 of the test) the temperature was lower than 34°C for about 2 hours (minimum value 32.7°C), this occurred during the assessment and treatment operations.
 Impact on the Study: None, the validity criteria were met, and the deviations are not considered to have affected the outcome of the study.

GLP: Yes
 Acceptability: Yes

Materials and methods

All samples were analysed by LC-MS/MS.

All standards were prepared in acetonitrile.

Results and discussions

Table A 14: Recovery results from method validation of Mandipropamid and Propamocarb-HCl using the analytical method

Matrix	Analyte	Fortification level (g/L) (n = x)	Mean recovery (%)	RSD (%)
Water stock solution	Mandipropamid	0.036 (5)	92.82	2.22
		2.47 (5)	89.92	5.18
	Propamocarb-HCl	0.21 (5)	102.07	1.43
		14.54 (5)	92.74	1.16

Table A 15: Characteristics for the analytical method used for validation of Mandipropamid and Propamocarb-HCl residues in water stock solution

	Mandipropamid	Propamocarb-HCl
Specificity	blank value < 30 % LOQ	blank value < 30 % LOQ
Calibration (type, number of data points)	Quadratic $y = 8.954328x^2 + 3133.163799x + 1090.941561$ $R^2 = 0.99891581$ number of data points: 5	Quadratic $y = -0.161143x^2 + 775.434994x + 1490.104357$ $R^2 = 0.99991399$ number of data points: 5
Calibration range	3.0784 – 133.3964 µg/L, corresponding to 0.0103 – 3.3349 g/L in matrix	8.9758 µg/L – 777.8993µg/L, corresponding to 0.0299 – 19.4475 g/L in matrix

	Mandipropamid	Propamocarb-HCl
Residuals analysis	Regression residuals are randomly distributed	Regression residuals are randomly distributed
Assessment of matrix effects is presented	Yes, < 20% Linearity solutions were prepared in matrix (Diluent) instead of pure solvent.	Yes, > 20% Linearity solutions were prepared in matrix instead of pure solvent..
Limit of detection/quantification	3.0784 µg/L / 0.036 g/L	8.9758 µg/L / 0.21 g/L
Extract and standard stability	The standard solutions and the extracts were prepared once and used in the same day of preparation, so no stability control was carried out.	

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 rev. 1 and can be used for analytical determination of Propamocarb-HCl and Mandipropamid in aqueous matrix.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.2 Analytical methods for Mandipropamid

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

Reference is made to 5.2.1 for a summary of the method used to determine the active substance in the formulated product.

A 2.2.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

No new or additional studies have been submitted.

A 2.2.1.1.1 Analytical methods in water used in aquatic toxicity studies

Please refer to A 2.1.1.1.2.

A 2.2.1.1.2 Analytical methods used in other ecotoxicological studies

Please refer to A 2.1.1.1.3.

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)

No new or additional studies have been submitted.

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 Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.