

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: **102000037599**

Product name(s): Active substance(s): **Prohexadione-Calcium OD 75 (75 g/L)**

Central Zone

Zonal Rapporteur Member State: **Poland**

CORE ASSESSMENT

(Authorisation)

Applicant: **Bayer CropScience Division**

Submission date: **30/04/2021**

MS Finalisation date: 29/04/2022



M-767195-01-1

Version history

When	What
April 2021	Original Bayer submission
July 2021	Dossier sent for evaluation
January 2022	zRMS finalised evaluation
April 2022	Final version prepared by zRMS after Commenting period

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The product prohexadione-calcium OD 75 (75 g/L) (PRL OD 75 / Product Code 102000037599) has not been previously evaluated at zonal level. It was not the representative formulation during the renewal of approval of prohexadione-calcium. All data and information assessed during the EU re-evaluation of prohexadione-calcium is considered EU peer-reviewed data.

zRMS comments:

This report has been completed by the Applicant.

The text highlighted in grey was provided by the evaluator.

5 Analytical methods

5.1 Conclusion and summary of assessment

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

Noticed data gaps are:

None

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

Noticed data gaps are:

None

Commodity/crop	Supported/ Not supported
Winter rape	Supported

State whether or not submitted data are sufficient for evaluation and whether enforcement of all relevant MRLs/residue levels is possible. Data gaps should be listed and conditions for registration presented, if appropriate. Provide an overview on the commodities/crops that are supported/ not supported as outcome of the evaluation on the available analytical methods.

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 prohexadione-calcium in plant protection product is provided as follows:

Comments of zRMS:	The method is accepted and may be used for analysing prohexadione-calcium in the PPP.
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Analytical method AM035319MF1 for the determination of prohexadione-calcium in formulations

Reference:	KCP 5.1.1/01
Title:	Determination of prohexadione-calcium in formulations - HPLC-UV, external standard
Report:	Jasak, J.; 2020; AM035319MF1; M-683049-01-1
Authority registration No:	
Guideline(s):	Commission Regulation (EU) 284/2013 in accordance with Regulation (EC) No 1107/2009 (10/2009), US EPA OCSPP Test Guideline No. 830.1800 (08/1996)
Deviations:	Not specified
GLP/GEP:	no
Acceptability:	
Duplication (if vertebrate study):	

Reference:	KCP 5.1.1/02
Title:	Validation of analytical method AM035319MF1 - Determination of prohexadione-calcium in the formulation prohexadione-calcium OD 75 (75 g/L)
Report:	Jasak, J.; Garcia Sanchez, M. T.; 2021; FM0408(MVF00)G01; M-765354-01-1
Authority registration No:	
Guideline(s):	SANCO/3030/99 rev. 5 (03/2019), Commission Regulation (EU) 284/2013 (03/2013) in accordance with Regulation (EC) No 1107/2009 (10/2009), US EPA OCSPP Test Guideline No. 830.1800 (08/1996)
Deviations:	None
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Materials and methods

The analytical method AM035319MF1 was developed for the determination of prohexadione-calcium in formulations.

The active substance prohexadione-calcium is separated from formulation components on a reversed phase column using isocratic elution. After UV detection, the quantitative evaluation is carried out by comparing the peak areas with those of reference items, using either a single-point calibration or a calibration curve.

Equipment and operating conditions are as follows:

Chromatographic conditions:

Column: XBridge Phenyl, 2.5 µm, 50 x 4.6 mm⁺
Supplier: Waters ⁺
Guard: n/a
⁺ or equivalent apparatus/configuration

Flow rate: see gradient program
Temperature: 40 °C
Injection volume: 3 µL
Solvent A: 0.5% 1 N sulfuric acid in purified water
Solvent B: acetonitrile
Gradient program:

	Time [min]*	A[%]	B[%]	flow rate [mL/min]
Separation	0.0	75	25	2.0
	1.4	75	25	2.0
Rinsing Gradient	1.6	5	95	3.0
	3.0	5	95	3.0
	3.1	75	25	2.0
	4.5	75	25	2.0

*adjust the equilibration time according to the pump- and injection system

Remark: Columns with alternative dimensions or particle size, but with the same stationary phase may also be used. The flow rate may be amended appropriately as required.

Total run time: 4.5 min
Retention time: prohexadione-calcium approx. 0.9 min
Measurement wavelength: 290 nm

An aliquot (0.275g, accurately weighed) of the formulation is diluted with acetonitrile: 0.5% sulphuric acid (1N) in water (30:70 v/v) using ultrasonication to aid dissolution.

Quantification of extracts was performed using external calibration standards over the nominal range 1.0 to 3 mg/L (equivalent to 3.6 to 10% w/w active content). Solvent standards were used.

The lowest level tested (LOQ) was 3.77% w/w prohexadione-calcium.

Validation of the analytical method AM035319MF1

The analytical method AM035319MF1 was validated for the determination of prohexadione-calcium in the test item prohexadione-calcium OD 75 (75 g/L). This was accomplished by evaluating specificity, calibration, recovery and precision.

Validation - Results and discussions

Table 5.2-1: Method suitable for the determination of the active substance prohexadione-calcium in plant protection product PRL OD 75

Analyte	prohexadione-calcium
Author(s), year	Jasak, J. Garcia Sanchez, M. T.
Principle of method	High Performance Liquid Chromatography (RP-HPLC)
Calibration I (50-150%) regression equation correlation coefficient type of regression function concentration range [mg/100 mL] concentration range [% (w/w)] ¹ concentration range [% DC] ²	n = 6 y = 0.071545 + 0.1589 x 1.00 linear (1st order) 10.37- 29.63 3.771– 10.77 51.9– 148
Calibration II (5-50%) regression equation correlation coefficient type of regression function concentration range [mg/100 mL] concentration range [% (w/w)] ¹ concentration range [% DC] ²	n = 7 y = 0.0024228 + 0.16443 x 1.00 linear (1st order) 1.091-10.37 0.3967-3.771 5.46-51.9
Precision mean value [% (w/w)] RSD [%] outliers detected Horwitz-Value RSDr(max) [%] Horrat value (Horwitz ratio) Hr	n = 6 7.48 0.35 no 1.98 0.18 The Horrat value (Horwitz ratio, Hr) is ≤ 1 and thus, the precision of the analytical method is assessed acceptable.
Recovery mean recovery [%] confidence interval [95%ile] RSD [%] concentration range [mg/100 mL] concentration range [% (w/w)] ¹ concentration range [% DC] ²	n = 6 101 ± 0.826 0.78 10.37-30.12 3.77-11.0 51.9-151
Specificity identity identity confirmed	Identity of prohexadione-calcium in test item was confirmed by comparison of UV spectra and retention times with those of certified reference items. yes
Interferences interferences identified	Comparison of chromatograms of reference item, test item and empty formulation with regards to interferences no
Comment	No deviation from guideline SANCO/3030/99 rev.5.

¹ Referred to a nominal test item concentration of 275 mg/100 mL.

² Referred to the declared contents of the active substances acc. to specification (declared content 75.0 g/L, corresponds to 7.28 % (w/w) prohexadione-calcium)

Conclusion

Analytical method AM035319MF1, has been shown to be valid when used for the determination of prohexadione-calcium in PRL OD 75 formulation.

5.2.1.2 Description of analytical methods for the determination of relevant

impurities (KCP 5.1.1)

PRL OD 75 does not contain any relevant impurities.

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

With respect to toxicological, eco-toxicological or environmental aspects the product PRL OD 75 does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of prohexadione-calcium in formulations.

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 prohexadione-calcium 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 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: Prohexadione-Calcium				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants, Plant products (Residues) oilseed rape whole plant Oilseed rape haulm Oilseed rape seed	Primary RES-00196	0.01 mg/kg	HPLC-MS/MS	Watson, G., 2020 Appendix 2
Plant products (residues) Apples, grapes, sunflower seeds, wheat	Primary 506248	0.01 mg/kg	HPLC-MS/MS	Baltussen, E., 2014, Appendix 2
Animal products, food of animal origin,... (Residues)	Not required			
Soil, water, sediment,... (Environmental fate)	Not required			
Soil, water,... (Efficacy)	Not required			
Feed, body fluids,... (Toxicology)	Not required			
Body fluids, air,... (Exposure)	Not required			
Water (Ecotoxicology)	Primary Method 01652	0.1 µg/L	HPLC-MS/MS	Ruttmann, F. 2021 M-761406-01-1

				Appendix 2 Used in support of study M-764734-01-1 , M-763760-01-1 , M-763594-01-1
Water, test solution (Ecotoxicology)	Primary Method AM035319MF1	2 g/L	HPLC-UV	Jasak, J. 2020 M-683049-01-1 Jasak, J., Garcia Sanchez, M. T., 2021 M-765354-01-1 Used in support of M-691184-02-1 , M-691185-01-1 , M-758398-01-1
Larval diet (Ecotoxicology)	Primary	0.01 mg/kg	HPLC-MS/MS	Kling, A. 2021 M-762844-01-1 Appendix 2
Application solution/ feeding solution (Ecotoxicology)	Primary	2 g/L	HPLC-UV	Chwiesko, D., Kowalczyk, F. 2021 M-761833-01-1 Appendix 2
Feeding solution (Ecotoxicology)	Primary	0.01 mg/kg	HPLC-MS/MS	Kling, A. 2021 M-762860-01-1 Appendix 2
Water (Ecotoxicology)	Primary	0.15 mg/L	HPLC-MS/MS	Armbruster, H., Emnet, P., 2021 M-764321-01-1 Appendix 2
Water, buffer solutions,... (Properties)	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.

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 prohexadione-calcium (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	prohexadione (prohexadione and its salts expressed as prohexadione-calcium)	0.01mg/kg	Reg. (EU) 2018/70 2021/976
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Muscle	prohexadione (prohexadione and its salts expressed as prohexadione-calcium)	0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Milk		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Eggs		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Fat		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Liver, kidney		0.01 mg/kg	Reg. (EU) 2018/70 2021/976
Soil (Ecotoxicology)	prohexadione (prohexadione and its salts expressed as prohexadione-calcium)	0.01 mg/kg	Common limit
Drinking water (Human toxicology)	prohexadione (prohexadione and its salts expressed as prohexadione-calcium)	0.05 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	prohexadione (prohexadione and its salts expressed as prohexadione-calcium)	0.05 µg/L	Lowest EC 50 from lemna aquatic study 363 µg/L
Air	prohexadione (prohexadione and its salts expressed as prohexadione-calcium)	6.1 µg/m ³	AOEL sys 0.35 mg/kg bw/d
Tissue (meat or liver)	not required	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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 Prohexadione-Ca in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies refer 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: prohexadione (prohexadione and its salts expressed as prohexadione calcium)				
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	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
High acid content	Primary	0.01 mg/kg	HPLC-MS/MS	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
High oil content	Primary	0.01 mg/kg	HPLC-MS/MS	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
High protein/high starch content (dry)	Primary	0.01 mg/kg	HPLC-MS/MS	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
Difficult (if required, depends on intended use)	Primary	-	Not required	-
	ILV	-	Not required	-
	Confirmatory (if required)	-	Not required	-

For any special comments or remarkable points concerning the analytical methods for the determination

of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Watson, G., 2020 RES-00196 see Appendix 2
	Watson, G., 2020 RES-00279, see Appendix 2

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

An overview on the acceptable methods and possible data gaps for analysis of prohexadione-calcium in animal matrices is given in the following tables.

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: prohexadione (prohexadione and its salts expressed as prohexadione calcium)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	HPLC-MS/MS	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
Eggs	Primary	0.01 mg/kg	HPLC-MS/MS	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed
	Confirmatory (if required)	-	Not required	-
Fat	Primary	-	Not required	-
	ILV	-	Not required	-
	Confirmatory (if required)	-	Not required	-
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	Lehmann, A., Mackenroth, C., 2005 and Oppinger, M., Kerl, W., 2005/EU agreed
	ILV	0.01 mg/kg	HPLC-MS/MS	Anspach, T., 2005/EU agreed

Component of residue definition: prohexadione (prohexadione and its salts expressed as prohexadione calcium)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	Confirmatory (if required)	-	Not required	-

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	The metabolism studies used acidified acetonitrile to extract residues of prohexadione-calcium which is the same solvent system used in the methods of analysis

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 prohexadione-calcium in soil is given in the following tables.

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

Component of residue definition: prohexadione (prohexadione and its salts expressed as prohexadione calcium)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/kg	HPLC-MS/MS	Richter, T., Laik, W, 2004/EU agreed
Confirmatory	-	Not required	-

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

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

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

Component of residue definition: prohexadione (prohexadione and its salts expressed as prohexadione calcium)				
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	Richter, T., Laik, W, 2004/ EU agreed
	ILV	-	Not required Not available	-
	Confirmatory	0.05 µg/L	HPLC-MS/MS	Richter, T., Laik, W, 2004/ EU agreed
Surface water	Primary	0.05 µg/L	HPLC-MS	Richter, T., Laik, W, 2004/ EU agreed

Component of residue definition: prohexadione (prohexadione and its salts expressed as prohexadione calcium)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	Confirmatory	0.05 µg/L	HPLC-MS	Richter, T., Laik, W, 2004/ EU agreed

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 prohexadione-calcium in air is given in the following tables.

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

Component of residue definition: prohexadione (prohexadione and its salts expressed as prohexadione calcium)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	6.1 µg/m ³	HPLC-UV	Zangmeister, W., 2000/ EU agreed
Confirmatory	6.1 µg/m ³	HPLC-MS/MS	Zangmeister, W., 2005/ EU agreed

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

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

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

Component of residue definition: not relevant			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/missing/EU agreed
Primary	-	Not required	EU agreed
Confirmatory	-	Not required	EU agreed

5.3.2.8 Other studies/ information

No other studies are required.

5.3.3 Description of analytical methods for the determination of residues of active substance 2 (KCP 5.2)

Not relevant, the plant protection product PRL OD75 only contains one active substance.

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 / 01	Jasak, J.	2020	Determination of prohexadione-calcium in formulations - HPLC-UV, external standard Report No.: AM035319MF1, Edition Number: M-683049-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: No unpublished	No	Bayer
KCP 5.1.1 / 02	Jasak, J.; Garcia Sanchez, M. T.	2021	Validation of analytical method AM035319MF1 - Determination of prohexadione-calcium in the formulation prohexadione-calcium OD 75 (75 g/L) Report No.: FM0408(MVF00)G01, Edition Number: M-765354-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
	Watson, G	2020	Prohexadione-calcium: Storage stability of residues of prohexadione-calcium in oilseed rape whole plant, haulm and seed stored frozen for up to 6 months and validation of the methodology used Report No.: RES-00196 ResChem Analytical Limited GLP, unpublished	N	Fine Agrochemicals Ltd
	Baltussen E	2014	Validation of an analytical method for the analysis of residues of Prohexadione calcium in Apples, grapes, sunflower seeds and wheat Report No : 506248 WIL Research Europe B.V. GLP, published	N	Fine Agrochemicals Ltd

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2.6 / 01	Ruttmann, F.	2021	Analytical method 01652 for the determination of prohexadione-calcium in test water from aquatic toxicity tests by HPLC-MS/MS Report No.: P 604 207016, Edition Number: M-761406-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: No unpublished	No	Bayer
KCP 5.1.2.6 / 02 ... also filed: KCP 10.2.2 / 01	Riebschläger, T.	2021	Acute toxicity of prohexadione-calcium OD 75 (75 g/L) to the waterflea Daphnia magna in a static renewal laboratory test system Report No.: EBPW0004, Edition Number: M-764734-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 03 ... also filed: KCP 10.2.2 / 02	Kuhl, K.	2021	Pseudokirchneriella subcapitata growth inhibition test with prohexadione-calcium OD 75 (75 g/L) Report No.: E 201 05583-4, Edition Number: M-763760-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 04 ... also filed: KCP 10.2.3 / 01	Schrader, D.	2021	Lemna gibba G3 - Growth inhibition test with prohexadione-calcium OD 75 (75 g/L) under static conditions Report No.: E 221 05564 - 5, Edition Number: M-763594-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 05 ... also filed: KCP 10.6.1 / 02 KCP 6.5.2 / 01	Köhler, P.	2020	Amendment no. 01: Effects on the vegetative vigor of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L) Report No.: VV20/021, Edition Number: M-691184-02-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany	No	Bayer

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
			... amended: 2020-09-07 GLP/GEP: Yes unpublished		
KCP 5.1.2.6 / 06 ... also filed: KCP 10.6.1 / 01 KCP 6.5.1 / 01	Köhler, P.	2020	Effects on the seedling emergence and growth of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L) Report No.: SE20/022, Edition Number: M-691185-01-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 07 ... also filed: KCP 10.6.2 / 01 KCP 6.5.2 / 02	Köhler, P.	2020	Effects on the vegetative vigor of the non-target terrestrial plant species Cucumis sativus (Tier 2); prohexadione-calcium OD 75 (75 g/L) Report No.: VV20/039, Edition Number: M-758398-01-1 Bayer AG, Crop Science Division, Frankfurt am Main, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 08 ... also filed: KCP 10.3.1.3 / 01	Kling, A.	2021	Prohexadione-calcium OD 75 (75 g/L): Honey bee (Apis mellifera L.) 22 day larval toxicity test (repeated exposure) - Final report - Report No.: S20-00850, Edition Number: M-762844-01-1 Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 09 ... also filed: KCP 10.3.1.1 / 02	Chwiesko, D.; Kowalczyk, F.	2021	Prohexadione-calcium OD 75 (75 g/L): Effects (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory - Final report Report No.: 152171105, Edition Number: M-761833-01-1 IBACON GmbH, Rossdorf, Germany GLP/GEP: Yes unpublished	No	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2.6 / 10 ... also filed: KCP 10.3.1.2 / 01	Kling, A.	2021	Prohexadione-calcium OD 75 (75 g/L): Honey bee (Apis mellifera L.) chronic oral toxicity test 10 day feeding test in the laboratory - Final report - Report No.: S20-00682, Edition Number: M-762860-01-1 Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 11 ... also filed: KCP 10.2.3 / 02	Armbruster, H.; Emnet, P.	2021	Prohexadione-calcium OD 75 (75 g/L): Toxicity to the aquatic plant Myriophyllum spicatum in a semi-static growth inhibition test with a prior rooting phase Report No.: 152171215, Edition Number: M-764321-01-1 IBACON GmbH, Rossdorf, Germany GLP/GEP: Yes unpublished	No	Bayer
	Watson, G	2021	Evaluation of extraction efficiency of incurred residues of prohexadione-calcium in oilseed rape whole plant Report No.: RES-00279 ResChem Analytical Limited GLP, unpublished.	No	Fine Agrochemicals Ltd

List of data submitted or referred to by the applicant and relied on are available via the Letter of Access from Fine Agrochemicals Ltd. All data mentioned as part of DAR, RAR, or EFSA journals are considered as relied on.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 4.1.2	Abdel-Baky S., Baumann S.	1997	Method for determination of BAS 125 W and its metabolite in animal tissues (liver, kidney, fat and muscle), milk, and apple commodities BASF Corp.; Research Triangle Park NC; United States of America 1997/5034 GLP, unpublished ⇒ RAR reference IIA 4.3/5	N	BASF
CA 4.1.2	Bacher R.	2004	Independent laboratory validation (ILV) of BASF analytical method D9608 used for the determination of Prohexadione calcium in plant material PTRL Europe GmbH; Ulm; Germany Fed.Rep. 2004/1015913 GLP, unpublished ⇒ RAR reference IIA 4.3/6	N	BASF
CA 4.2	Anspach, T	2005	Independent laboratory validation of BASF method 564/0: Method for the determination of Prohexadione-Ca in plant and animal matrices (BASF study code 134612) Dr. Specht & Partner Chemische Laboratorien GmbH; Hamburg; Germany Fed.Rep. 2005/1006484 GLP, unpublished ⇒ RAR reference IIA 4.3/4	N	BASF
CA 4.2	Lehmann A., Mackenroth C	2005	Validation of the analytical method 564/0: Method for the determination of Prohexadione-Ca in plant and animal matrices BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep.	N	BASF

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
			2005/1007581 GLP, unpublished ⇒ RAR reference IIA 4.3/2		
CA 4.2	Oppinger M., Kerl W.	2005	Technical procedure: Method for the determination of Prohexadione-Ca in plant and animal matrices BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2005/1007580 Not-GLP, unpublished ⇒ RAR reference IIA 4.3/3	N	BASF
CA 4.2	Richter T., Laik W.	2004a	Validation of analytical method 548/0 for the determination of Prohexadione - (Calcium) in tap and surface water by HPLC/MS including storage stability investigations in solvents BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2004/1009167 GLP, unpublished ⇒ RAR reference IIA 4.5/1	N	BASF
CA 4.2	Richter T., Laik W.	2004b	Validation of method No. 569/0 for the determination of BAS 125 W (Prohexadione calcium) residues in soil by HPLC/MS/MS BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2004/1022530 Not-GLP, unpublished ⇒ RAR reference IIA 4.4/1	N	BASF
CA 4.2	Zangmeister W.	2000	Validation of analytical method 459. Determination of BAS 125 W (Reg.No. 285 342) in air by HPLC- UV BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep.	N	BASF

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
			2000/1000126 GLP, unpublished ⇒ RAR reference IIA 4.7/1		
CA 4.2	Zangmeister W.	2005	Validation of the confirmatory method to analytical method 459: Determination of BAS 125 W (Reg.No. 285 342) in air by HPLC - UV BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2005/1004788 GLP, unpublished ⇒ RAR reference IIA 4.7/2	N	BASF

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for the active substance prohexadione-calcium

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

A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

A 2.1.1.1.1 Analytical method RES-00196

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	The study is accepted.
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Reference:	RES-00196
Report	Watson, G (2020). Storage stability of residues of prohexadione-calcium in oilseed rape whole plant, haulm and seed stored frozen for up to 6 months and validation of the methodology used. Unpublished Report No.: RES-00196
Guideline(s):	SANCO/825/00 rev.8.1, SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method referenced for the determination of prohexadione-calcium in oilseed rape whole plant, haulm and seed stored frozen for up to 6 months was validated. The method was based on method 506248.

To 9 g seed and whole plant or 4.5g haulm, prohexadione-calcium is extracted with 0.1% formic acid in 80/20 (v/v) acetonitrile/water (60ml, minus crop moisture content). The samples are extracted by shaking at 150 rpm for 30 minutes. The sample is filtered through cotton wool and 10 mL of the resulting solution is evaporated using a dri-block concentrator at 50°C. The volume is then adjusted to 2 mL with deionized water. Hexane (2 mL) is added and mixed gently. The sample is centrifuged at 1500 rpm for 1 minute before the hexane layer is discarded. The partition with hexane is repeated once more and again, the hexane layer discarded. Residual hexane is removed by placing back on the dri-block concentrator at 40°C. Hydrochloric acid (10N), 20 µL and ethyl acetate, 2 mL are added, and the sample mixed gently. The sample is centrifuged at 1500 rpm for 1 minute before transferring the ethyl acetate to a separate tube. The partition with ethyl acetate is performed twice more, collecting all extracts in the same tube. The ethyl acetate sample is evaporated to dryness at 45°C and re-dissolved in 0.1% formic acid in water (2 mL for seed and whole plant, 1ml for haulm).

Analysis was by LC-MS/MS employing a Fortis H20 column. ESI ionisation mode is used with two MRM transitions for quantification and confirmation.

A comparison of the peak areas obtained from matrix-matched standards against the peak areas obtained from standards prepared in solvent showed matrix effects of detector response in the presence for all matrices (% difference >20%). It was therefore appropriate to use matrix-matched standards for calibration and quantification. Quantification was performed using matrix matched calibration standards over the range 0.00225 to 0.1 µg/mL equivalent to 0.003 to 0.133 mg/kg.

Results and discussions

The analytical method for the determination of prohexadione-calcium in oilseed rape whole plant, haulm

and seed was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. No additional confirmatory method is required.

Table A 1: Recovery results from method validation of prohexadione-calcium using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=x)	Mean recovery (%)	RSD (%)	Comments
Oilseed rape whole plant	Prohexadione- calcium	0.01 (n=5)	84	1.2	Range 83-85% MRM 1 (213→157)
		0.1 (n=5)	87	1.7	Range 84-88% MRM 1 (213→157)
		0.01 (n=5)	80	2.6	Range 77-82% MRM 2 (213→139)
		0.1(n=5)	87	2.4	Range 84-88% MRM 2 (213→139)
oilseed rape haulm		0.01 (n=5)	91	2.3	Range 89-93% MRM 1 (213→157)
		0.1 (n=5)	88	3.8	Range 84-92% MRM 1 (213→157)
		0.01 (n=5)	77	3.0	Range 74-79% MRM 2 (213→139)
		0.1 (n=5)	92	4.0	Range 87-93% MRM 2 (213→139)
oilseed rape seed		0.01 (n=5)	95	4.7	Range 89-100% MRM 1 (213→157)
		0.1 (n=5)	92	4.8	Range 89-96% MRM 1 (213→157)
		0.01 (n=5)	92	3.5	Range 88-95% MRM 2 (213→139)
		0.1 (n=5)	97	5.5	Range 91-103% MRM 2 (213→139)

Table A 2: Characteristics for the analytical method used for validation of prohexadione-calcium residues in oilseed rape whole plant, haulm and seed.

	MRM 1	MRM 2
Specificity	blank value < 30 % LOQ)	blank value < 30 % LOQ)
Calibration (type, number of data points)	individual calibration data presented linear $r \geq 0.9993$ $n = 6$	individual calibration data presented linear $r \geq 0.9993$ $n = 6$
Calibration range	0.00225 to 0.1 µg/mL equivalent to 0.003 to 0.133 mg/kg	0.00225 to 0.1 µg/mL equivalent to 0.003 to 0.133 mg/kg
Assessment of matrix effects is presented	Yes (significant)	Yes (significant)
Limit of determination/quantification	LOQ = 0.01 mg/kg	LOQ = 0.01 mg/kg

Conclusion

The analytical method for the determination of prohexadione-calcium in oilseed rape whole plant, haulm and seed was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. The data meet the acceptance criteria of SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.

A 2.1.1.1.2 Analytical method Report No.: 506248

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	The study is accepted.
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Reference:	Report No.: 506248
Report	Baltussen, E. (2014) Validation of an analytical method for the analysis of residues of Prohexadione Calcium in apples, grapes, sunflower seeds and wheat. Unpublished Report No.: 506248
Guideline(s):	Yes: SANCO/825/00 rev.8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method referenced for the determination of prohexadione-calcium in apples (a high water matrix), grapes (a high acid matrix), sunflower seeds (a high oil matrix) and wheat (a dry matrix) was validated.

Prohexadione-calcium is extracted from 1.5 g of crop matrix. For each 1 g of matrix, 6.67 mL 0.1% formic acid in 80/20 (v/v) acetonitrile/water was added. The samples are extracted by shaking at 250 rpm for 30 minutes. 10 mL of the resulting solution is filtered through an Isolute ENV+ cartridge (Biotage, Uppsala, Sweden). The extracts are diluted in a 1:1 (v:v) ratio with water.

Analysis by LC-MS/MS using Acquity UPL BEH C18 column. ESI ionisation mode is used with two MRM transitions for quantification and confirmation. An MS scan for prohexadione calcium was presented in the report.

A comparison of the peak areas obtained from matrix-matched standards against the peak areas obtained from standards prepared in solvent showed matrix effects of detector response in the presence for all matrices (% difference >20%). It was therefore appropriate to use matrix-matched standards for calibration and quantification. Linear regression with $1/x^2$ weighting was applied.

Results and discussions

The analytical method for the determination of prohexadione-calcium in apples (a high water matrix), grapes (a high acid matrix), sunflower seeds (a high oil matrix) and wheat (a dry matrix) was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. No additional confirmatory method is required and an ILV report is available (refer to KCP 5.2/02).

Table A 3: Recovery results from method validation of prohexadione-calcium using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Apples	Prohexadione-calcium	0.01 (n = 5)	86	3.9	Range 83-91% MRM 1 (213.0→138.9)

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
		0.1 (n = 4)	94	1.8	Range 92-96% MRM 1 (213.0→138.9)
		0.01 (n = 5)	91	7.0	Range 85-100% MRM 2 (213.0→156.9)
		0.1 (n = 4)	93	3.6	Range 90-96% MRM 2 (213.0→156.9)
Grapes	Prohexadione-calcium	0.01 (n = 5)	86	4.0	Range 83-92% MRM 1 (213.0→138.9)
		0.1 (n = 5)	90	4.0	Range 87-94% MRM 1 (213.0→138.9)
		0.01 (n = 5)	84	4.5	Range 80-89% MRM 2 (213.0→156.9)
		0.1 (n = 5)	90	3.6	Range 87-95% MRM 2 (213.0→156.9)
Sunflower seeds	Prohexadione-calcium	0.01 (n = 5)	105	17	Range 78-122% MRM 1 (213.0→138.9)
		0.025 (n = 5)	103	2.7	Range 98-106% MRM 1 (213.0→138.9)
		0.1 (n = 5)	96	3.0	Range 93-101% MRM 1 (213.0→138.9)
		0.01 (n = 5)	87	17	Range 75-110% MRM 2 (213.0→156.9)
		0.025 (n = 5)	96	7.2	Range 85-103% MRM 2 (213.0→156.9)
		0.1 (n = 5)	96	2.3	Range 95-100% MRM 2 (213.0→156.9)
Wheat	Prohexadione-calcium	0.01 (n = 5)	88	6.2	Range 80-93% MRM 1 (213.0→138.9)
		0.1 (n = 5)	81	6.0	Range 77-88% MRM 1 (213.0→138.9)
		0.01	95	10	Range 81-103%

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
		(n = 5)			MRM 2 (213.0→156.9)
		0.1 (n = 5)	80	6.2	Range 77-87% MRM 2 (213.0→156.9)

Table A 4: Characteristics for the analytical method used for validation of prohexadione-calcium residues in apples, grapes, sunflower seeds and wheat

	MRM I	MRM II
Specificity	mass spectrum is provided blank value < 30 % LOQ	mass spectrum is provided blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data presented linear, 1/x ² weighting r ≥ 0.99 n = 6 matrix matched	individual calibration data presented linear, 1/x ² weighting r ≥ 0.99 n = 6 matrix matched
Calibration range	Apples, grapes and wheat Calibration range 0.15 to 19.6 µg/L Equivalent to 0.002 to 0.26 mg/kg in injected samples. Sunflower seeds Calibration range 0.399 to 19.6 µg/L Equivalent to 0.005 to 0.26 mg/kg in injected samples.	Apples, grapes and wheat Calibration range 0.15 to 19.6 µg/L Equivalent to 0.002 to 0.26 mg/kg in injected samples. Sunflower seeds Calibration range 0.399 to 19.6 µg/L Equivalent to 0.005 to 0.26 mg/kg in injected samples.
Assessment of matrix effects is presented	Yes (significant)	Yes (significant)
Limit of quantification	LOQ = 0.01 mg/kg	LOQ = 0.01 mg/kg

Conclusion

The analytical method for the determination of prohexadione-calcium in apples (a high water matrix), grapes (a high acid matrix), sunflower seeds (a high oil matrix) and wheat (a dry matrix) was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. The data meet the acceptance criteria of SANCO/3029/99 rev. 4 and SANCO/825/00 Rev. 8.1.

A 2.1.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.3 Description of analytical methods for the determination of residues in support to environmental fate studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.4 Description of analytical methods for the determination of residues in support to toxicological studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.5 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.6 Description of analytical methods for the determination of residues in ecotoxicology studies (KCP 5.1)

A 2.1.1.6.1 Analytical method 01652

A 2.1.1.6.1.1 Method validation

Comments of zRMS:	The study is accepted.
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Reference:	KCP 5.1.2.6/01
Title:	Analytical method 01652 for the determination of prohexadione-calcium in test water from aquatic toxicity tests by HPLC-MS/MS
Report:	Ruttman, F.; 2021; P 604 207016; M-761406-01-1
Authority registration No:	
Guideline(s):	None
Deviations:	None
GLP/GEP:	no
Acceptability:	
Duplication (if vertebrate study):	

The analytical method 01652 describes the determination of prohexadione-calcium in test water from aquatic toxicity tests by HPLC-MS/MS using two MRM transitions.

The water samples are determined by direct injection into the HPLC-MS/MS instrument using the negative ion mode without further clean-up. Concentrations were quantified using external matrix-matched standard solutions.

Materials and methods

Water samples are analysed without further dilution by method 01652.

All samples are determined using LC-MS/MS employing an Ascentis Express column with two mass transitions.

Quantification of extracts was performed using external calibration standards over the range 0.03 to 10 µg/L. Solvent standards were used.

The lowest level tested (LOQ) was 0.1 µg/L prohexadione-calcium in test water.

Results and discussions

Due to the direct measurement of the test samples, accuracy was not performed. Precision data based on 10 injections of a standard solution was generated. The results of the method validation are summarized in the tables below.

Table A 5: Precision results from method validation of prohexadione-calcium using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n=x)	Mean recovery	RSD (%)	Comments
Test water	Prohexadione-calcium	0.10 (n=10)	NA	4.0	MRM 1 (211→111)
		1.0 (n=10)	NA	1.6	MRM 1 (211→111)
Test water		0.10 (n=10)	NA	3.2	MRM 2 (211→123)
		1.0 (n=10)	NA	1.6	MRM 2 (211→123)

NA – Not applicable. Recovery not determined as the method was direct injection

Table A 6: Characteristics for the analytical method used for validation of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	mass spectrum is provided blank value < 30 % LOQ) No residues detected in control samples.
Calibration (type, number of data points)	r = 0.9998 linear (1/x weighted) n = 6
Calibration range	Calibration range: 0.03 – 10 µg/L
Assessment of matrix effects is presented	Not required, direct analysis
Limit of determination/quantification	LOQ= 0.04 0.1 µg/L

Conclusion

In conclusion, the results summarized in the tables above show that the assay method presented here is satisfactory for determining prohexadione-calcium in test water within the range used in the study.

A 2.1.1.6.2 Analytical method 01652 in support of study [M-764734-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/02
Title:	Acute toxicity of prohexadione-calcium OD 75 (75 g/L) to the waterflea Daphnia magna in a static renewal laboratory test system
Report:	Riebschläger, T.; 2021; EBPW0004; M-764734-01-1
Authority registration No:	
Guideline(s):	OECD Guideline No. 202 (Guideline for Testing of Chemicals, "Daphnia sp., Acute Immobilisation Test (April 13, 2004") OCSPP Guideline 850.1010: Aquatic invertebrate acute toxicity test, freshwater daphnids (October 2016 (modified)
Deviations:	None
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentrations of the prohexadione-calcium in test water from aquatic toxicity tests. The analytical method 01652 describes the determination

of prohexadione-calcium in test water from aquatic toxicity tests by HPLC-MS/MS using two MRM transitions.

Materials and Methods

Water samples were diluted by a factor of 10 or 100 (as appropriate) with test water prior to analysis following method 01652 (which is fully validated, Ruttman, F.; 2021; [M-761406-01-1](#)).

All samples were analysed using LC-MS/MS employing an Ascentis Express column with two mass transitions.

Quantification of extracts was performed using external calibration standards over the range 0.76 to 20 µg/L. Solvent standards were used.

The lowest level tested (LOQ) was 0.76 µg/L prohexadione-calcium in test water.

Results and discussion

As the method was validated under [M-761406-01-1](#), no further accuracy or precision was performed.

Table A 7: Characteristics for the analytical method 01652 used for determination of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	blank value < 30 % LOQ) No residues detected in control samples.
Calibration (type, number of data points)	r = 0.9998 linear (1/x weighted) n = 5
Calibration range	Calibration range: 0.76 – 20 µg/L
Assessment of matrix effects is presented	Not required
Limit of determination/quantification	LOQ= 0.76 µg/L

Conclusion

In conclusion, the results summarized in the tables above show that the assay method presented here is satisfactory for determining prohexadione-calcium in test water within the range used in the study.

A 2.1.1.6.3 Analytical method 01652 in support of study [M-763760-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/03
Title:	Pseudokirchneriella subcapitata growth inhibition test with prohexadione-calcium OD 75 (75 g/L)
Report:	Kuhl, K.; 2021; E 201 05583-4; M-763760-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 201: “Freshwater Alga and Cyanobacteria, Growth Inhibition Test” (July 28, 2011) OCSPP Guideline 850.4500: “Algal Toxicity” (January 2012)
Deviations:	None
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentrations of the prohexadione-calcium in test water from aquatic toxicity tests. The analytical method 01652 describes the determination of prohexadione-calcium in test water from aquatic toxicity tests by HPLC-MS/MS using two MRM

transitions.

Materials and Methods

Water samples were diluted by a factor of 100 or 1000 (as appropriate) with test water prior to analysis following method 01652 (which is fully validated, Ruttman, F.; 2021; [M-761406-01-1](#)).

All samples were analysed using LC-MS/MS employing an Ascentis Express column with two mass transitions.

Quantification of extracts was performed using external calibration standards over the range 0.2 to 20 µg/L. Solvent standards were used.

The lowest level tested (LOQ) was 0.2 µg/L prohexadione-calcium in test water.

Results and discussion

As the method was validated under [M-761406-01-1](#), no further accuracy or precision was performed.

Table A 8: Characteristics for the analytical method 01652 used for determination of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	blank value < 30 % LOQ) No residues detected in control samples.
Calibration (type, number of data points)	r = 0.9997 linear (1/x weighted) n = 5
Calibration range	Calibration range: 0.2 – 20 µg/L
Assessment of matrix effects is presented	Not required
Limit of determination/quantification	LOQ= 0.2 µg/L

Conclusion

In conclusion, the results summarized in the tables above show that the assay method presented here is satisfactory for determining prohexadione-calcium in test water within the range used in the study.

A 2.1.1.6.4 Analytical method 01652 in support of study [M-763594-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/04
Title:	Lemna gibba G3 - Growth inhibition test with prohexadione-calcium OD 75 (75 g/L) under static conditions
Report:	Schrader, D.; 2021; E 221 05564 - 5; M-763594-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 221 (March 23, 2006) US EPA OCSPP 850.4400
Deviations:	None
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentrations of the prohexadione-calcium in test water from aquatic toxicity tests. The analytical method 01652 describes the determination of prohexadione-calcium in test water from aquatic toxicity tests by HPLC-MS/MS using two MRM transitions.

Materials and Methods

Water samples were diluted by a factor of 10, 100 or 1000 (as appropriate) with test water prior to analysis following method 01652 (which is fully validated, Ruttman, F.; 2021; [M-761406-01-1](#)).

All samples were analysed using LC-MS/MS employing an Ascentis Express column with two mass transitions.

Quantification of extracts was performed using external calibration standards over the range 0.5 to 20 µg/L. Solvent standards were used.

The lowest level tested (LOQ) was 0.5 µg/L prohexadione-calcium in test water.

Results and discussion

As the method was validated under [M-761406-01-1](#), no further accuracy or precision was performed.

Table A 9: Characteristics for the analytical method 01652 used for determination of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	blank value < 30 % LOQ) No residues detected in control samples.
Calibration (type, number of data points)	r = 0.9996 linear (1/x weighted) n = 5
Calibration range	Calibration range: 0.5 – 20 µg/L
Assessment of matrix effects is presented	Not required
Limit of determination/quantification	LOQ= 0.5 µg/L

Conclusion

In conclusion, the results summarized in the tables above show that the assay method presented here is satisfactory for determining prohexadione-calcium in test water within the range used in the study.

A 2.1.1.6.5 Analytical Method AM035319MF1 in support of study [M-691184-02-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/05
Title:	Amendment no. 01: Effects on the vegetative vigor of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L)
Report:	Köhler, P.; 2020; VV20/021; M-691184-02-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4150(2012) OECD 227 (2006)
Deviations:	Yes (see report)
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentrations of the prohexadione-calcium in test water from non-target terrestrial plant tests.

Materials and Methods

For the determination of prohexadione-calcium, the analytical method AM035319MF1 was used in the present study which is fully validated (Jasak, J., Garcia Sanchez, M. T., 2021. [M-765354-01-1](#)).

To water samples (10 mL), acetonitrile (15 mL) and 0.5% 1N sulphuric acid in water (70 mL) was added. The sample was ultrasonicated for 15 minutes prior to dilution to 100 mL with acetonitrile. Samples were filtered prior to analysis.

All samples were analysed using HPLC-UV employing an XBridge Phenyl column at 290nm.

Quantification of extracts was performed using an external calibration standard at 2 g/L.

The lowest level tested (LOQ) was 2 g/L prohexadione-calcium in test water.

Results and discussion

As the method was validated under [M-765354-01-1](#), no further precision or calibration was performed.

Table A 10: Characteristics for the analytical method AM035319MF1 used for determination of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	blank value < 30 % LOQ) No residues detected in control samples.
Accuracy	2 g/L mean recovery = 102% RSD = 0.14 n = 2
Limit of determination/quantification	LOQ= 2 µg/L

Conclusion

In conclusion, the results summarized in the tables above show that the assay method presented here is satisfactory for determining prohexadione-calcium in test water within the range used in the study.

A 2.1.1.6.1 Analytical Method AM035319MF1 in support of study [M-691185-01-1](#)

Comments of zRMS: The analytical part of the study is accepted.

Reference:	KCP 5.1.2.6/06
Title:	Effects on the seedling emergence and growth of 10 species of non-target terrestrial plants (Tier 1); prohexadione-calcium OD 75 (75 g/L)
Report:	Köhler, P.; 2020; SE20/022; M-691185-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4100 (2012) OECD 208 (2006)
Deviations:	Yes (see report)
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentrations of the prohexadione-calcium in test water from non-target terrestrial plant tests.

Materials and Methods

For the determination of prohexadione-calcium, the analytical method AM035319MF1 was used in the present study which is fully validated (Jasak, J., Garcia Sanchez, M. T., 2021. [M-765354-01-1](#)).

To water samples (10 mL), acetonitrile (15 mL) and 0.5% 1N sulphuric acid in water (70 mL) was added. The sample was ultrasonicated for 15 minutes prior to dilution to 100 mL with acetonitrile. Samples were filtered prior to analysis.

All samples were analysed using HPLC-UV employing an XBridge Phenyl column at 290nm.

Quantification of extracts was performed using an external calibration standard at 2 g/L.
 The lowest level tested (LOQ) was 2 g/L prohexadione-calcium in test water.

Results and discussion

As the method was validated under [M-765354-01-1](#), no further precision or calibration was performed.

Table A 11: Characteristics for the analytical method AM035319MF1 used for determination of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	blank value < 30 % LOQ) No residues detected in control samples.
Accuracy	2 g/L mean recovery = 102% RSD = 0.14 n = 2
Limit of determination/quantification	LOQ= 2 µg/L

Conclusion

In conclusion, the results summarized in the tables above show that the assay method presented here is satisfactory for determining prohexadione-calcium in test water within the range used in the study.

A 2.1.1.6.2 Analytical Method AM035319MF1 in support of study [M-758398-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/07
Title:	Effects on the vegetative vigor of the non-target terrestrial plant species Cucumis sativus (Tier 2); prohexadione-calcium OD 75 (75 g/L)
Report:	Köhler, P.; 2020; VV20/039; M-758398-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4150(2012) OECD 227 (2006)
Deviations:	Yes (see report)
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentrations of the prohexadione-calcium in test water from non-target terrestrial plant tests.

Materials and Methods

For the determination of prohexadione-calcium, the analytical method AM035319MF1 was used in the present study which is fully validated (Jasak, J., Garcia Sanchez, M. T., 2021. [M-765354-01-1](#)). To water samples (10 mL), acetonitrile (15 mL) and 0.5% 1N sulphuric acid in water (70 mL) was added. The sample was ultrasonicated for 15 minutes prior to dilution to 100 mL with acetonitrile. Samples were filtered prior to analysis.

All samples were analysed using HPLC-UV employing an XBridge Phenyl column at 290nm.

Quantification of extracts was performed using an external calibration standard at 2 g/L.

The lowest level tested (LOQ) was 2 g/L prohexadione-calcium in test water.

Results and discussion

As the method was validated under [M-765354-01-1](#), no further precision or calibration was performed.

Table A 12: Characteristics for the analytical method AM035319MF1 used for determination of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	blank value < 30 % LOQ) No residues detected in control samples.
Accuracy	2 g/L mean recovery = 100% RSD = 0.56 n = 2
Limit of determination/quantification	LOQ= 2 µg/L

Conclusion

In conclusion, the results summarized in the tables above show that the assay method presented here is satisfactory for determining prohexadione-calcium in test water within the range used in the study.

A 2.1.1.6.3 Analytical method for the determination of prohexadione-calcium in larval diet to support [M-762844-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/08
Title:	Prohexadione-calcium OD 75 (75 g/L): Honeybee (Apis mellifera L.) 22 day larval toxicity test (repeated exposure) - Final report -
Report:	Kling, A.; 2021; S20-00850; M-762844-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 (Oct. 2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD Guidance Document 239 (2016)
Deviations:	Yes, but acceptable
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

An analytical method for the determination of prohexadione-calcium in larval diet was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection.

Materials and methods

Larval diet samples (50% royal jelly + 50% aqueous solution containing yeast, glucose and fructose), 2 mL are diluted with acetonitrile:water (1:1 v/v), plus 1% formic acid, 44 mL. The samples are shaken on a horizontal flat bed shaker for 10 minutes. An aliquot (10 mL) is transferred to a centrifuge tube and one QuEChERS-Citrat-Kit-02 is added. The samples are shaken and centrifuged at 4000rpm for 5 minutes. 500 µL of the acetonitrile phase is diluted with water (500 µL). The samples were further diluted as required.

All samples are determined using LC-MS/MS employing an Atlantis T3 column with three mass transitions.

Quantification of extracts was performed using matrix matched external calibration standards over the

range 0.15 to 20 ng/mL, corresponding to 0.003 to 0.400 mg/kg. Solvent standards were used.

The lowest level tested (LOQ) was 0.01 mg/kg prohexadione-calcium in larval diet.

Results and discussions

The analytical method for the determination of prohexadione-calcium in larval diet was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. No additional confirmatory method is required.

Table A 13: Recovery results from method validation of prohexadione-calcium using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=x)	Mean recovery (%)	RSD (%)	Comments
Larval diet	Prohexadione-calcium	0.01 (n=5)	101	11	Range 95-120%
		400 (n=5)	106	2	Range 104-110%

Table A 14: Characteristics for the analytical method used for validation of prohexadione-calcium residues in larval diet.

	prohexadione-calcium
Specificity	blank value < 30 % LOQ)
Calibration (type, number of data points)	individual calibration data presented linear, 1/x-weighting $r \geq 0.999$ $n = 9$
Calibration range	0.15 to 20 ng/mL equivalent to 0.003 to 0.4 mg/kg
Assessment of matrix effects is presented	No, however matrix matched standards used
Limit of determination/quantification	LOQ = 0.01 mg/kg

Conclusion

The analytical method for the determination of prohexadione-calcium in larval diet was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. The data meet the acceptance criteria of SANCO/3029/99 rev. 4.

A 2.1.1.6.4 Analytical method to support study [M-761833-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/09
Title:	Prohexadione-calcium OD 75 (75 g/L): Effects (acute contact and oral) on bumblebees (<i>Bombus terrestris</i> L.) in the laboratory - Final report
Report:	Chwiesko, D.; Kowalczyk, F.; 2021; 152171105; M-761833-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 246 and 247 (2017)
Deviations:	None
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentrations of the active ingredient prohexadione-calcium in the contact application solution and in the highest and lowest concentrated oral feeding solutions.

Materials and methods

Samples of application solution (tap water with 0.1% v/v Triton X-100) or feeding solution (50% w/v sucrose solution) are diluted with acetonitrile/water + 0.1% orthophosphoric acid (50/50 v/v) as necessary.

All samples are determined using HPLC-UV employing a Kinetex C18 column.

Quantification of extracts was performed using external reference calibration standards over the range 2.5 to 30 mg/L prohexadione-calcium. Solvent standards were used.

The lowest level tested (LOQ) in the contact test was 200 g/L test material, equivalent to 6.1 mg/L after dilution (by factor of 2500).

The lowest level tested (LOQ) in the oral test feeding solution was 2 g/L test material, equivalent to 7.6 mg/L prohexadione-calcium after dilution (by factor of 20).

Results and discussions

The analytical method for the determination of prohexadione-calcium in application and feeding solutions was successfully validated with a limit of quantification (LOQ) of 200 g/L in application solution and 2 g/L in feeding solution. No additional confirmatory method is required.

Table A 15: Recovery results from method validation of prohexadione-calcium using the analytical method

Matrix	Analyte	Fortification level (g/L) (n=x)	Mean recovery (%)	RSD (%)	Comments
Application solution	Prohexadione- calcium	200 (n=5)	87	1	Range 86-88%
		800 (n=5)	86	2	Range 85-88%
Feeding solution		2 (n=5)	77	4	Range 72-81%
		50 (n=5)	78	11	Range 67-88%

Table A 16: Characteristics for the analytical method used for validation of prohexadione-calcium residues in application and feeding solutions.

	prohexadione-calcium
Specificity	blank value < 30 % LOQ) confirmed by reference standard and UV spectra
Calibration (type, number of data points)	individual calibration data presented linear $r \geq 0.9998$ $n = 6$
Calibration range	2.5 to 30 mg/L reference material equivalent to 32 to 382 mg/L test material after dilution
Assessment of matrix effects is presented	No, however acceptable recoveries show matrix effects not significant
Limit of determination/quantification	Application solution LOQ = 200 g/L Feeding solution LOQ = 2 g/L

Conclusion

The analytical method for the determination of prohexadione-calcium in application solution and feeding solution was successfully validated with a limit of quantification (LOQ) of 200 g/L in application solution and 2 g/L in feeding solution. The data meet the acceptance criteria of SANCO/3029/99 rev. 4.

A 2.1.1.6.5 Analytical method to support [M-762860-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/10
Title:	Prohexadione-calcium OD 75 (75 g/L): Honeybee (<i>Apis mellifera</i> L.) chronic oral toxicity test 10 day feeding test in the laboratory - Final report -
Report:	Kling, A.; 2021; S20-00682; M-762860-01-1
Authority registration No:	
Guideline(s):	Commission Regulation (EU) No 283/2013 (Mar. 2013) and No 284/2013 (Mar. 2013) in accordance with Regulation (EC) No 1107/2009 (Oct. 2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD Guideline No. 245 (2017)
Deviations:	None
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentration of the test item prohexadione-calcium in the feeding solutions.

Materials and methods

Feeding solution samples (50% aqueous sucrose solution containing 0.1% xanthan and 0.5% Tween), 5 mL are diluted with acetonitrile:water (1:1 v/v), plus 1% formic acid, 25 mL. The samples are shaken on a horizontal flat bed shaker for 10 minutes. An aliquot (10 mL) is transferred to a centrifuge tube and one QuEChERS-Citrat-Kit-02 is added. The samples are shaken and centrifuged at 4000 rpm for 5 minutes. 500 µL of the acetonitrile phase is diluted with water (500 µL). The samples were further diluted as required.

All samples are determined using LC-MS/MS employing an Atlantis T3 column with three mass transitions.

Quantification of extracts was performed using matrix matched external calibration standards over the range 0.5 to 20 ng/mL, corresponding to 0.00210 to 0.0840 mg/kg. Solvent standards were used.

The lowest level tested (LOQ) was 0.01 mg/kg prohexadione-calcium in feeding solution.

Results and discussions

The analytical method for the determination of prohexadione-calcium in feeding solution was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. No additional confirmatory method is required.

Table A 17: Recovery results from method validation of prohexadione-calcium using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n=x)	Mean recovery (%)	RSD (%)	Comments
Feeding solution	Prohexadione Calcium	0.01 (n=5)	107	2	Range 104-110%
		1000 (n=5)	102	5	Range 96-108%

Table A 18: Characteristics for the analytical method used for validation of prohexadione-calcium residues in feeding solution.

	prohexadione-calcium
Specificity	blank value < 30 % LOQ)
Calibration (type, number of data points)	individual calibration data presented linear, 1/x-weighting r = 1.000 n = 8
Calibration range	0.5 to 20 ng/mL equivalent to 0.00210 to 0.0840 mg/kg
Assessment of matrix effects is presented	No, however acceptable recoveries show matrix effects not significant
Limit of determination/quantification	LOQ = 0.01 mg/kg

Conclusion

The analytical method for the determination of prohexadione-calcium in feeding solution was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. The data meet the acceptance criteria of SANCO/3029/99 rev. 4.

A 2.1.1.6.6 Analytical method in support of study [M-764321-01-1](#)

Comments of zRMS:	The analytical part of the study is accepted.
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Reference:	KCP 5.1.2.6/11
Title:	Prohexadione-calcium OD 75 (75 g/L): Toxicity to the aquatic plant <i>Myriophyllum spicatum</i> in a semi-static growth inhibition test with a prior rooting phase
Report:	Armbruster, H.; Emnet, P.; 2021; 152171215; M-764321-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 239: Water-Sediment <i>Myriophyllum spicatum</i> Toxicity Test, September 26, 2014 SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of Directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

The purpose of the analytical part of this study was to verify the concentration of the test item prohexadione-calcium in the test medium.

Materials and methods

Overlying water samples, 10 mL are acidified with formic acid (10 µL). The samples were diluted as appropriate with test water containing 0.1% formic acid. Samples were centrifuged prior to analysis.

All samples are determined using LC-MS/MS employing a Kinetex C18 column with two mass transitions.

Quantification of extracts was performed using external calibration standards over the range 4 to 300 µg/L (reference item), 1st analysis and 2 to 300 µg/L (reference item) 2nd analysis.

The lowest level tested (LOQ) was 11 µg/L prohexadione-calcium in test water.

Results and discussions

Due to the direct measurement of the test samples, accuracy was not performed. Precision data based on 10 injections of a standard solution was generated. The results of the method validation are summarized in the tables below.

Table A 19: Recovery results from method validation of prohexadione-calcium using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n=x)	Mean recovery (%)	RSD (%)	Comments
Test water	Prohexadione-calcium	0.15 (n=5)	84	14	Range 72-99%
		130 (n=5)	85	5	Range 79-90%

Table A 20: Characteristics for the analytical method used for validation of prohexadione-calcium residues in test water

	prohexadione-calcium
Specificity	mass spectrum is provided blank value < 30 % LOQ No residues detected in control samples.
Calibration (type, number of data points)	r = >0.999 linear (1/x weighted) n >5

Calibration range	Calibration range: 4 – 300 µg/L and 2 – 300 µg/L
Assessment of matrix effects is presented	Not required, direct analysis
Limit of determination/quantification	LOQ= 0.15 mg/L

Conclusion

The analytical method for the determination of prohexadione-calcium in test water was successfully validated with a limit of quantification (LOQ) of 0.011 mg/L. The data meet the acceptance criteria of SANCO/3029/99 rev. 4.

A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1)

No specific method developed for this purpose.

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 Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.7 Other Studies/ Information

Additional studies have been submitted for extraction efficiency.

A 2.1.2.7.1.1 Extraction Efficiency

Comments of zRMS:	Extraction efficiency study, as discussed at recent SCPAFF-residue section, should be assessed at Community level. Its absence at the PPP assessment stage should not be a reason for refusing authorization in situation where this study was not assessed at the approval/renewal of an active substance.
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Reference:	RES-00279
Report	Watson, G (2020). Evaluation of extraction efficiency of incurred residues of prohexadione-calcium in oilseed rape whole plant. Unpublished Report No.: RES-00279
Guideline(s):	SANCO/825/00 rev.8.1, SANCO/3029/99 rev.4

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method referenced for the extraction efficiency of prohexadione-calcium in oilseed rape whole plant, was demonstrated.

Method 1

To 9 g whole plant, prohexadione-calcium is extracted with 0.1% formic acid in 80/20 (v/v) acetonitrile/water (60ml, minus crop moisture content). The samples are extracted by shaking at 150 rpm for 30 minutes. The sample is filtered through cotton wool and 10 mL of the resulting solution is evaporated using a dri-block concentrator at 50°C. The volume is then adjusted to 2mL with deionized water. Hexane (2 mL) is added and mixed gently. The sample is centrifuged at 1500rpm for 1 minute before the hexane layer is discarded. The partition with hexane is repeated once more and again, the hexane layer discarded. Residual hexane is removed by placing back on the dri-block concentrator at 40°C. Hydrochloric acid (10N), 20 µL and ethyl acetate, 2 mL are added and the sample mixed gently. The sample is centrifuged at 1500 rpm for 1 minute before transferring the ethyl acetate to a separate tube. The partition with ethyl acetate is performed twice more, collecting all extracts in the same tube. The ethyl acetate sample is evaporated to dryness at 45°C and re-dissolved in 0.1% formic acid in water (2 mL).

Method 2

To 10 g whole plant, prohexadione-calcium is extracted with acetonitrile (10 mL). The samples are extracted by shaking at by hand for 1 minute. QuEChERS extraction salts are added to the tubes and shaken for a for 1 minute. The samples are then centrifuged at 4000 rpm for 2 minutes. The upper layer is transferred to a separate tube and diluted by a factor of 25 with 0.1% formic acid in water.

Method 3

To 9 g whole plant, prohexadione-calcium is extracted with acetone/1.5M sulphuric acid 90/10 (v/v) (60ml, minus crop moisture content) and the samples extracted by maceration. The sample is filtered through cotton wool and 2.5 mL of the resulting solution is evaporated using a dri-block concentrator at 50°C and re-dissolved in 0.1% formic acid in water (10 mL).

Method 4

To 9 g whole plant, prohexadione-calcium is extracted with 0.01M sodium hydroxide (60ml, minus crop moisture content) and the samples extracted by maceration. The sample is filtered through cotton wool and diluted by a factor of 4 with 0.1% formic acid in water (10 mL).

Analysis was by LC-MS/MS employing a Fortis H20 column. ESI ionisation mode is used with two MRM transitions for quantification and confirmation.

Quantification was performed using matrix matched calibration standards over the range 0.005 to 0.2 µg/mL.

Results and discussions

The extraction efficiency of the methods used to extract prohexadione-calcium from oilseed rape whole plant was successfully achieved for 3 out of the 4 methods.

Table A 21: Procedural recovery results from each extraction method for prohexadione-calcium using the analytical method, primary transition only

Method	Fortification level (mg/kg)	n	Recovery (%)	Mean Recovery (%)	Comments
1	0.5	1	108	98	acceptable
	2.0	1	87		
2	0.5	1	74	62	not acceptable
	2.0	1	51		
3	0.5	1	98	91	acceptable
	2.0	1	84		
4	0.5	1	103	94	acceptable
	2.0	1	85		

Table A 22: Characteristics for the analytical method used for extraction efficiency of prohexadione-calcium residues in oilseed rape whole plant

	MRM 1
Specificity	blank value < 30 % LOQ)
Calibration (type, number of data points)	individual calibration data presented linear $r \geq 0.9997$ $n = 6$
Calibration range	0.005 to 0.2 µg/mL
Assessment of matrix effects is presented	No

Conclusion

The extraction efficiency of the methods used to extract prohexadione-calcium from oilseed rape whole plant was successfully achieved for 3 out of the 4 methods. QuEChERS is not appropriate for analysis of prohexadione-calcium in oilseed rape whole plant.