European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

LENACIL

Volume 3 – B.5 (AS)

Rapporteur Member State : Belgium
Co-Rapporteur Member State : Austria

Version History

When	What
November 2007 – July 2009	Draft Assessment Report (DAR) – prepared by RMS BE in the context of the inclusion of the a.s. in Annex I to Council Directive 91/414/EEC. Updated versions of the initial DAR, as well as addenda to the initial DAR, were issued in the period February 2009 – May 2009 (before and after experts' meetings) and were compiled by EFSA in a final 'addendum' dated July 2009.
December 2012 – March 2013	Addenda to DAR Vol.3, B.8 and B.7 (Environmental Fate & Behaviour and Residues), respectively – prepared by RMS BE in the context of the evaluation of confirmatory information requested by Commission Directive 2010/39/EU.
May 2016	Update of DAR Vol.3, B.6 (Toxicology and metabolism) – prepared by RMS BE in the context of the evaluation of confirmatory data on the relevance of ground water metabolites (following classification of lenacil according to Reg. (EC) No 1272/2008).
May 2019	Draft Renewal Assessment Report (DRAR) – prepared by RMS BE in the context of the application for renewal of approval of the a.s. according to Reg. (EU) No 844/2012. *Note: The DRAR is a stand-alone document containing the evaluations already displayed in the initial DAR (incl. its addenda and updated versions), as well as the new assessments. The revision of the initial DAR has been done in accordance with SANCO/10180/2013 rev.1 (March 2013), with changes to the original text – resulting from assessment of new studies (or reconsideration of old studies or studies that were not yet previously peer-reviewed) – being highlighted by means of yellow shading. Changes to the original conclusions have been highlighted in level 2 of Vol.1.*

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS.

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B.5. METHODS OF ANALYSIS

Samples of the active substance as manufactured, analytical standards for the pure active substance, analytical standards for relevant metabolites including any other components included in the residue definition, and samples of reference substances for quantitatively significant impurities (>1 g/kg) are available and can be provided upon request.

Unless specifically indicated, all reports in this section are submitted to address mandatory data requirements for the approval of active substance.

Note: references are provided as follows : i.e. CA 4.1.1(a)/xx is data point according to Reg. (EC) 283/2013 followed by the data point as initially referred by notifier in CADDY for renewal or in the initial monograph given in brackets.

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

B.5.1.1. Methods for the analysis of the active substance as manufactured

B.5.1.1.1. Methods for the analysis of the active substance content

Previous evaluation:	*Initial monograph November 2007*

Study No. 1

Report:	**CA 4.1.1(a)/01 (IIA 1.11-02)** Hansen, S.W. (1998) Technical grade lenacil (DPX-B0634). Analysis and certification of product ingredients E.I. Du Pont De Nemours and Company, Delaware DuPont Report No.: AMR 3747-96
Guidelines:	Not stated.
GLP:	Yes

Description of the method B0634.220.03.ES:
The sample is dissolved in a methanol solution containing the internal standard (N,N-diphenyl-N'-methylurea). Following further dilution with methanol, determination of lenacil is done by reversed-phase HPLC (Zorbax SB-C8, 250mm x 4.6mm, 5µm; 10 µL injected, column temperature of 40°C, mobile phase is 65% HPLC water/35% acetonitrile, flow rate is 1.5 mL/min) with UV detection (at wavelength 254nm, rt lenacil ~ 6.7 min and rt internal standard ~ 7.5 min.).

Findings:

Specificity - interferences:	The HPLC-UV chromatograms showed no obvious interferences that would affect the determination of lenacil or the internal standard. Chromatograms were provided for a calibration standard and a batch sample.
Linearity:	Linear range: 0.59 – 0.99 mg/mL (covering a purity range of 66 – 110%); R^2 = 1.000 (n=3). A calibration curve was constructed using linear regression (least-squares). Calibration solutions were prepared in methanol.
Accuracy:	Not required (however results were presented in the study report by analyzing standard material as surrogate samples at levels above and below the nominal concentration, average was 99.9% with RSD = 0.21%); see linearity and precision.
Repeatability:	The repeatability (precision) of the method was demonstrated by determination of 8 replicate samples of lenacil technical. The relative standard deviation (RSD) was 0.3%.

RMS conclusion 2018 (renewal):
The method was considered suitable for the determination of pure a.s. content in technical lenacil in the initial monograph and was the method used to analyse one of the old 5-BA studies which was considered not relied upon at the time of the first approval. The study is therefore not relied upon.

Study No. 2

Report:	**CA 4.1.1(a)/02 (IIA 1.11-01)** Wittig, (2000) Lenacil technical determination of purity and content in five batches UCL Umwelt Control Labor GmbH, Köln, Germany DuPont Report No.: PR00/015
Guidelines:	Not stated.
GLP:	Yes

Description of the method:
The sample is dissolved in methanol and determination of lenacil is done by reversed-phase HPLC (LiChrospher, 100RP-18, 250mm x 4mm, 5µm; column temperature : room temperature; mobile phase: 0.1% phosphoric acid/acetonitrile: 30%70% with isocratic elution, flow of 1 mL/min, 10 µL injected) with UV detection (at wavelength 270 nm). External calibration was used.

Findings:

Specificity - interferences: The HPLC-UV chromatograms showed no interferences that would affect the determination of lenacil. In addition the report states that specificity is guaranteed through comparison of retention time and comparison of the UV spectrum against the reference material. DAD is considered to be a highly specific technique.
Chromatograms were provided for calibration solutions and a batch sample.

Linearity: Linear range: 0.35 – 0.65 mg/mL (covering a purity range of 70 – 130%); R^2 = 0.9997 (n=3x2 [duplicate injection]). A typical calibration curve with the corresponding regression equation were provided within the study report. Calibration solutions were prepared in methanol.

Accuracy: Not required; see linearity and precision.

Repeatability: The repeatability (precision) of the method was demonstrated by determination of 5 replicate samples of lenacil technical. The relative standard deviation (RSD) was 0.5% (mean = 99%).

RMS conclusion 2018 (renewal):
The method was considered suitable for the determination of pure a.s. content in technical lenacil in the initial monograph and was the method used to check the lenacil content in the old 5 batches.
The method used to analyse the current 5-BA study is in fact a revised version of the method presented here where new validation were generated (see studies No. 3 - 5).

New methods for the determination of lenacil in technical material are available and relied upon in the frame of the renewal dossier. These methods are validated in accordance with SANCO/3030/99. These new methods are summarized here below.

Previous evaluation:	*No, submitted for the purpose of renewal but the study was already considered for the zonal level*

Study No. 3

Report:	**CA 4.1.1(a)/03 (KCA 4.1.1/04)**

	Nagaraj K., (2012) Validation of the analytical method for determination of lenacil (DPX-B0634) in technical grade lenacil and lenacil end-use products Syngene International Limited, Bangalore, India Syngene International Limited study No. G11165 DuPont Report No.: DuPont-34533
Guidelines:	SANCO/3030/99 rev.4
GLP:	Yes

Note: Benzophenone internal standard was purchased commercially and was characterized by the manufacturer but characterization was not conducted under GLP.

Description of the method (DuPont Method No. B0634.220.03.ES.Second Revision):

The technical grade sample was dissolved in methanol. The sample solutions were analysed by reversed-phase liquid chromatography with UV detection. The method used an internal standard (benzophenone). The weight percent of lenacil (DPX-B0634), in each sample, is determined by comparison to a calibration curve (area ratio vs. amount ratio) prepared from the analysis of standard solutions.

Note: validation of the method occurred also for the determination of lenacil in the end-use product Venzar 80 WP but the results are not presented here.

Chromatographic conditions for UPLC:

Column:	Zorbax RRHD-C18, 100×2.1 mm, 1.8 µ particle size
Column temperature:	50°C
Injection volume:	5 µL
Flow rate:	0.8 mL/min
Diluent:	Methanol
Mobile phase composition:	55% (v/v) 0.1% trifluoroacetic acid in water (pH should nominally be 2.0)
	45% (v/v) Methanol
UV Detection:	272 nm
Retention time:	Lenacil: 2.5 min.; benzophenone: 5.0 min.

Chromatographic conditions for HPLC:

Column:	Zorbax Eclipse plus C18, 50×4.6 mm, 1.8µ particle size
Column temperature:	50°C
Injection volume:	5 µL
Flow rate:	1.0 mL/min
Diluent:	Methanol
Mobile phase composition:	55% (v/v) 0.1% trifluoroacetic acid in water (pH should nominally be 2.0)
	45% (v/v) Methanol
UV Detection:	272 nm
Retention time:	Lenacil: 4.0 min.; benzophenone: 8.2 min.

Findings:

Specificity - interferences:

The selectivity of the chromatographic separation was established by comparison of the retention times of a standard of the active substance and the technical material and by demonstrating the purity of the UPLC and HPLC peak(s) of interest. Confirmation of identity also occurred by HPLC/MS (50°C, 272 nm, 5 µL injected, methanol as diluent, mobile phase: 55% (v/v) 0.1% trifluoroacetic acid in water 45% (v/v) methanol, ESI – ion trap – positive) by comparison of the retention times of a standard of the active substance and the technical material and by comparison of spectral data.

Individual solutions of each component were prepared using standard materials and injected in duplicate. Chromatograms were examined to determine if there was complete separation between the active ingredient and the internal standard. The report mentions that no interferences were observed but beside the chromatogram of the blank sample (methanol), chromatograms were only provided for a lenacil formulation 80 WP, not for the technical material. However, in the 6th revision of the method, chromatograms of the reference standard, blank sample and technical material were provided (see study No. 4).

Linearity:

The linearity of the method proposed for determination of the pure active substance as manufactured was demonstrated. The least square fit equations are y = 36.7899x + 0.0020 (for HPLC) and y = 36.9122x + 0.0033 (for UPLC). R^2 for seven different concentrations of lenacil standards (duplicate injection) over the range of 0.1 – 1.2 mg/mL for lenacil (corresponding to 50 to 150% of the assay level) are 0.9996 (HPLC) and 1.0000 (UPLC). The range is from 0.1 to 0.6 mg/mL for the internal standard. Calibration curves for UPLC and HPLC were provided within the report. The concentration of the samples (~ 1 mg/mL) meet the calibration range.

Accuracy:

Not required. However, the accuracy of this method for the analysis of lenacil as manufactured samples was evaluated by analysing standard material as surrogate samples in duplicate at three levels that bracket the analyte target concentration for the analysis of real samples (75 – 125%). The average percent recovery obtained for HPLC was 99.4% with a standard deviation of 0.3% and for UPLC was 99.0% with a standard deviation of 0.4% (see Table below).

Repeatability:

Repeatability testing of the assay method was determined by calculating the standard deviation of the average percent lenacil obtained from the analysis of eight replicate test portions of the same sample of lenacil as manufactured DPX-B0634-108. The relative standard deviation was systematically below the maximum allowable relative standard deviation calculated from the modified Horwitz equation. Therefore, this method fulfils the EU repeatability criteria. There were no outliers during this testing.

Table B.5.1.1-1 : UPLC and HPLC accuracy for lenacil by DuPont Method No. B0634.220.03.ES.Second Revision

Analyte	Concentration levels (% of nominal)	% recovery*	RSD (%)	n**
HPLC				
Lenacil	75%	99.5	-	2
	100%	99.6	-	2
	125%	99.4	-	2
	Overall 75 – 125%	99.4	0.3	6
UPLC				
Lenacil	75%	99.4	-	2
	100%	99.1	-	2
	125%	98.9	-	2
	Overall 75 – 125%	99.0	0.4	6

* Mean recovery at each level re-calculated by RMS based on the results available within the study report.
** Duplicate sample at each level, each injected in duplicate.

Table B.5.1.1-2 : UPLC and HPLC repeatability for lenacil by DuPont Method No. B0634.220.03.ES.Second Revision

Analyte	Mean content (% w/w)	Repeatability – RSD (%)	n
HPLC			
Lenacil	100.1	0.4 (< 1.34*)	8
UPLC			
Lenacil	99.7	0.2 (< 1.34*)	8

* Modified Horwitz Limit (%)

RMS conclusion 2018 (renewal):

The method is validated according to SANCO/3030/99 and suitable for the determination of pure a.s. content in technical lenacil. The method is an earlier version of the method used in support of the current 5-BA analysis.

Study No. 4

Report:	**CA 4.1.1(a)/04 (KCA 4.1.1/05)** Anonymous, (2016) Lenacil (DPX-B0634); Determination of DPX-B0634; Reversed-Phase High Performance Liquid Chromatographic (RPLC) and/or Ultra Performance Liquid Chromatography Assay Method DuPont Report No.: B0634.220.03.ES (6th revision)
Guidelines:	SANCO/3030/99 rev.4
GLP:	No

Note : the 6th revision supersedes the 5th revision of method B0634.220.03.ES. The reason for the revision was to fix a typographical error in section 5.2 with the correct column information and add the final run time for both parameters.

Description of the method (No. B0634.220.03.ES – 6th revision):

The technical grade sample was dissolved in methanol. The sample solutions were analysed by reversed-phase liquid chromatography, using a 1.8 µm particle size, 2.1 mm x 100 mm Zorbax® RRHD C18 (for UPLC) or a 1.8 µm particle size, 4.6 mm x 50 mm Zorbax® Eclipse plus C18 (for HPLC) with UV detection at 272 nm. The internal standard was benzophenone. The weight percent of lenacil (DPX-B0634) in each sample is determined by comparison to a calibration curve (area ratio vs. amount ratio) prepared from the analysis of standard solutions.

The chromatographic conditions are the same as those mentioned under Study No. 3.

Notes:

- the report stated that no column substitution can be made unless the method is revalidated.
- validation of the method occurred also for the determination of lenacil in the end-use products Venzar 80 WP and Venzar 500 SC but only the results for the determination of lenacil in the technical material are presented here.

Findings:

Specificity - interferences:	The selectivity of the chromatographic separation was established by demonstrating the purity of the HPLC peak(s) of interest. Confirmation of identity also occurred by HPLC/MS. Individual solutions of each component were prepared using standard materials and injected in duplicate. Chromatograms were examined to determine if there was complete separation between the active ingredient and the internal standard. No interferences were observed for each separation. HPLC and UPLC chromatograms were provided for a solvent blank solution, a standard solution of lenacil, and a test sample (technical material).

Linearity:	The linearity of the method proposed for determination of lenacil in the test materials using HPLC and UPLC was demonstrated. Correlation coefficients R > 0.999 for 8 different concentrations of lenacil standards over the range of 0.09 to 1.49 mg/mL (corrected for purity) were obtained. Calibration solutions were prepared in methanol. The concentration of the samples (~ 1 mg/mL) meet the calibration range.
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Accuracy:	Not required. Nevertheless, accuracy was determined by measuring lenacil content of representative weights of analytical standard. The weights were selected to bracket the expected results for technical samples. The average percent recoveries obtained were 99.4% and 99.2% for the HPLC and UPLC analyses respectively.
Repeatability:	Repeatability testing of the assay method was determined by calculating the standard deviation of the average percent lenacil obtained from the analysis of eight replicate test portions of lenacil as manufactured. The relative standard deviation was 0.4% for a mean of 100.1% (HPLC) and 0.77% for a mean of 98.26% (UPLC) for lenacil as manufactured. Those values are below the maximum allowable relative standard deviation calculated from the modified Horwitz equation.

RMS conclusion 2018 (renewal):
The method is validated according to SANCO/3030/99 and suitable for the determination of pure a.s. content in technical lenacil. The method is an update of the method used to analyse the current 5-BA data.
The method is also proposed to determine the lenacil content in the formulated product. For details on the validation in the formulation, please refer to Vol.3 CP-B.5.1.1.1 (CP 5.1.1.1/02).

Study No. 5

Report:	**CA 4.1.1(a)/05 (KCA 4.1.1/06)** Matthew C. Repp and J. Gregory McAlpin (2015) Validation of the Analytical Method for Determination of Lenacil (DPX-B0634) in Technical Grade Lenacil and Lenacil End-Use Products E.I. Du Pont de Nemours and Company, Delaware, USA. DuPont Report No.: 44061
Guidelines:	SANCO/3030/99 rev.4
GLP:	Yes

Reference to that report was provided in the 5-BA study by Repp, M.C. (2016) considered for the purpose of the renewal (see Vol.4 C.1.2.3).

Description of the method (No. B0634.220.03.ES):
The technical grade sample was dissolved in methanol. The sample solutions were analysed by reversed-phase liquid chromatography with UV detection. The method used an internal standard (benzophenone). The weight percent of lenacil (DPX-B0634), in each sample, is determined by comparison to a calibration curve (area ratio vs. amount ratio) prepared from the analysis of standard solutions.

The chromatographic conditions are the same as those presented under Study No.3 and only the HPLC was used in the present study.

Findings:

Specificity - interferences:	The selectivity of the chromatographic separation was established by comparison of the retention times of a standard of the active substance and the technical material and by demonstrating the purity of the HPLC peak(s) of interest. Confirmation of identity also occurred by HPLC/MS by comparison of the retention times of a standard of the active substance and the technical material and by comparison of spectral data. Individual solutions of each component were prepared using standard materials and injected in duplicate. Chromatograms were examined to determine if there was complete separation between the active ingredient and the internal standard. The report mentions that no interferences were observed. Chromatograms were provided for a standard solution and a test sample containing the internal standard. A chromatogram of the blank solution was not reported but available in the revision 6 of the method.
Linearity:	The linearity of the method proposed for determination of the pure active substance as manufactured was demonstrated. The correlation coefficient r for eight different concentrations of lenacil standards over the range of 0.09 – 1.5 mg/mL for lenacil (corresponding to ~50 to ~150% of the assay level) was 0.999. A typical calibration curve was not displayed within the study report but the regression equation was provided. The concentration of the samples (~ 1 mg/mL) meet the calibration range.
Accuracy:	Not required. However, the accuracy of this method for the analysis of lenacil as manufactured samples was evaluated by analysing standard material as surrogate samples in duplicate at three levels that bracket the analyte target concentration for the analysis of real samples (75 – 125%). The average percent recovery obtained for HPLC was 99.21% (see Table below).
Repeatability:	Repeatability testing of the assay method was determined by calculating the standard deviation of the average percent lenacil obtained from the analysis of 10 replicate test portions of the same sample of lenacil as manufactured DPX-B0634-136. The relative standard deviation was systematically below the maximum allowable relative standard deviation calculated from the modified Horwitz equation (% RSD = 0.77, mean = 98.26%).

RMS conclusion 2018 (renewal):
The method is validated according to SANCO/3030/99 and suitable for the determination of pure a.s. content in technical lenacil. The method is the method used in the 5-BA study by Repp, M.C. (2016) considered for the purpose of the renewal (see Vol.4 C.1.2.3 – Study No. 2).

Applicability of existing CIPAC methods

A CIPAC analytical method is not available for lenacil.

B.5.1.1.2. Methods for the analysis of significant and/or relevant impurities in the active substance as manufactured

Since the information on the significant impurities present in lenacil technical material is considered as confidential, the analytical methods for the determination of quantitatively significant impurities (>1 g/kg) in lenacil as manufactured (included in the batch analytical report DuPont-44060) can be found in the confidential part of the monograph (Vol. 4). The validation reports DuPont-44062 and DuPont-45930 for the respective impurities can also be found in that confidential part Vol. 4.
According to the notifier, no relevant impurities are present in lenacil technical material. However, RMS identified impurities potentially relevant (please refer to Vol. 4).

B.5.1.1.3. Methods for the analysis of additives (e.g. stabilizers) in the active substance as manufactured

There are no additives considered of toxicological or environmental significance in lenacil as manufactured which would justify submission of analytical methods within this part. Information is available in the Vol.4.

B.5.1.2. Methods for risk assessment

B.5.1.2.1. Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Soil

The proposed environmental monitoring methods summarised in B.5.2.3 were also used to support the environmental fate studies.
The following method was used to analyse samples collected during the adsorption desorption study for the possible metabolite IN-KE121.

Previous evaluation:	*Initial monograph November 2007*

The study was considered for the first approval of the active substance but details and validation data on the analytical method were not reported. Those are now summarized here for the sake of completeness.

Study No. 1

Report:	**CA 4.1.2(a)/01 (IIA 7.1.2-04)** Kane, T. (2004) IN-KE121 Adsorption desorption on soil Huntingdon Life Sciences Ltd, England DuPont Report No.: ACD 063/042264
Guidelines:	Not stated
GLP:	Yes

The analytical method and validation were reported in Appendix 4 of the study report.

Matrices: aqueous supernatant (calcium chloride solution) and soil.

Characteristics of the test soils:

Parameter	**Sheringham**	**Wick 285**	**Elmton**
Textural class	Loamy sand	Loamy sand	clay loam
0.063-2 mm(sand)	83.24	82.52	46.33
2 µm-63 µm (silt)	7.63	8.38	29.26
<2 µm (clay)	9.13	9.1	24.41
pH in 0.01 M $CaCl_2$	6.4	5.6	7.3
Organic carbon (%)	1.0	1.0	3.2
$CaCO_3$ (g/kg)	0.8	1.1	263.1
CEC (meq/100 g)	12.2	9.5	28.7

Description of the method:
Soil and (Calcium Chloride) Soil Extract Analyses
IN-KE121 residues were extracted sequentially from soil samples soaked in 0.01M $CaCl_2$ with acetonitrile (2X) and acetonitrile: water (3:1 v:v) using an ultrasonic bath and shaker followed by centrifugation after each extraction in order to separate the acetonitrile layer from the aqueous layer with the soil pellet. The acetonitrile layer with IN-KE121 residues was removed from each extract and pooled together, evaporated under nitrogen at 40°C until

the aqueous layer remained, and diluted with water: methanol (50:50 v:v). Samples were analysed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). External calibration was used.

Calcium chloride soil extracts were analysed for IN-KE121 directly using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Any further dilutions were performed using 0.01 M calcium chloride solution. External calibration was used.

Chromatographic conditions:
Column: Phenomenex Luna C18 (15 cm × 2 mm)
Mobile phase and gradient program:

	Mobile phase A (%)	Mobile phase B (%)
Time (min)	Water/methanol 90/10 (v/v) + 0.01 M ammonium formate and 0.1% formic acid	Water/methanol 10/90 (v/v) + 0.01 M ammonium formate and 0.1% formic acid
0	100	0
6	0	100
12	0	100
13	100	0

Flow: 0.2 mL/min.

Injection volume: 20 µL
Ion monitored: m/z 249.2 → 153.1
Ionistation mode: positive electrospray
Retention time: ~ 13 min

Note: alternative isocratic HPLC conditions were also used for the analysis of some samples. The conditions were as above except the mobile phase used was 60 % mobile phase A : 40% mobile phase B (pumped isocratically), resulting in a retention time of ~ 5 min. (some variation was observed when using columns of differing ages).

Findings:

Specificity - interferences: There was no apparent response in the region in the chromatograms corresponding to the retention time of IN-KE121 in all control (untreated) soil samples. Chromatograms were provided for calibration standard solutions (with gradient and isocratic HPLC conditions), control soils and supernatant from adsorption/desorption test for the three soils. Chromatograms from fortified samples used for validation were not provided. A product ion spectrum was missing within the study report.

Linearity: Calibration curves showed good linearity in the ranges of 0.2 to 50 ng/mL (n = 11; soil analysis; gradient HPLC conditions; corresponding ~ 1 – 250 ng/g) and 1 to 100 ng/mL (n = 9; soil extract analysis; isocratic HPLC conditions) for IN-KE121, i.e. r^2 = 0.9973 and 0.9986, respectively. Typical calibration curves with the corresponding regression equations were provided within the study report. The samples meet the calibration range (i.e. samples fortified at 5000 ng/mL were diluted by a factor 1000, samples fortified at 250 and 10000 ng/g were diluted by a factor 10 and 50, respectively). All calculations were performed using standards prepared in solvent.
The matrix effect was not assessed. However, the average recoveries for IN-KE121 at different fortification levels were generally in the range of 80% - 110%, demonstrating that matrix effect was not significant when using standards in neat solvents. It must be noted that all control samples showed also no detectable residues of IN-KE121.
There was only one exception for the metabolite IN-KE121 in Wick 285 Soil at the LOQ fortification level of 0.0025 mg/kg which gave an average recovery of 77% (n=5; still within the 70-110% acceptability criterion). Nevertheless, the overall recovery of the three fortification levels (0.0025, 0.25 and 10 mg/kg) for Wick 285 Soil was 86%. These results show that the matrix effects from soil are insignificant and allow the use of calibration standards in solvent.

Accuracy:	The fortification data reported in the method are summarised in Table B.5.1.2.1-1 below. Several procedural recoveries were also performed in the course of the study showing acceptable results.
Repeatability:	Repeatability of the methods are determined from the recovery results and addressed by the data in Table B.5.1.2.1-1. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix, as well as at higher levels. Therefore, the repeatability of this method would seem to be adequate for the purposes of detecting metabolite IN-KE121 residues in soil.
Reproducibility:	The methods used to generate these data are not proposed as environmental monitoring methods. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	The limit of quantification of the method for metabolite IN-KE121 in soil is 2.5 µg/kg and in calcium chloride extract is 0.005 mg/L.

Table B.5.1.2.1-1: Validation data for analytical method for the determination of the potential metabolite IN-KE121 in soil (LC-MS/MS)

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a] (mg/L) [b] [c]	Average recovery (%)	Standard deviation	% Relative standard deviation
Kane, T. (2004), ACD 063/042264	**IN-KE121 in soil (monitored ion transition 249.2→153.1)**					
	Elmton Soil	5 5 5 Total = 15	0.0025 0.25 10	93 105 107 Overall = 102	5.4 5.0 4.1 Overall = 7.8	5.7 4.7 3.9 Overall = 7.6
	Sheringham Soil	5 5 5 Total = 15	0.0025 0.25 10	86 87 94 Overall = 89	4.0 3.2 7.3 Overall = 5.9	4.6 3.7 7.8 Overall = 6.7
	Wick 285 Soil	5 5 5 Total = 15	0.0025 0.25 10	77 88 93 Overall = 86	5.4 3.4 5.2 Overall = 8.0	7.0 3.9 5.6 Overall = 9.4
	IN-KE121 in calcium chloride soil extract (monitored ion transition 249.2→153.1)					
	Elmton Soil	5 5 5 Total = 15	0.005 0.05 5	91 94 98 Overall = 95	5.4 5.6 2.0 Overall = 5.2	5.9 5.9 2.1 Overall = 5.5
	Sheringham Soil	5 5 5 Total = 15	0.005 0.05 5	83 96 87 Overall = 89	2.9 2.6 5.5 Overall = 6.8	3.5 2.7 6.3 Overall = 7.6
	Wick 285 Soil	5 5 5 Total = 15	0.005 0.05 5	88 89 95 Overall = 89	4.1 4.9 1.7 Overall = 4.6	4.7 5.5 1.8 Overall = 5.1

[a] Soil analysis

[b] Soil supernatant analysis

[c] Fortifications were performed with analyte reference standard solutions

Two control per type soil were analysed in the course of the study.

RMS conclusion 2018 (renewal):

The LC-MS/MS appears to be validated according to SANCO/3029/99 and suitable to determine the IN-KE121 residue in different soil types with a validated LOQ of 2.5 µg/kg and in calcium chloride extracts with a validated LOQ of 0.005 mg/L. Specificity/absence of interferences, linearity, accuracy (all mean recoveries were within the 70 – 110% range with sufficient replicates) and repeatability (all < 20% with sufficient replicates) have been successfully demonstrated with the three different soil types.

The following method is new and was used to analyse samples collected during the adsorption desorption study for the possible metabolite IN-KF313.

Previous evaluation:	*No, submitted for the purpose of renewal*

Study No. 2

Report:	**CA 4.1.2(a)/02 (KCA 4.1.2/01 also under CA 7.1.3.1.2/01)** Wright, D., Gilbert, J., Heslop, D. (2011) IN-KF313: Adsorption/desorption study in three soil. Covance Laboratories Ltd., UK DuPont Report No.: 8224952
Guidelines:	OECD guideline 106 (January 2000)
GLP:	Yes

The analytical method and validation were reported in Appendices of the study report.

Matrices: aqueous supernatant (calcium chloride solution) and soil.

Characteristics of the test soils:

Parameter	TL78517229	SK961089	SK920191
Textural class	Loamy sand	Clay loam	clay
pH (water)	8.1	7.8	7.7
Organic carbon (%)	0.7	5.0	4.8
Organic matter (%)	1.2	8.6	8.3
Sand (63 – 2000 µm) (%)	86	38	37
Silt (2 – 63 µm) (%)	6	34	27
Clay (< 2 µm) (%)	8	28	36
CEC (mEq/100g)	6.9	40.9	37.6

Description of the method:
Soil (methods CLE 8224952-01V.S and CLE 8224952-02V.S) and (Calcium Chloride) Soil Extract (method CLE 8224952-01V.A) Analyses:
IN-KF313 residues were extracted sequentially from soil samples (soil TL78517229) by sonicating and shaking with acetonitrile (twice) followed by a further extraction with acetonitrile:water (3:1, v/v). Liquid extracts were separated by centrifugation then combined and analysed for IN-KF313 using UPLC-MS/MS. External calibration was used.

For the determination of IN-KF313 in the soils SK961089 and SK920191 (clay loam and clay) the residues were extracted by sonicating with acetonitrile (twice) followed by acetonitrile: water (3:1, v/v), followed by further extracted with acetonitrile: 0.1 M ammonium acetate (1:1, v/v) using an ultrasonic probe and shaking. Liquid extracts were separated by centrifugation then combined and analysed for IN-KF313 content using UPLC-MS/MS. External calibration was used.

Calcium chloride soil extracts were diluted with water in order to be within the calibration range and analysed for IN-K F313 using liquid chromatography with tandem mass spectrometric detection (UPLC-MS/MS). External calibration was used.

Chromatographic conditions for the 3 CLE procedures:
UPLC Column: Acquity, BEH C18, 1.7 µm, 50 × 2.1 mm
Mobile phase and gradient program:

	Mobile phase A (%)	Mobile phase B (%)
Time (min)	10 mM ammonium formate (aq.):formic acid (100:0.2; v/v)	Acetonitrile

0	90	20
0.7	90	20
3.5	60	95
3.6	2	95
4.1	2	98
4.2	90	20

Flow: 0.5 mL/min

Injection volume: 5 µL
Ion monitored: m/z 249.3 → 167.2
Ionisation mode: Turbo Ion Spray (positive)

Findings:

Specificity - interferences: There was no apparent response in the region in the chromatograms corresponding to the retention time of IN-KF313 in all control (untreated) soil samples.
For the analysis of the supernatant solution (calcium chloride solution) and for each soil type, chromatograms were provided for calibration standard solutions at the lowest and highest levels, control 0.01 M calcium chloride solutions and the fortified samples at the lowest level.
For the analysis of soil extracts and for each soil type, chromatograms were provided for controls and fortified samples at the lowest level (procedures CLE 8224952-01V.S and CLE 8224952-02V.S). Chromatograms for calibration standard solutions were however not provided in this latter case. A product ion spectrum was missing within the study report.

Linearity: Calibration curves showed good linearity in the ranges of 0.25 to 16 ng/mL (n = 10; soil analysis; for both procedures CLE 8224952-01V.S and CLE 8224952-02V.S; this range corresponds to ~ 0.6 – 38.4 µg/g) and 0.2 to 18 ng/mL (n =8; soil extract analysis, procedure CLE 8224952-01V.A) for IN-KF313, i.e. the correlation coefficients were 0.9955, 0.9982 and 0.999, respectively. Typical calibration curves with the corresponding regression equations were provided within the study report. All calculations were performed using standards prepared in solvent. The concentration of the samples meet the calibration ranges.
The matrix effect was not assessed. However, the average recoveries for IN-KF313 at different fortification levels were generally in the range of 80% - 110%, demonstrating that matrix effect was not significant when using standards in neat solvents. It must also be noted that all control samples showed no detectable residues of IN-KF313.
These results show that the matrix effects from soil are insignificant and allow the use of calibration standards in solvent.

Recovery: The fortification data reported in the method are summarised in Table B.5.1.2.1-2 below.

Repeatability: Repeatability of the method is determined from the recovery experiments and addressed by the data in Table B.5.1.2.1-2. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix, as well as at higher levels. Therefore, the repeatability of this

method would seem to be adequate for the purposes of detecting metabolite IN-KF313 residues in soil.

Reproducibility: The methods used to generate these data are not proposed as environmental monitoring methods. Independent laboratory validations are not required for data collection methods.

Limit of quantification: The limit of quantification of the methods for IN-KF313 in soil and in calcium chloride solutions is 2.5 µg/g and 0.005 µg/mL, respectively.

Table B.5.1.2.1-2: Validation data for analytical method for the determination of the potential metabolite IN-KF313 in soil (UPLC-MS/MS)

Reference	Matrix	Number of tests	Fortification level (µg/g) [a] (µg/mL) [b] [c]	Average recovery (%)	Standard deviation	% Relative standard deviation
Wright, D., Gilbert, J., Heslop, D. (2011); 8224952	**IN-KF313 in soil (*m/z* 249.3→167.2)**					
	Soil TL78517229, SK961089 and SK920191 (procedure CLE 8224952-01V.S)	6* 6* 6* Total = 18	2.5 4 8	104.2 103.3 102,5 Overall = 103.3	2.40 2.16 2.35	2.30 2.09 2.29 Overall = 2.21
	Soils SK961089 and SK920191 (procedure CLE 8224952-02V.S)	4** 4** 4** Total = 12	2.5 4 8	100.5 106.0 100.0 Overall = 102.2	4.2 10.03 2.94	4.18 9.46 2.94 Overall = 6.39
	IN-KF313 in calcium chloride solution (*m/z* 249.3→167.2)					
	Calcium chloride solution (0.01 M)	6* 6* 6* Total = 18	0.005 0.1 10	101.7 102.8 103.2 Overall = 102.6	5.47 3.66 3.87	5.38 3.56 3.75 Overall = 4.08

[a] Soil analysis

[b] Calcium chloride solution

[c] Fortifications were performed with analyte reference standard solutions

* the number of tests is 6 considering the 2 replicates at each concentration levels and the three soil types.

** the number of tests is 4 considering the 2 replicates at each concentration levels and the two soil types concerned by procedure CLE 8224952-02V.S.

A control for each soil type was analysed for each validation set of each analytical procedure.

RMS conclusion 2018 (renewal):

The LC-MS/MS appears to be validated according to SANCO/3029/99 and suitable to determine the IN-KE121 residue in the different soil types with a validated LOQ of 2.5 µg/g and in calcium chloride extracts with a validated LOQ of 0.005 mg/L. Specificity/absence of interferences, linearity, accuracy (all mean recoveries were within the

70 – 110% range with sufficient replicates taking into account the results of the three soil types together) and repeatability (all < 20% with sufficient replicates taking into account the results of the three soil types together) have been successfully demonstrated with the three different soil types.

Water

The following method can be used for data generation purposes. The proposed environmental monitoring method for lenacil summarised in section B.5.2.4 below can be used to support the environmental fate studies.

Previous evaluation:	*No, submitted for the purpose of renewal*

Study No. 3

Report:	**CA 4.1.2(a)/03 (KCA 4.1.2.2/01)** Jooß, S., (2015) Development and validation of an LC/MS/MS method for the determination of the lenacil metabolite IN-KF 313 in surface water PTRL Europe Germany DuPont Report No.: P3463G
Guidelines:	SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1
GLP:	Yes

Description of the method:
Surface water was analysed by direct injection (DI-) LC-MS/MS in the positive ionization mode monitoring the parent-daughter ion transitions 249 m/z → 167 m/z for quantification and 247 m/z → 124 m/z for confirmation. External calibration was used.

Chromatographic conditions:
Column: Thermo Aquasil, 3.0 µm particle size, 150 mm × 3.0 mm i.d.; pre-column Phenomenex C18, 4 mm × 3.0 mm i.d.
Injection volume: 10 µL
Column temperature: 35°C
Mobile phase:

Time (min)	Flow rate (mL/min)	Water containing 0.1% formic acid	Acetonitrile containing 0.1% formic acid
0.00	0.4	80	20
1.00	0.4	80	20
4.00	0.4	5	95
8.00	0.4	5	95
8.10	0.4	80	20
10.00	0.4	80	20

Retention time: ~ 4.7 min.
Detection: Applied Biosystems MDS Sciex API 5500 QTrap triple quadrupole, Turbo IonSpray ESI.

Characteristics of the surface water:

pH	8.07
Total hardness	2.18 mmol/L corresponding to 12.2°dH
Conductivity at 25°C	495 µS/cm
Mg	16.7 mg/L
Ca	60.0 mg/L
TOC (total organic carbon)	1.9 mg/L
DOC (dissolved organic carbon)	1.8 mg/L
Silt content	9.4 mg/L

Findings:	
Specificity - interferences:	LC-MS/MS is highly specific by monitoring to mass transitions to ensure unambiguous identification and is therefore self-confirmatory. Both mass transitions were validated. No significant interferences from the specimen matrix or reagent blank (residues below 20 % of the LOQ) were detected at the retention times corresponding IN-KF313 in any of the control specimens. Chromatograms were provided for a standard in solvent (0.02 µg/L), blank control, and water sample fortified at 0.1 µg/L, in each case for both mass transitions. A product ion spectrum was presented in the study report.
Linearity:	Calibration curves for IN-KF313for both mass transitions were linear in the range of 0.02 to 1.2 ng/mL (n = 7), with a correlation coefficient of 0.9995 for both m/z transitions. All calculations were performed using standards prepared in solvent. Typical calibration curves (peak area vs. concentration) for both mass transitions together with the corresponding regression equation were provided within the study report. The concentration of the samples meet the calibration range (concentration of the sample was ~ 0.1 and 1.0 ng/mL according to the method). The matrix effect of surface water on the LC-MS/MS response was tested for both mass transitions and no influence of the matrix was found (≤ 3%, 0.05 ng/mL). Consequently, calibration in solvent (water containing 0.1% of formic acid) was used.
Recovery:	The fortification data reported in the method proposed for the metabolite IN-KF313 residues in water are summarised in Table B.5.1.2.1- Table B.5.1.2.1-3 below.
Repeatability:	Repeatability of the method is addressed by the data in Table B**.5.1.2.1-3**. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit and 10x the quantification limit for each matrix. Therefore, the repeatability of this method would seem to be adequate for the purposes of detecting IN-KF313 residues in water.
Reproducibility:	The method used to generate these data is not proposed as an environmental monitoring method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	0.1 µg/L (0.0001 mg/L) (LOD set to 0.02 µg/L). The EU guidelines for drinking water methods specify that methods must be capable of measuring

levels at or above 0.1 µg/L, which is the maximum allowable level of any crop protection chemical in drinking water.

Table B.5.1.2.1-3 : Validation data for analytical methods for the determination IN-KF313 in water (LC-MS/MS)

Reference	Matrix	Number of tests	Fortification level (µg/L) [a, b]	Average recovery (%) [range]	% Relative standard deviation
Jooß (2015), DuPont-P3463G	IN-KF313 MRM 249 →167 (quantification)				
	Surface Water	5 5 Total = 10	0.1 1.0	107 [102 – 116] 99 [98 – 99] Overall = 103	5 1 Overall = 6
	IN-KF313 MRM 249 → 124 (confirmation)				
	Surface Water	5 5 Total = 10	0.1 1.0	107 [102 – 117] 100 [99 – 101] Overall = 104	6 1 Overall = 6

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Note: One reagent blank and two blank controls were analysed.

Stability of standard solutions and extracts:
Stability of selected standard solutions and extracts was assessed after refrigerated storage for at least 21 days for both mass transitions. Stability of calibration solutions (1 ng/mL) was demonstrated by comparing calibration solutions diluted from an old and from a freshly prepared stock solution. The deviation between the solutions (old and freshly prepared) was less than 6%.
Selected fortified samples (at 0.1 and 1.0 µg/L fortification levels) were re-injected after at least 21 days of refrigerated storage. Recoveries were still within the 70 – 110 % range (minimum recovery obtained was 98%).

RMS conclusion 2018 (Renewal):
The LC-MS/MS method used to determine metabolite IN-KF313 in water samples is fully validated according to SANCO/3029/99 but also according to SANCO/825/00 rev. 8.1 (ILV however not investigated). The method is however not proposed for monitoring purposes since metabolite is not part of the residue definition for monitoring. Specificity/absence of interferences, linearity, accuracy and repeatability have been successfully demonstrated for both mass transitions (method is self-confirmatory). Although some individual recoveries were found to be slightly above 110%, the mean recovery at each fortification level remains within the acceptable range of 70 – 110% with % RSD systematically below 20%. The proposed LOQ of 0.1 µg/L corresponds to the lowest concentration at which acceptable recovery and % RSD were found and is therefore supported.
This method has not been specifically used in any risk assessment studies. This method was developed as a water enforcement method for the major e-fate metabolite, IN-KF313.

Air

The active substance lenacil is non-volatile. The vapour pressure of lenacil is 1.7 x 10^{-9} Pa. Therefore, no environmental fate studies in air were conducted. A monitoring method in air is however available (see B.5.2.5).

B.5.1.2.2. Methods in soil, water and any additional matrices used in support of efficacy studies

No new data on efficacy are submitted. The efficacy trials conducted and already evaluated for the Annex I inclusion did not require the analysis of soil or water samples. Therefore a method is not submitted.

B.5.1.2.3. Methods in products of animal origin and feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Products of animal origin and feed

Previous evaluation:	*Initial monograph November 2007*

The following studies (studies No. 1 to 5) were considered for the first approval of the active substance but details and validation data on the analytical methods were not reported. Those are now summarized here for the sake of completeness.

Study No. 1

Report:	**CA 4.1.2(c)/01 (IIA 5.3.1.1-01)** [redacted] (2002) Lenacil technical: preliminary study by dietary administration to Han Wistar rats for 4 weeks DuPont Report No.: ACD 001/010098
Guidelines:	Not stated.
GLP:	Yes

Study No. 2

Report:	**CA 4.1.2(c)/02 (IIA 5.3.2.2-01)** [redacted] (1991) Subchronic oral toxicity: 90 day study with DPX-B634-91 (Lenacil) feeding study in mice DuPont Report No.: HLR 293-91
Guidelines:	Not stated.
GLP:	Yes

Study No. 3

Report:	**CA 4.1.2(c)/03 (IIA 5.5.2-01)** [redacted] (1994) Oncogenicity study with DPX-B634-91 (Lenacil) eighteen-month feeding study in mice DuPont Report No.: HLR 336-93
Guidelines:	Not stated.
GLP:	Yes

Description of the method:
Lenacil residues were extracted from diet feed samples with methanol. The extracts were filtered and diluted to volume with methanol. Quantitation was performed using reversed phase HPLC with UV detection at 269 nm.

Chromatographic conditions:

HPLC system:	HP 1050 with variable wavelength UV detector and HP ChemStation.
Analytical column:	Hypersil 5ODS (10cm x 4.6mm id) with C18 Security Guard (3.0mm id)
Column temperature:	40°C
Mobile phase:	water (3.1mM H_3PO_4) : acetonitrile (65 : 35 v/v)
Flow rate:	1.0mL/min
Detector wavelength:	269nm
Injection volume:	10µL
Retention time:	approximately 3.6min

Findings:

Specificity:	Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil. There was, however, a peak which eluded just

before the peak of lenacil technical (at 2.5ppm). A chromatogram of the control diet fortified with lenacil technical (at 7.69ppm) gave a response that was clearly distinguishable from baseline and this is considered the limit of detection. Chromatograms of control and fortified samples were provided within the study report.

Linearity: The calibration curves showed good linearity in the range of 5 µg/mL to 25 µg/mL (n=5) for lenacil, i.e. the correlation coefficient r > 0.9999. All calculations were performed using standards prepared in solvent. Two sets of standards were prepared on different days and chromatographed alongside each other. Typical calibration curves (peak area vs. concentration) together with the corresponding regression equation were provided within the study report.

Recovery: Procedural recoveries were prepared by fortifying samples of control diet with known amounts of lenacil technical, added as the solid substance. Recovery results were within guideline requirements (70-120%, RSD ≤20%). The results obtained are summarized in Table 5.1.2.3-1.

Repeatability: Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in the table above. The relative standard deviations of recovery data obtained is within the guideline of ≤20%. This method is adequate for determining lenacil residues in the feed item used in the study.

Reproducibility: The methods used to generate these data are not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: The limit of quantification of the method for lenacil in feed is 100mg/kg.

Table 5.1.2.3-1 : Validation data for analytical methods for the determination of lenacil in feed samples

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a]	Average recovery (%) [range]	Standard deviation	% Relative standard deviation
[redacted] (2002), ACD 001/010098	Feed	6 6 Total = 12	100 20000	103.1 [102.5 – 103.8] 96.2 [94.4 – 97.5] Overall = 99.6	0.50 1.46 Overall = 3.75	0.5 1.5 Overall = 3.7
[redacted] (1991) HLR 293-91 [b]	Feed	2 2 Total = 4	100 10000	97.5 [94 – 101] 95.4 [93.6 – 97.1] Overall = 96.5	- - Overall = 2.6	- - Overall = 2.7
[redacted] (1994) HLR 336-93 [c]	Feed	1 5 2 1 1 1 1 1 Total = 13	48.8 100 6960 6980 7350 7700 7500 7780	104.5 96.6 [88.3 – 107] 98 [93.1 – 102.9] 91.0 99.4 96.0 90.7 103.5 Overall = 97.3	- 8.00 - - - - - - Overall = 6.5	- 8.28 - - - - - - Overall = 6.6

[a] Fortifications were performed with analyte reference standard solutions

b Duplicate injections per test

c Concurrent recoveries; duplicate injections per test

Confirmatory method

The methods used to generate these data are not proposed as an enforcement method. Confirmatory methods are not required for data collection methods.

Overall suitability for data collection

These methods are suitable to generate data used for toxicological risk assessment. The instrumentation required to perform the analysis is available in most well equipped analytical laboratories. No toxic or hazardous reagents are required to prepare the samples, and all of the sample preparation equipment is commercially available.

RMS conclusion 2018 (renewal):
The reversed phase HPLC-UV method used to determine lenacil in diet feed samples is fully validated according to SANCO/3029/99. Specificity/absence of interferences, linearity, accuracy and repeatability have been successfully demonstrated. The mean recovery at each fortification level remains within the acceptable range of 70 – 110% with % RSD systematically below 20%. At 48.8mg/kg only one recovery is available, and no % RSD determination is possible, therefore no full validation can be done at this level and it cannot be accepted as LOQ. An LOQ of 100mg/kg corresponds to the lowest concentration at which acceptable recovery and % RSD were found and is therefore supported.
The method has been used in support of different toxicological studies and is considered "fit for purpose".

Study No. 4

Report:	**CA 4.1.2(c)/04 (IIA 5.6.1.2-01)** [redacted] (2003a) Lenacil technical: Study of effects on reproductive performance in Han Wistar Rats treated continuously through two successive generations by dietary administration DuPont Report No.: ACD 020/023865
Guidelines:	Not stated.
GLP:	Yes

Description of the method:

Chromatographic conditions:

HPLC system:	Agilent 1050 (Quaternary Pump, Autosampler, V W Detector) and Agilent Chemstation
Analytical column:	Hypersil 5ODS (100m x 4.6mm id) with C18 Security Guard (3.0mm id)
Column temperature:	40°C
Mobile phase:	water : acetonitrile (65 : 35 v/v)
Flow rate:	1.0mL/min
Detector wavelength:	269nm
Injection volume:	10µL
Retention time:	approximately 3.6min

Feed Sample Analysis:

Lenacil residues were extracted from diet feed samples with methanol. The extracts were filtered and diluted to volume with methanol. Quantification was performed using reversed phase HPLC with UV detection at 269 nm.

Findings:

Specificity: Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil. It can therefore be concluded that few, if any,

	apparent residues or false positive values would arise. Chromatograms of control and fortified samples were provided within the study report.
Linearity:	The calibration curves showed good linearity in the range of 5 µg/mL to 25 µg/mL (n=5) for lenacil, i.e. the correlation coefficient r = 1.0000. All calculations were performed using standards prepared in solvent. A typical calibration curve (peak area vs. concentration) together with the corresponding regression equation was provided within the study report.
Recovery:	Procedural recoveries were prepared by fortifying samples of control diet with known amounts of lenacil technical, added as the solid substance. Recovery results were within guideline requirements (70-120%, RSD ≤20%). The results obtained are summarized inTable 5.1.2.3-2.
Repeatability:	Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in the table above. The relative standard deviations of recovery data obtained is within the guideline of ≤20%. This method is adequate for determining lenacil residues in the feed item used in the study.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	The limit of quantification of the method for lenacil in feed is 50 mg/kg.

Table 5.1.2.3-2 : Validation data for analytical methods for the determination of lenacil feed samples

Reference	Matrix	Number of tests	Fortification level (mg/kg)	Average recovery (%) [range]	Standard deviation	% Relative standard deviation
[redacted] (2003a) ACD 020/023865	Feed	5 3 Total = 8	50 50000	99.2 [95.5 – 101.1] 94.6 [92.9 – 96.9] Overall = 97.5	2.4 2.1 Overall = 3.2	2.4 2.2 Overall = 3.3

Confirmatory method

The method used to generate this data is not proposed as an enforcement method. Confirmatory methods are not required for data collection methods.

Overall suitability for data collection

This method is suitable to generate data used for toxicological risk assessment. The instrumentation required to perform the analysis is available in most well equipped analytical laboratories. No toxic or hazardous reagents are required to prepare the samples, and all of the sample preparation equipment is commercially available.

RMS conclusion 2018:

The reversed phase HPLC-UV method used to determine lenacil in diet feed samples is fully validated according to SANCO/3029/99. Specificity/absence of interferences, linearity, accuracy and repeatability have been successfully demonstrated. The mean recovery at each fortification level remains within the acceptable range of 70 – 110% with % RSD systematically below 20%. The proposed LOQ of 50mg/kg corresponds to the lowest concentration at which acceptable recovery and % RSD were found and is therefore supported.
The method has been used in support of the reproductive toxicological study in rats (two successive generations) and is considered "fit for purpose".

Study No. 5

Report:	**CA 4.1.2(c)/05 (IIA 5.6.2.1-04)** [redacted] (2003b)

	Lenacil technical: Study of effects on embryo-fetal development in CD rats treated continuously by gavage administration DuPont Report No.: ACD 058/032316
Guidelines:	Not stated.
GLP:	Yes

Description of the method:

Chromatographic conditions:

HPLC system:	Agilent HP 1050 (79852 Quaternary Pump, 798555 Autosampler, V W 79853 Detector) and Agilent HP 1050 Chemstation
Analytical column:	Hypersil 5ODS (100m x 4.6mm id, Hichrom) with C18 Security Guard cardridge system
temperature:	40°C
Mobile phase:	water (pH 2.6) : acetonitrile (65 : 35 v/v)
Flow rate:	1.0mL/min
Detector wavelength:	269nm
Injection volume:	10µL
Retention time/volume:	3.5 – 4 minutes / 3.5 – 4 mL

Feed Sample Analysis:

Sample aliquots were dissolved with the methanol. The extract was diluted, initially using methanol and finally using the mobile phase (acetonitrile/water, 35/65 v/v) filtered and analyzed by reversed phase HPLC with UV detection at 269 nm.

Findings:

Specificity:	Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil. It can therefore be concluded that few, if any, apparent residues or false positive values would arise. Chromatograms of control and fortified samples were provided within the study report.
Linearity:	Calibration curves showed good linearity in the range of 5 µg/mL to 25 µg/mL for lenacil (n=5x2), i.e. the correlation coefficient R >0.99. All calculations were performed using standards prepared in solvent. A typical calibration curve (peak area vs. concentration) together with the corresponding regression equation was provided within the study report.
Recovery:	Recovery results were within guideline requirements (70-120%, RSD ≤20%). The results obtained are summarized in Table 5.1.2.3-3.
Repeatability:	Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in the table above. The relative

	standard deviations of recovery data obtained is within the guideline of ≤20%. This method is adequate for determining lenacil residues in the feed item used in the study.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	The limit of quantification of the method for lenacil in feed is 1 mg/mL.

Table 5.1.2.3-3 : Validation data for analytical methods for the determination of lenacil in 0.5% w/v methylcellulose formulations

Reference	Matrix	Number of tests	Fortification level (mg/mL)	Average recovery (%)	Standard deviation	% Relative standard deviation
[redacted] (2003a) ACD 058/032316	0.5% w/v methylcellulose formulations	6 6 Total = 12	1 100	94.4 97.9 Overall = 96.1	3.3 0.4 Overall = 2.9	3.5 0.4 Overall = 3.0

Confirmatory method

The method used to generate this data is not proposed as an enforcement method. Confirmatory methods are not required for data collection methods.

Overall suitability for data collection

The method is suitable to generate data used for toxicological risk assessment. The instrumentation required to perform the analysis is available in most well equipped analytical laboratories. No toxic or hazardous reagents are required to prepare the samples, and all of the sample preparation equipment is commercially available.

RMS Conclusion 2018 (renewal):

The reversed phase HPLC-UV method used to determine lenacil in methylcellulose formulation samples is fully validated according to SANCO/3029/99. Specificity/absence of interferences, linearity, accuracy and repeatability have been successfully demonstrated. The mean recovery at each fortification level remains within the acceptable range of 70 – 110% with % RSD systematically below 20%. The proposed LOQ of 1mg/mL corresponds to the lowest concentration at which acceptable recovery and % RSD were found and is therefore supported.
The method has been used in support of the on embryo-fetal development in CD rats treated continuously by gavage administration and is considered "fit for purpose".

In total, only 3 main methods have been described to support the toxicological studies. These methods are clearly linked to particular toxicological studies. However, for all the other existing and considered toxicological studies, there was/is no summary of the methods used under section 4 – analytical methods of the summary dossier.
The method used in each toxicological study should be clearly identified with providing details of the method and its available validation data. The methods described here above are maybe also used in other toxicological studies or eventual bridging could be made. In this case, this should be clearly mentioned and identified with a clear cross-reference between the toxicological study and the method used.
Consequently, the notifier has been asked to provide a summary of each method not yet reported here and used in support of toxicological studies or, where appropriate, to provide a rationale that the methods presented here are representative and sufficient to support the other toxicological studies.

The notifier therefore submitted, in the course of the assessment, the following summary tables. RMS added some remarks within the tables.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Analytical methods	DKC
KCA, 5.1.1/01	Pineiro Costas, N	2016	Interspecies comparison of *in vitro* metabolism of [pyrimidine-2-^{14}C]Lenacil in mouse, rat, dog and human hepatocytes WIL Research Europe B.V,'s-Hertogenbosch 512721 GLP: Yes Published: No	N	Y	Analytical methods were applied to prove radiochemical purity, metabolite identification. Provided are linearity plots, mass spectra, chromatograms. No validation data relevant for M-CA Section 4 available.	No requirement
KCA, 5.2.7/01	Westerink, W.M.A.	2016	Evaluation of *in vitro* phototoxicity of lenacil TGAI in 3T3 fibroblasts using the neutral red uptake assay WIL Research Europe B.V. 511052 GLP: Yes Published: No	N	Y	Report contains no analytical data.	No requirement
KCA, 5.8.1/01	Kurubaran , S.	2016	Expert statement: Assessment of the toxicological relevance of the groundwater metabolites of lenacil and proposal with a view to a human health-based risk assessment Dr Knoell Consult Limited DuPont-47276 GLP: No Published: No	N	N	Expert statement, no analytical data.	No requirement
KCA, 5.8.1/01	Tier, G.T.	2014	Position paper title: A non-testing approach to evaluate the relevance of specific groundwater metbaolites of lenacil E.I. du Pont de Nemours and Company DuPont-39162 GLP: No Published: No	N	N	Expert position paper, no analytical data.	No requirement

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Analytical methods	DKC
KCA, 5.9.1	Klotzbach, K	2016	Medical expertise for the lenacil production E.I. du Pont de Nemours and Company 101581-5-9-1-01 GLP: No Published: No	N	Y	Expert statement, no analytical data.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.1.1	[redacted]	1996	Absorption, distribution, metabolism and excretion of [2-^{14}C]-lenacil ([2-^{14}C]-DPX-B634) in the rat [redacted] HLR 62-94 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Study conducted with radioactive labelled lenacil. Standard procedures for an ADME study were applied. No analytical method validation data (recoveries) are provided in the report.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.2.1	[redacted]	2001	Lenacil technical acute oral toxicity to the rat (acute toxic class method) [redacted] ACD 004/013224/AC Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical data provided. Administration by oral gavage. Test was substance prepared at the day of dosing. **RMS remark:** Matrix : 1% w/v aqueous methylcellulose (dose level 5000 mg/kg). Homogeneity, stability and purity of the active substance were not undertaken within the study.	No requirement
CA, 5.2.2	[redacted]	2001	Lenacil technical acute dermal toxicity to the rat [redacted] ACD 005/013220/AC Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical data provided. Administration by spreading equally over prepared skin. Test substance was prepared at the day of dosing. **RMS remark:** Matrix : 1% w/v aqueous methylcellulose. Homogeneity, stability and purity of the active substance were not undertaken within the study.	No requirement
CA, 5.2.3	[redacted]	2001	Lenacil technical acute (four-hour) inhalation study in rats [redacted] ACD 021/013229 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical data provided. Administration by a Wright Dust Feed mechanism. Chamber concentration of lenacil technical was tested, gravimetric analysis of the test aerosol. No analytical method validation data provided. Aerosol target: conc. 5 mg/L.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.2.4	[redacted]	2001	Lenacil technical skin irritation to the rabbit [redacted] ACD 006/013201/SE Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical data provided. Administration by gauze pads to the skin. **RMS remark:** Homogeneity, stability and purity of the active substance were not undertaken within the study.	No requirement
CA, 5.2.5	[redacted]	2001	Lenacil technical eye irritation to the rabbit [redacted] ACD 007/013273/SE Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical data provided. Administration by instillation using a syringe. **RMS remark:** Homogeneity, stability and purity of the active substance were not undertaken within the study.	No requirement
CA, 5.2.6	[redacted]	1992	Closed-patch repeated insult dermal sensitization study (maximization method) with DPX-B634-91 in guinea pigs [redacted] HLO 34-92 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical data provided.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.3.1	[redacted]	2001	Lenacil technical preliminary study by dietary administration to Han Wistar rats for 4 weeks [redacted] ACD 001/010098 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Analytical data already summarised in K-CA Section 4. **RMS remark:** Matrix: diet sample. Please refer to the present volume – section B.5.1.2.3 – Study No. 1 where the method has been considered validated and "fit for purpose".	No requirement
CA, 5.3.1	[redacted]	2001	Lenacil technical preliminary study by dietary administration to beagle dogs for 4 weeks [redacted] ACD 003/013230 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical data provided. This is a preliminary study. Page 14: It is stated that results of homogeneity and stability analyses will be included in the 13 week report (study number ACD/002).	No requirement

CA, 5.3.2	[redacted]	2002	Lenacil technical toxicity study by dietary administration to Han Wistar rats for 13 weeks followed by a 4 week recovery period [redacted] ACD 002/013903 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Reference to analytical procedure is provided: • AMR 3747-96 Rev No. 1 • HLR 293-91 Validation data already summarised (see HLR 293-91). **RMS remark:** Matrix: diet sample. Reference is indeed done to analytical method validated in ACD 001/010098 and HLR 293-91. Please refer to the present volume – section B.5.1.2.3 – Studies No. 1 (ACD 001/010098) and No. 2 (HLR 293-91), where the method was validated in the 100 – 20000 ppm and 100 -10000 ppm, respectively. The method was however modified to comply with the current laboratory standard procedures and instrumentation. The homogeneity (mean = 102%, CV = 1.05%, at 50 ppm and mean = 90%, CV = 1.43 % at 50000 ppm, each with n = 6 taking into account duplicate sample at bottom, middle and top) and stability (recoveries between 98 and 108%) were assessed within the study at 50 and 50000 ppm. Procedural recoveries were also performed in the course of the study at 50, 500, 5000 and 50000 ppm with recoveries between 95.1 and 107.2%. The method was found to be linear in the 5 -25 µg/mL range (n = 5 + origin point), with a r = 0.9999 (typical calibration curve and regression equation provided). Chromatograms were provided for control and test samples at the different concentrations.	No requirement
CA, 5.3.2	[redacted]	2004	Lenacil technical additional histopathological investigations to a toxicity study by dietary administration to han wistar rats for 13 weeks	Yes	No analytical data provided. Additional histopathological investigations to study ACD 002/013903.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
			followed by a 4-week recovery period [redacted] ACD 055/024499 Study previously reviewed for EU approval in the 2007 DAR Published: No		**RMS remark:** Matrix : diet sample. Please refer to the line here above.	
CA, 5.3.2	[redacted]	1991	Subchronic oral toxicity: 90-day study with DPX-B634-91 (lenacil) feeding study in mice [redacted] HLR 293-91 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Analytical part already summarised in K-CA Section 4. **RMS remark:** Matrix: diet sample. Please refer to the present volume – section B.5.1.2.3 Study No. 2 : the method has been considered validated and "fit for purpose".	No requirement

CA, 5.3.2	[redacted]	2002	Lenacil technical toxicity study by dietary administration to beagle dogs for 13 weeks [redacted] ACD 022/014297 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Reference to analytical procedure is provided: • ACD/001 Validation data already summarised. Acceptable procedural recovery available in report. **RMS remark:** Matrix: diet sample (1000 – 25000 ppm). Reference is indeed done to analytical method validated in ACD 001/010098. Please refer to the present volume – section B.5.1.2.3 – Study No. 1 (ACD 001/010098), where the method was validated in the 100 – 20000 ppm. Reference was also made to AMR 3747-96 revision No. 1 and HLR 293-91 (B.5.1.2.3 – Study No. 2). The method was however modified to comply with the current laboratory standard procedures and instrumentation. The homogeneity (mean = 102%, CV = 1.06%, at 50 ppm and mean = 97%, CV = 0.73 % at 50000 ppm, each with n = 6 taking into account duplicate sample at bottom, middle and top) and stability (recoveries between 99 and 100%) were assessed within the study ACD 022/014297 at 50 and 50000 ppm. Procedural recoveries were also performed in the course of the study at 50, 1000, 5000 and 25000 ppm with recoveries between 94.5 and 104.7%. The method was found to be linear in the 10 -20 µg/mL range (n = 5 + origin point), with a r = 0.9999 (typical calibration curve and regression equation provided). Chromatograms were provided for control and test samples at the different concentrations. Additionally, further validation to confirm linearity, specificity and accuracy and precision was conducted within the study and concurrent with	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
					similar validation of the method conducted in ACD 019. The linearity was demonstrated in the 5 – 25 µg/mL range (n = 5 + origin point) with r = 0.9999 (regression equation provided). The results of recovery and precision are presented here below:	

Nominal fortification (ppm)	Recovery (%)	Accuracy and precision data			
				Overall	
		Mean	CV	Mean	CV
100	99.8	101.4	1.09		
100	101.0				
100	102.1				
100	101.2				
100	103.1				
100	101.3			97.8	3.99
50,000	93.1	94.2	1.10		
50,000	93.9				
50,000	94.8				
50,000	93.7				
50,000	93.7				
50,000	96.0				

CV Coefficient of variation

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.4.1	May, K.	2001	Lenacil technical bacterial mutation assay Huntingdon Life Sciences Ltd ACD 016/013217 Study previously reviewed for EU approval in the 2007 DAR Published: No	No	No analytical validation data provided. *In vitro* study. **RMS remark:** Matrix: DMSO. The stability of the test substance and the stability and homogeneity of the test substance in the vehicle were not determined as part of the study. Analysis of achieved concentration was not performed as part of the study.	No requirement
CA, 5.4.1	Russel, J.F.	1977	Mutagenic activity of uracil, 3-cyclohexyl-5,6,-trimethylene in the *Salmonella*/microsome assay DuPont Haskell Laboratory HLR 601-77 Study previously reviewed for EU approval in the 2007 DAR Published: No	No	No analytical validation data provided. *In vitro* study.	No requirement
CA, 5.4.1	D'Amico, S.W.	1994	Mutagenicity testing of DPX-B634-107 (lenacil) in the *Salmonella typhimurium* plate incorporation assay DuPont Haskell Laboratory HLR 413-94 Study previously reviewed for EU approval in the 2007 DAR Published: No	No	No analytical validation data provided. *In vitro* study.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.4.1	Grancharov, K., Gorneva, G., Mladenova, J., Norpoth, K., Golovinsky, E.	1986	Lack of genotoxic and cytotoxic effects of the herbicide lenacil on mouse tumor cells and on some Salmonella typhimurium strains Arzneimittelforschung 369110, 1660-1663 Study previously reviewed for EU approval in the 2007 DAR Published: Yes	N	No analytical validation data provided. *In vitro* study.	No requirement
CA, 5.4.1	Reynolds, V.L.	1989	Mutagenicity testing of IN E1512-2 in the Salmonella typhimurium plate incorporation assay DuPont Haskell Laboratory HLR 550-89 Study previously reviewed for EU approval in the 2007 DAR Published: No	No	No analytical validation data provided. In vitro study.	No requirement
CA, 5.4.1	Allais, L.	2001	Lenacil technical in vitro mammalian chromosome aberration test in human lymphocytes Huntingdon Life Sciences Ltd ACD 017/013707 Study previously reviewed for EU approval in the 2007 DAR Published: No	No	No analytical validation data provided. In vitro study. **RMS remark:** The stability of the test substance and the stability and homogeneity of the test substance in the vehicle were not determined as part of the study. Analysis of achieved concentration was not performed as part of the study.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.4.1	Clare, G.	2003	Lenacil technical in vitro mammalian cell gene mutation test Huntingdon Life Sciences Ltd ACD 053/023530 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical validation data provided. In vitro study. **RMS remark:** The stability of the test substance and the stability and homogeneity of the test substance in the vehicle were not determined as part of the study. Analysis of achieved concentration was not performed as part of the study.	No requirement
CA, 5.4.1	Riach, C.G.,Mohammed, R.	1990	Lenacil: assessment of genotoxicity in an unscheduled DNA synthesis assay using adult rat hepatocyte primary cultures Inveresk Research International IRI 6135 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical validation data provided. In vitro study. Matrix : DMSO	No requirement
CA, 5.4.2	[redacted]	2001	Lenacil technical mouse micronucleus test [redacted] ACD 018/013472 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical validation data provided. In vitro study. **RMS remark:** Matrix : 0.5% w/v aqueous methylcellulose (25 – 100 mg/mL). Stability and homogeneity of the test substance and of the test substance in the vehicle as well as the chemical analysis of dosing formulations for achieved concentrations were not performed within the study.	No requirement

CA, 5.5	[redacted]	2002	Lenacil technical combined chronic toxicity and carcinogenicity study by dietary administration to han wistar rats over 104 weeks interim report: 0-52 weeks [redacted] ACD 045/024288 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Reference to analytical procedure is provided: • ACD/001 • ACD/002 Validation data already summarised. Acceptable procedural recovery data available in report. **RMS remark:** Matrix: diet sample (250 – 25000 ppm). The samples were analysed in accordance with the analytical procedure ERC/FCH/M15/01. The analytical procedure was validated as part of two earlier studies ACD 001 (ACD 001/010098, levels: 100 and 20000 ppm) and ACD 002 (ACD 002/013903, level: 50 ppm) for which the analytical part is already summarised in the present volume – section B.5.1.2.3 – Studies No. 1 and 2 (HLR 293-91). The method has been considered validated and "fit for purpose" (see above). Reference was also made to AMR 3747-96 Revision No. 1 and HLR 293-91. Homogeneity and stability were demonstrated at 50 and 50000 ppm in study ACD 002. Procedural recoveries were also performed in study ACD 045/024288 at 250, 2500 and 25000 ppm with obtained recoveries between 98.1 and 103.0%. Linearity of the method was shown in the 5 – 25 µg/mL range (n = 5 + origin point) with r = 0.9999 (typical calibration line and regression equation were provided) and chromatograms of a standard solution, a control and test samples and procedural recoveries were provided.	No requirement
CA, 5.5	[redacted]	2004	Lenacil technical combined chronic toxicity and carcinogenicity study by dietary administration to han wistar rats over 104 weeks	Yes	Reference to analytical procedure is provided: • ERC/FCH/M15/01 issue 01230401 • AMR 3747-96 Rev No. 1 • HLR 293-91	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
			[redacted] ACD 045/042214 Study previously reviewed for EU approval in the 2007 DAR Published: No		Validation data already summarised. Acceptable procedural recovery data available in report. **RMS remark:** Matrix: diet feed sample (250 – 25000 ppm). The samples were analysed in accordance with the analytical procedure ERC/FCH/M15/01. The analytical procedure was validated as part of two earlier studies ACD 001 (ACD 001/010098, levels: 100 and 20000 ppm) and ACD 002 (ACD 002/013903, level: 50 ppm) for which the analytical part is already summarised in the present volume – section B.5.1.2.3 – Studies No. 1 and 2 HLR 293-91). The method has been considered validated and "fit for purpose". Homogeneity and stability were demonstrated at 50 and 50000 ppm in study ACD 002. Procedural recoveries were also performed in study ACD 045/042214 at 250, 2500 and 25000 ppm with obtained recoveries between 95.3 and 103.5%. Linearity of the method was shown in the 5 – 25 µg/mL (n = 5 + origin point) with r = 0.9999 (typical calibration line and regression equation were provided) range and chromatograms of a standard solution, a control and test samples and procedural recoveries were provided.	

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.5	[redacted]	1994	Oncogenicity study with DPX-B634-91 (lenacil) eighteen-month feeding study in mice [redacted] HLR 336-93 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Analytical part already summarised in K-CA Section 4. **RMS remark:** Please refer to the present Volume – section B.5.1.2.3 Study No. 3 : the method is considered validated and "fit for purpose".	No requirement

<table>
<tr>
<td>CA, 5.6.1</td>
<td>[redacted]</td>
<td>2002</td>
<td>Lenacil technical preliminary study of effects on reproductive performance in Han Wistar rats by dietary administration
[redacted]
ACD 019/010186
Study previously reviewed for EU approval in the 2007 DAR
Published: No</td>
<td>Yes</td>
<td>Preliminary report. Reference to analytical procedure is provided:
• ACD/001
• AMR 3747-96 Rev No. 1
• HLR 293-91

Validation data are available, see Appendix 1D on page 82.

Validation procedural recovery data for Lenacil technical in VRF-1 diet form
<table>
<tr><th rowspan="2">Nominal fortification (ppm)</th><th rowspan="2">Recovery (%)</th><th colspan="4">Accuracy and precision data</th></tr>
<tr><th></th><th></th><th>Overall</th><th></th></tr>
<tr><th></th><th></th><th>Mean</th><th>CV</th><th>Mean</th><th>CV</th></tr>
<tr><td>10,000</td><td>100.0</td><td>99.0</td><td>1.09</td><td></td><td></td></tr>
<tr><td>10,000</td><td>98.0</td><td></td><td></td><td></td><td></td></tr>
<tr><td>10,000</td><td>98.9</td><td></td><td></td><td></td><td></td></tr>
<tr><td>10,000</td><td>98.3</td><td></td><td></td><td></td><td></td></tr>
<tr><td>10,000</td><td>98.3</td><td></td><td></td><td></td><td></td></tr>
<tr><td>10,000</td><td>100.7</td><td></td><td></td><td>96.9</td><td>2.52</td></tr>
<tr><td>50,000</td><td>94.1</td><td>94.9</td><td>1.26</td><td></td><td></td></tr>
<tr><td>50,000</td><td>95.9</td><td></td><td></td><td></td><td></td></tr>
<tr><td>50,000</td><td>94.8</td><td></td><td></td><td></td><td></td></tr>
<tr><td>50,000</td><td>96.5</td><td></td><td></td><td></td><td></td></tr>
<tr><td>50,000</td><td>94.6</td><td></td><td></td><td></td><td></td></tr>
<tr><td>50,000</td><td>93.2</td><td></td><td></td><td></td><td></td></tr>
</table>

Acceptable procedural recovery data available in report.

RMS remark:
Matrix: diet sample (10000 – 50000 ppm).

The samples were analysed in accordance with the analytical procedure ERC/FCH/M15/01. The analytical procedure was validated as part of two earlier studies ACD 001 (ACD 001/010098, levels: 100 and 20000 ppm) and ACD 002 (ACD 002/013903, level: 50 ppm) for which the analytical</td>
<td>Data to be summarised.</td>
</tr>
</table>

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
					part is already summarised in the present volume – section B.5.1.2.3 – Studies No. 1 and 2 (HLR 293-91). The method is considered validated and "fit for purpose". Homogeneity and stability were also demonstrated in study ACD 020 (at 50 and 50000 ppm). The method was modified to comply with the laboratory standard procedures and instrumentation. Procedural recoveries were also performed in study ACD 019/010186 at 10000, 25000 and 50000 ppm with obtained recoveries between 91.8 and 99.8%. Linearity of the method was shown in the 5 – 25 µg/mL (n = 5 + origin point) with r = 0.9999 (typical calibration line and regression equation were provided) range and chromatograms of a control and test samples were provided. Further validation to confirm the specificity, linearity, and method accuracy and precision were also performed at 10000 and 50000 ppm within study ACD 019/010186. For accuracy and precision results, please refer to the table provided by the notifier.	

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.6.1	[redacted]	2003	Lenacil technical study of effects on reproductive performance in han wistar rats treated continuously through two successive generations by dietary administration [redacted] ACD 020/023865 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Analytical part already summarised in K-CA Section 4. **RMS remark:** Please refer to the present Volume – section B.5.1.2.3 Study No. 4 : the method is considered validated and "fit for purpose".	No requirement
CA, 5.6.2	[redacted]	1978	Embryotoxic and teratogenic study in rats with lenacil (INB-634) [redacted] HLR 405-78 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	**RMS remark:** Matrix: diet sample (500 – 5000 ppm). No analytical data available, no reference provided to analytical methods.	
CA, 5.6.2	[redacted]	1996	DPX-B634-91 (lenacil): Pilot developmental toxicity study in rats [redacted] HLR 996-96 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	**RMS remark :** Matrix : 0.5 % w/v aqueous methylcellulose (500 - 4000 mg/kg, 50 – 400 mg/mL). The study protocol mentioned that stability, homogeneity and dose verification will occur but no analytical data was available within th study report, and no reference was provided to analytical methods.	

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.6.2	[redacted]	2003	Lenacil technical preliminary study of effects on embryo-fetal development in CD rats treated by oral gavage administration [redacted] ACD 057/030001 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	**RMS remark:** Matrix : 0.5 % w/v aqueous methylcellulose. The samples were analysed in accordance to the analytical procedure ERC/FCH/M39/02. The analytical procedure was validated as part of another study ACD 058 (ACD 058/032316) for which the analytical part is already summarised in the present volume – section B.5.1.2.3 – Study No. 5. The method has been considered validated and "fit for purpose". Procedural recoveries were also performed in study ACD 057/030001 at 10, 30 and 100 mg/mL with obtained recoveries between 96.4 and 103.1%.	
CA, 5.6.2	[redacted]	2003	Lenacil technical study on effects on embryo-fetal development in CD rats treated by oral gavage administration [redacted] ACD 058/032316 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Analytical part already summarised in K-CA Section 4. **RMS remark:** Matrix : 0.5 % w/v aqueous methylcellulose. Please refer to the present volume – section B.5.1.2.3 – Study No. 5. The method is considered validated and "fit for purpose".	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.6.2	[redacted]	1991	Teratogenicity study of DPX-B634-91 in rabbits [redacted] HLR 626-91 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	Samples of dosing solutions were analysed using HPLC-UVD at 269 nm. Hypersil ODS column: ACN/water pH 2.6 : 35/65, 1 ml/min, 40°C, 5 µL injected). Acceptable measured concentrations were obtained, with acceptable mean recoveries and RSDs (RMS: Although based on the results the RSDs would be acceptable, their values were not displayed in the report). Matrix : 0.5 % w/v aqueous methylcellulose (5, 20 , 100 and 400 mg/mL corresponding to daily dose levels of 50, 200, 1000 and 4000 mg/kg).	No requirement
CA 5.8.1	[redacted]	1989	Approximate lethal dose (ALD) of IN E1512-2 in rats, HLR564-89 [redacted] HLR 564-89 Study previously reviewed for EU approval in the 2007 DAR Published: No	No	No analytical data available, no reference provided to analytical methods.	No requirement

Data Requirement No	Author(s)	Year	Title Source Company Report No. Indication of the reason not submitted Published or not	Vertebrate study Y/N	Analytical methods	DKC
CA, 5.8.2	[redacted]	2004	Lenacil technical Investigation into potential effects on thyroid function after 20 weeks of treatment in female han Wistar rats using the "perchlorate discharge test" [redacted] ACD 060/033946 Study previously reviewed for EU approval in the 2007 DAR Published: No	Yes	No analytical validation data provided. **RMS remark:** Matrix: diet sample (250 and 50000 ppm). Homogeneity and stability of the test substance in the diet sample were performed as part of another study ACD 002/013903 (see above). Only the mean concentrations of lenacil in test formulations were analysed during the study and were within +/- 4 % of the nominal concentration. The samples were analysed in accordance with the analytical procedure ERC/FCH/M15/01. The analytical procedure was validated as part of another study ACD 001 (ACD 001/010098) for which the analytical part is already summarised in the present volume – section B.5.1.2.3 – Study No. 1. Some procedural recoveries were also reported at 250 and 50000 ppm with recoveries between 98 and 104.7 %.	No requirement
CA 5.9.1	Klotzbach, K.	2003	Medical expertise for the Lenacil production. Unpublished letter report, B·A·D Gesundheitsvorsorge und Sicherheitstechnick GmbH, not detailed. Not GLP, Published: No	No		

Body fluids and tissues

Body fluids and tissues were not analysed in cause of the studies. Therefore, no methods are provided here.

In the area of identity, physical/chemical/technical properties and methods of analysis, data gaps were identified for monitoring analytical methods for plants and body fluids and tissues.

Air

The active substance lenacil is non-volatile. The vapour pressure of lenacil is 1.7 x 10^{-9} Pa (see Vol.3 CA-B.5). Therefore, no further toxicological studies in air were conducted.

B.5.1.2.4. Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No additional analytical methods were developed to collect operator, worker, resident or bystander exposure data.

B.5.1.2.5. Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residue trials

B.5.1.2.5.1. Plant matrices

Previous evaluation:	*Initial monograph November 2007*

The studies No. 1 to 5 were already considered for the first approval of lenacil and are yet summarised here for the sake of completeness.

Study No. 1

Report:	**CA 4.1.2(e)/01 (IIA 4.2.1.1-01 and IIA 6.3-02b)** Mende, P. (2002) Analytical Final Report – Generation of Samples for the determination of Residues of Venzar 80 % WP (containing 80 % Lenacil) in Sugar Beets. Five Sites in Europe, 2001 GAB Biotechnology GmbH, Germany DuPont Report No.: 20011048/E1-FPSB
Guidelines:	SANCO/3029/99 rev.4
GLP:	Reported as GLP in initial monograph but no GLP certificate or GLP compliance statement

Study No. 2

Report:	**CA 4.1.2(e)/02 (IIA 4.2.1.1-02)** Turnbull G. (2003) Validation of Analytical Methodology for the Determination of Lenacil in Sugar Beet Pesticides and Veterinary Medicines Team D, Central Science Laboratory (CSL), UK DuPont Report No.: PDG-107
Guidelines:	SANCO/825/00 rev. 6
GLP:	Yes (GLP compliance statement)

Study No. 3

Report:	**CA 4.1.2(e)/03 (IIA 6.3-03b)**

	Hamberger, R, (2002) Analytical Report – Generation of samples for the determination of residues of Venzar (80% WP (containing 08% lenacil) in sugar beets, one site in Europe, 2002. GAB Biotechnology GmbH, Germany DuPont Report No.: 20011048/E2-FPSB
Guidelines:	SANCO/3029/99 rev.4
GLP:	Yes

Description of the method (modified DFG S19 – LC-MS/MS):
20011048/E1-FPSB (and ILV PDG-107) and 20011048/E2-FPSB follow a modified multi-residue method DFG S19 (specht et al. 1995).
Lenacil was extracted from the sample material with acetone (after adjustment of water content in sample, so that the acetone/water ratio was 2:1 in v/v), and the aqueous phase was saturated with sodium chloride. Subsequently, liquid-liquid partitioning into an ethylacetate/cyclohexane mixture (1/1) was performed, the extract of which was then concentrated and cleaned-up on a GPC column (Bio-Beads S-X3). Extracts were analysed by HPLC-MS/MS. Quantitation of extracts was performed by monitoring the MS/MS transition of 233 amu to 151 amu and using peak areas of external calibration standards.

ILV has been conducted for sugar beet (roots and leafs), using essentially the same analytical procedure. A second MS/MS transition (233 amu to 107 amu) was also monitored for additional confirmation (ILV).

Chromatographic conditions (studies 20011048/E1-FPSB and 20011048/E2-FPSB):

Column:	ThermoHypersil HyPurity C8 column, 150mm x 3mm i.d., 5µm mean particle size (No. 22205-037), 1 cm guard column.

Mobile phase:

Time (min)	Flow (mL/min)	% MeOH/water (20/80, v/v)	ACN/water (50/50, v/v)	ACN	Gradient
0.00	0.5	100	0	0	-
5.00	0.5	0	100	0	linear
10.00	0.5	0	50	50	linear
10.01	0.5	0	0	100	-
12.00	0.5	0	0	100	
13.00	0.5	100	0	0	linear

Temperature: 40°C
Injection volume: 30 µL
Retention time: 7.3 min (7.5 min in study 20011048/E2-FPSB)
Detection: ESI negative
Parent ion m/z = 233
Fragment ion m/z = 151 (note: the more intense fragment ion at 173 was not included in the quantification since it was responsible for a high background).

Chromatographic conditions (study PDG-107 [ILV]):
Column: ThermoHypersil HyPurity C8 column, 150mm x 3mm i.d., 5µm mean particle, 1 cm guard column.
Mobile phase:

Time (min)	Flow (mL/min)	% MeOH/water (20/80, v/v)	ACN/water (50/50, v/v)
0.00	0.5	100	0
10.00	0.5	0	100
12.00	0.5	0	100
13.00	0.5	100	0
16.00	0.5	100	0

Temperature: not reported.
Injection volume: 30 µL
Retention time: 6.5 – 11.5 min to MS
Detection: TurboIonspray negative mode
Parent ion m/z = 233
Fragment ion m/z = 151
Fragment ion m/z = 107

Findings:

Specificity - interferences:

HPLC-MS/MS is a highly specific technique by monitoring two m/z transitions but only one m/z transition was followed here. Both methods showed that lenacil was not detectable (< 30 % of LOQ) in all control (untreated) samples of sugar beet leaves and roots; no other interferences were observed at the characteristic retention time of lenacil. Chromatograms were provided for a standard solution, a control sample (untreated), a fortified sample and a test sample (treated) for sugar beet beets and sugar beet leaves in both studies 20011048/E1-FPSB and 20011048/E2-FPSB. A MS spectrum and product ion spectrum (MS/MS) were also presented within the reports.

ILV: The specificity of the method was tested using control (untreated) samples of sugar beet root and leaf. The lenacil concentrations in the controls were <30 % of the LOQ.
The peak area ratios of the two ion transitions monitored for standards and fortified samples were provided and compared within the study. There was good agreement between the mean peak area ratio in the fortified samples

	compared with that of the standards, the difference being ≤ 8%, indicating that the residues are confirmed. Chromatograms were provided for a calibration standard, control samples and fortified samples at 0.02 mg/kg for both matrices.
Linearity:	Calibration curves for lenacil showed good linearity in the range of 0.03 – 1 µg/mL (n =8, quadratic regression line), i.e., R^2 = 0.9964 (R^2 = 0.9991 in study 20011048/E2-FPSB); approximate corresponding residue concentration range: 0.01 – 0.2 mg/kg (dependent on final extract volume). The calibration curve and the corresponding equation were provided within the study. All standard solutions were prepared in methanol/water (30:70, v/v). The absence of matrix effects was not demonstrated.
ILV:	Calibration range: 0.02 – 0.6 µg/mL (n=5, duplicate determination); linear regression line (R^2 = 0.9652); approximate corresponding residue concentration range: 0.01 – 0.25 mg/kg. The calibration curve and the corresponding equation were provided within the study for the m/z transition 233 → 151. All standard solutions were prepared in methanol/water (30:70, v/v). The absence of matrix effects were not demonstrated.
Recovery:	Recovery samples were prepared by fortification of control samples from the current trial with the reference substance prior to extraction. The full method validation has been performed in study 200110481E1-FPSB. Mende, 2002: only 3 replicates per fortification level. Insufficient information was provided in the report for the fortification level 4.0 mg/kg. In study 20011048/E2-FPSB, procedural recoveries were analysed to cover the LOQ (0.02 mg/kg). Recovery results were within guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.5-1.
Repeatability:	Repeatability of the method(s) was demonstrated by the standard deviations/relative standard deviations of the recovery values given in the table above. The relative standard deviations of recovery data obtained are within the guideline of ≤20%. The method is adequate for determining lenacil residues in the sugar beet root and leaf samples used in the study.
Limit of quantification:	The limit of quantification of the method for lenacil in sugar beet is 0.020 mg/kg.

Table B.5.1.2.5-1 : Validation data for analytical methods for the determination of lenacil in sugar beet (HPLC-MS/MS)

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a, b]	Average recovery (%) [range]	% Relative standard deviation
Mende (2002), 20011048/ E1-FPSB*	Sugar beet leaves (high water)	3 3 2 Total = 8	0.02 0.20 4.0	83 [80 – 85] 92 [84 -100] 95 [95 – 95] Overall = 89	3 9 - Overall = 8
	Sugar beets roots (high starch)	3 3 Total = 6	0.02 0.20	100 [90 – 109] 95 [88 – 100] Overall = 98	10 7 Overall = 8
Turnbull (2003)*	Sugar beet Leaves (high water)	5 5 Total = 10	0.02 0.2	91 [88 – 97] 75 [73 – 79] 83	4 3 11
	Sugar beet roots (high starch)	5 5 Total = 10	0.02 0.2	98 [80 – 111] 86 [82 – 93] 92	15 5 12.5
Hamberger **, (2002), 20011048/ E2-FPSB*	Leaves	1	0.02	84	-
	Beets	1	0.02	96	-

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

* Validation results for the m/z transition 233 → 151.

** Procedural recoveries.

RMS Conclusion – initial monograph:

Although the validation data provided in the analytical report by Mende (2002) do not fully comply with the guidelines described in SANCO/825/00 rev. 7 (only 3 replicates were performed at each fortification level), the overall data package provided by primary validation and ILV is considered to be sufficient to demonstrate that the HPLC-MS/MS method is suitable for the determination of lenacil in sugar beet (leafs and roots) with a LOQ of 0.02 mg/kg. Hence, the method is considered acceptable as enforcement method in sugar beet.

Furthermore, it is noted that the method follows the approach of multi-residue method DFG S19, as far as extraction and clean-up (GPC) is concerned.

RMS Conclusion 2018 (renewal):

The HPLC-MS/MS (modified multi-residue method DFG S19) method was considered for 1st approval. The validation data presented (incl. absence of quantitative validation data for a confirmative m/z transition) are not sufficient to consider the method fully validated according to the current requirements of SANCO/825/00 rev. 8.1. The method has been used in support of the residues Vol.3 CA-B.7.3.1 Storage stability (Hamberger 2002) and B.7.3 Magnitude of residues (Pollmann, 2002) still relied on for renewal and is considered sufficiently validated according to SANCO/3029/99 and "fit for purpose" taking into account the overall data obtained within the three above mentioned studies. All the mean recoveries and % RSD meet the requirements of SANCO/3029/99 (70 – 110% range for recovery and % RSD < 20%). The method is suitable to determine the residues of lenacil in sugar beet roots and leaves with an LOQ of 0.02 mg/kg which corresponds to the lowest fortification level showing an acceptable recovery and repeatability.

As mentioned above and although the method seems to be suitable to determine lenacil in sugar beet leaves and roots with an LOQ of 0.02 mg/kg, the method is not fully validated according to SANCO/825/00 rev. 8.1 to be recommended as monitoring method as it was the case for 1st approval. However, in the frame of the renewal a new validation data set has been generated for HPLC-MS/MS still based on DFG S19 (using different chromatographic conditions) in high water, high acidic, oily and dry (high starch) matrices (therefore covering the GAP uses) and found acceptable (see B.5.2.1 below).

Study No. 4

Report:	**CA 4.1.2(e)/04 (IIA 4.2.1.1-03)** Tillkes, M. (1998) Magnitude of residue of lenacil and trisulfuron methyl in sugar beet grown in France following application of Venzar and DPX-MX843-1 – Season 1995 DuPont Report No.: F-95-001-RES → Analytical Part: Gas-Chromatographic Determination of Pesticides Residues after Clean-up by Gel-Permeation Chromatography and Mini-Silica Gel-Column Chromatography. 6. Communication: Replacement of dichloromethane by ethyl acetate/cyclohexane in liquid-liquid partition and simplified conditions for extraction and liquid – liquid partition (Specht *et al.*, 1995 – *Fresenius J. Anal. Chem.* 353, 183-190) Dr. Specht & Partner, Germany Specht & Partner No. DUP-9701/az. 56301/97 Report No. F-95-001-RES
Guidelines:	SANCO/3029/99 rev.4
GLP:	Yes (GLP compliance statement)

Description of the method (DFG S19 – GC-MSD, with modified extraction):
100 g sample material is extracted with 200 mL acetone. Water is added beforehand in amount that takes full account of the natural water content so that the acetone/water ration remains constant at 2:1 (v:v). After addition of ethyl acetate/ cyclohexane (1:1) and repeated mixing excess water is separated. The evaporated residue of an aliquot of the organic phase is cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1:1) as eluent. The residue-containing fraction is concentrated and analysed by GC using a fused silica capillary column (XTI-5, 30m x 0.25mm, 0.25µm) and a mass selective detector (m/z = 153). External calibration.

Chromatographic conditions:

Column:	30 m fused silica capillary column XTI-5, internal diameter 0.25 mm, fil thickness 0.25 µm
Gas flow rates:	Carrier: He 1.0 mL/min
Temperature:	Oven: initial 60°C, hold for 1 min With a rate of 40°C/min to 100°C With a rate of 10°C/min to 250°C, hold for 4 min. Injector: 250°C Interface: 280°C
Injection volume:	1 µL, splitless
Retention time:	~ 19.1 min

Findings:

Specificity- interferences: No interfering peaks occurred; No lenacil detected (< 30% LOQ) in control (untreated) samples. Chromatograms were provided for standard solution of lenacil, control samples (untreated), fortified samples at 0.01 and 0.1 mg/kg and treated samples.

Linearity: Calibration range: 0.02 – 2.03 µg/mL (n=8); linear regression line (R^2 = 0.998); approximate corresponding residue concentration range: 0.004 – 0.4 mg/kg. A linearity curve together with the regression equation were provided within the study report. Calibrations were prepared in ethyl acetate. The absence of matrix effects was not demonstrated.

Recovery: see Table B.5.2.1.5-2. Only 1 recovery experiment per fortification level.

Repeatability: see Table B.5.2.1.5-2. Only 1 recovery experiment per fortification level.

Reproducibility: ILV not addressed.

Limit of quantification: The limit of quantification of the method for lenacil in sugar beet is 0.010 mg/kg.

Table B.5.2.1.5-2: Recovery rates and limits of quantification for lenacil in sugar beet samples (Specht *et al.*, 1998) (GC-MSD)

Matrix	Analyte	Method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values ± RSD
Sugar beet roots	lenacil	GC-MSD	0.01	1	-	86
Sugar beet roots	lenacil	GC-MSD	0.1	1	-	81
Sugar beet leaf	lenacil	GC-MSD	0.01	1	-	87
Sugar beet leaf	lenacil	GC-MSD	0.1	1	-	79
Sugar beet leaf and roots	lenacil	GC-MSD	0.01 - 0.10	4	79 – 87	83 (4.7)

RMS Conclusion – initial monograph:
Applicability of multi-residue method DFG S19 (GC-MSD) for determination of lenacil residues in sugar beet was only partly addressed, i.e. validation data package does not comply with SANCO/825/00 (only 1 mass fragment ion monitored, only 1 sample per fortification level, no ILV).

RMS Conclusion 2018 (Renewal):
As already mentioned for 1st approval, the GC-MSD method (DFG S19) is not validated according to SANCO/3029/99 (or SANCO/825/00). The number of replicates at each fortification level was insufficient for a primary method. The method was used in support of the magnitude residue study (Tillkes 1998) (Vol. 3 CA-B.7.3) considered for renewal but the validation data are insufficient to allow to consider the method as "fit for purpose".

Study No. 5

Report:	**CA 4.1.2(e)/05 (IIA 4.2.1.1-04 and IIA 6.3-04)** Anderson I., Kakkonen J.E. (2006) Decline of lenacil residues in sugar beet (root and tuber vegetables) following a single application of Venzar® 80WP (Lenacil)- Southern Europe, Season 2005 Charles River Laboratories, DuPont Report No.: CRL 688479 Witte (2006) Analytical Phase Report – Decline of lenacil residues in sugar beet (root and tuber vegetables) following a single application of Venzar 80 WP (lenacil) – Southern Europe, season 2005 GAB Analytik GmbH, Germany Report No. 20051414/01-RSB
Guidelines:	SANCO/3029/99
GLP:	Yes (also for the analytical phase)

Description of the method:
In the frame of a residue decline study the following method for the determination of lenacil residues in sugar beet was validated. The method is a modified version of the method of Fillion et al. 2000 : Multiresidue Method for the Determination of Residues of 251 Pesticides in Fruits and Vegetables by Gas Chromatography/Mass Spectrometry and Liquid Chromatography with Fluorescence Detection. J. of AOAC Int. 83, 698-713.
Lenacil residues were extracted from homogenised samples (roots and leaves) with acetonitrile/water (50:50, v:v). The extracts were filtered through 0.45 µm PTFE filters and analysed by reversed-phase HPLC-MS/MS. If necessary, final sample extracts were diluted further with acetonitrile/water (1:1, v/v) to achieve analyte concentration within the calibration concentration range. The detection was performed in the negative ESI ionisation mode monitoring the mass transition 233→151.

Chromatographic conditions:

Column:	Thermo HyPurity Aquastar C18 column, 150mm x 3mm i.d., 5µm mean particle size (No. 22505-153030), 1 cm guard column.
Mobile phase:	80% water/20% ACN, linear gradient within 4 min to 90% ACN. Purge with 90% CAN for 2 min. 0.5 mL/min flow rate.
Temperature:	40°C
Injection volume:	20 µL
Retention time:	4.3 min
Detection:	Triple quadrupole, ESI negative Parent ion m/z = 233 Fragment ion m/z = 151

Findings:

Specificity- interferences:
- HPLC-MS/MS is a highly specific technique.
- No interfering peaks occurred; No lenacil detected (< 30% LOQ) in control (untreated) samples.
- Chromatograms were provided for a standard solution in sugar beet plant and in sugar beet root, control samples of both matrices, fortified samples at LOQ and treated samples.

Linearity: Matrix-matched calibration range: 0.5 to 500 ng/mL (n=8); linear regression line (R^2 = 0.9995 for sugar beet plant and 0.9999 for sugar beet root); approximate corresponding residue concentration range: 0.005 – 5 mg/kg. Calibration curves for sugar beet plants and sugar beet roots (matrix-matched) together with the corresponding regression equations were provided within the study report.

Recovery: see Table B.5.1.2.5-3. Recovery results were within the respective guideline requirements for all fortification levels (70-120%, RSD ≤20%).

Repeatability: see Table B.5.1.2.5-3.

Reproducibility: ILV not addressed. The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: The limit of quantification of the method for lenacil in sugar beet is 0.02 mg/kg.

Table B.5.1.2.5-3 : Validation data for analytical HPLC-MS/MS method for the determination of lenacil in sugar beet samples (Witte, 2006)

Reference	Matrix	Number of tests	Fortification level (mg/kg) [(a)]	Average recovery (%) [range]	% Relative standard deviation
Anderson I., Kakkonen J.E. (2006) – Witte (2006) for the analytical part	Sugar beet root (high starch)	5 5 Total = 10	0.02 0.20	103 [100 – 107] 103 [95 – 108] Overall = 103	3 5 Overall = 4
	Sugar beet plant (high water)	5 3 5 5 Total=18	0.02 0.20 2.0 20.0	105 [93 - 110] 105 [101 – 107] 104 [103 – 105] 100 [96 – 107] Overall = 103	7 3 1 5 Overall = 5

[a] Fortifications were performed with analyte reference standard solutions

Stability of solution
Since all samples were analysed within one day, testing the stability of extract, standards or stock solution was not necessary.

RMS Conclusions – initial monograph:
The HPLC-MS/MS method described above appears to be suitable for the determination of lenacil in sugar beet (leafs and root) with a LOQ of 0.02 mg/kg. An independent laboratory validation of this method has not been provided. It should be noted that this method only differs from the method by Mende, 2002 and Turnbull, 2003 (see further above) in that it uses another HPLC-column (C18 instead of C8).

RMS Conclusion 2018 (renewal):
The HPLC-MS/MS method is sufficiently validated according to SANCO/3029/99. Specificity/absence of interferences, linearity, accuracy and repeatability were demonstrated. The mean recovery is within the 70 – 110% range at each fortification level and the % RSD is systematically below 20% The number of replicates at each fortification level, although not always at the recommended number of 5, is considered sufficient. The LOQ of 0.02 mg/kg in sugar beet plant and roots is substantiated by the experimental data and corresponds to the lowest fortification level with acceptable recovery and RSD.
The method was used in support of the magnitude residue study of Anderson I., Kakkonen J.E. (2006) (Vol.3 CA-B.7.3) considered for the purpose of renewal and the method can be declared “fit for purpose”.

It is noted that the extraction efficiency of the above mentioned methods was not addressed within the renewal dossier. However for studies of Mende, Turnbull, Hamberger and Tillkes, the methods used also the modified DFG S19 technique and a mixture of acetone/water : 2/1 as extraction solvent as in the method recommended for monitoring. Therefore, the same reasoning as the one presented under section B.5.2.1 can apply.
For the modified version of method of Fillion et al. (study by Witte 2006), the extraction solvent is acetonitrile/water : 50/50 which remains comparable to the metabolism study (see B.5.2.1) using acetonitrile/water : 2/1.

B.5.1.2.5.2. Animal matrices

No feeding studies for lenacil were conducted. Therefore no analytical method is submitted here.

B.5.1.2.6. Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Soil

In the cause of the ecotoxicology studies soil samples were not analysed. Therefore, no methods are submitted here.

Water

Previous evaluation:	*Initial monograph November 2007*

The following ecotoxicity studies (studies No. 1 to 8 were considered for first approval but the details and assessment of the analytical method were not reported in the initial DAR. Those are now summarized here below for the sake of completeness.

Study No. 1

Report:	**CA 4.1.2(f)/01 (CA8.1.1.1/01, previously IIA 8.1.1-01 in the old dossier)** [redacted] (2002a) Lenacil technical Acute oral toxicity (LD_{50}) to the mallard duck [redacted] DuPont Report No.: ACD 048/022425
Guidelines:	Not stated.
GLP:	Yes

Note: details of the analytical phase was reported in Appendix to the study report.

Study No. 2

Report:	**CA 4.1.2(f)/02 (CA8.1.1.1/02, previously IIA 8.1.1-02 in the old dossier)** [redacted] (2002b) Lenacil technical acute oral toxicity (LD50) to the bobwhite quail [redacted] DuPont Report No.: ACD 049/022426
Guidelines:	Not stated.
GLP:	Yes

Note: details of the analytical phase was reported in Appendix to the study report but validation data were reported in report ACD 048/022425.

Matrix: aqueous 1% w/v methylcellulose formulations

Description of the method (analytical procedure HRC/FCH/M64/01 issue 01/121201):
Sample of test formulation were dissolved in a suitable volume of tetrahydrofuran. The extracted aliquots were diluted with the LC mobile phase (32/68 acetonitrile/water) (final expected concentrations: 10 – 20 µg/mL), filtered and analysed by reversed phase HPLC with UV detection at 254 nm. External calibration.

Chromatographic conditions:

Column:	Omnispher C18, 150 × 4.6 mm, 5 µm, Varian (guard column: SecurityGuard C18, 4 × 3.2 mm, 5 µm, Phenomenex)
Column temperature:	40°C
Mobile phase:	Acetonitrile/water (32/68, v/v)
Flow rate:	1 mL/min
Injection volume:	20 µL
Detection:	UV, 254 nm
Retention time:	~ 8.2 min

Findings:

Specificity – absence of interferences:	Analysis of control samples resulted in no detectable apparent residues of lenacil; the response in the area of the lenacil peaks were always corresponded to less than 20% of the limit of determination.

	It can therefore, be concluded that few, if any, apparent residues or false positive values would arise. Chromatograms were provided for an analytical standard solution, a control sample, test samples at 100, 200 and 400 mg/mL and procedural recoveries samples at the same concentrations. A chromatogram at the 50 mg/mL fortification level was not provided in the study.
Linearity:	Good linearity was observed in the range of 5 µg/mL to 25 µg/mL for lenacil (n = 5, each in duplicate). R^2 was found to be > 0.9993 within report ACD 048/022425, R^2 > 0.9999 within report ACD 049/022426. A typical calibration curve and the corresponding regression equation were provided within the study report. Calibration solutions were prepared in the mobile phase. The concentration of the samples meet the calibration range. The matrix effects were not assessed.
Recovery:	Samples of control matrix (1% MC) have been fortified with lenacil at two different levels. The fortification data reported in the method for lenacil residues in 1% w/v methylcellulose formulations are summarised in Table B.5.1.2.6-1 below. Dose verification, homogeneity and stability were also reported. Additionally, some procedural recoveries were performed at the 100, 200 and 400 mg/mL levels in study ACD 048/022425 and at 50, 100 and 200 mg/mL levels in study ACD 049/022426. Results are presented in Tables below.
Repeatability:	Repeatability of the method is addressed by the data in Tables below. Relative standard deviations of less than 20% were obtained for the tested fortifications. Therefore, the repeatability of this method would

seem to be adequate for the purposes of detecting lenacil residues in the formulation.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: 50 mg/mL (LOD was set at 0.15 mg/mL (signal to noise ratio of 3)).

Table B.5.1.2.6-4 : Validation data for analytical method for the determination of lenacil in 1% w/v methylcellulose formulations by HPLC-UV

Reference	Matrix	Number of tests	Fortification level (mg/mL)	Average recovery (%) [range]	% Relative standard deviation
Validation data					
[redacted] (2002a), ACD 048/022425	1% w/v methylcellulose formulations	3 3 Total = 6 (days 1 + 2)	50	99.5 [99.2 – 99.6] 97.6 [95.8 – 98.6] 98.5	0.24 1.60 1.45
		3 3 Total = 6 (days 1 + 2)	400	99.3 [99.1 – 99.6] 98.4 [97.4 – 99.0] 98.9	0.29 0.91 0.76
		12	50 - 400	98.7	1.10
Procedural recoveries					
[redacted] (2002a), ACD 048/022425	1% w/v methylcellulose formulations	1*	100	96.7	-
		1*	200	98.9	-
		1*	400	98.1	-
[redacted] (2002a), ACD 049/022426	1% w/v methylcellulose formulations	1*	50	98.1	-
		1*	100	97.1	-
		1*	200	98.2	-

*Duplicate analyses.
Note: the system precision was evaluated by determining the precision of six replicate injections of both the lowest and highest standard solutions and found to be < 1%.
A chromatogram of a control sample was provided within the study report but it is not indicated if two control samples were analysed during validation.

Table B.5.1.2.6-2 : Dose verification

Reference	Matrix	Analyte	Nominal Dose level [mg/mL]	Number of Replicates**	Mean analysed conc. In mg/mL (% of nominal dose level*)	RSD [%]
[redacted] (2002a), ACD 048/022425	1% w/v methylcellulose formulations	Lenacil	100	1	101 (101)	-
			200	1	203 (101.5)	-
			400	1	425 (106.3)	-
[redacted] (2002a), ACD 049/022426	1% w/v methylcellulose formulations	Lenacil	50	1	49.4 (98.8)	-
			100	1	99.4 (99.4)	-
			200	1	199 (99.5)	-

* Re-calculated by RMS based on the results available in the report.
** Duplicate injection.
Note: the report mentions that analytical results were corrected for the appropriate procedural recoveries determined at analysis.

Table B.5.1.2.6-3 : Homogeneity and stability of lenacil in 1% w/v MC formulations

Reference	Matrix	Analyte	Dose level [mg/mL]	Number of Replicates	Analysed concentration [mg/mL] (% of nominal dose level**)	RSD [%]
[redacted] (2002a), ACD 048/022425	1% w/v methylcellulose formulations	Lenacil	50	3* (0 hour)	50.5 (101.0)	0.41
				3* (1 hour)	50.2 (100.4)	0.33
				3* (4 hours)	50.4 (100.8)	0.24
			400	3* (0 hour)	396 (99.0)	0.23
				3* (1 hour)	395 (98.8)	0.38
				3* (4 hours)	395 (98.8)	0.37

* n = 3 when considering samples from top, middle and bottom.
** re-calculated by RMS based on the concentration level reported.
Note: the report mentions that analytical results were corrected for the appropriate procedural recoveries determined at analysis.

RMS conclusion 2018 (renewal):
The method is sufficiently validated according to SANCO/3029/99: specificity – absence of interferences, linearity, accuracy and repeatability were demonstrated. Mean recoveries and % RSD (where available) at each fortification levels are within the acceptable limits of 70 – 110 % and < 20%, respectively. Homogeneity and stability were also demonstrated.
An LOD of 0.15 mg/mL was stated in the report, but is proposed to set the LOQ at 50 mg/mL corresponding to the lowest fortification level at which an acceptable recovery and repeatability were obtained. This LOQ and the working range support the concentrations tested within the ecotoxicity study.

Study No. 3

Report:	**CA 4.1.2(f)/03 (CA8.2.6.1/01, previously IIA 8.2.6-02 in the old dossier)** Flatman, D. (2003c) Lenacil technical; algal growth inhibition assay *Selenastrum capricornutum* Huntingdon Life Sciences Ltd. England

	Huntingdon Life Sciences project identity: ACD 034 DuPont Report No.: ACD 034/022511
Guidelines:	Not stated
GLP:	Yes

Study No. 4

Report:	**CA 4.1.2(f)/04 (CA8.2.1/01, previously IIA 8.2.1-03 in the old dossier)** Flatman, D. (2003a) Lenacil technical – Acute toxicity to fish (*Cyprinus carpio*) Huntingdon Life Sciences Ltd. England Huntingdon Life Sciences project identity: ACD 035 DuPont Report No.: ACD 035/022512
Guidelines:	Not stated
GLP:	Yes

Study No. 5

Report:	**CA 4.1.2(f)/05 (CA8.2.6.2/01, previously IIA 8.2.6-01 in the old dossier)** Flatman, D. (2003) Lenacil technical; algal growth inhibition assay *Navicula pelliculosa* Huntingdon Life Sciences Ltd. England Huntingdon Life Sciences project identity: ACD 036 DuPont Report No.: ACD 036/024694
Guidelines:	Not stated
GLP:	Yes

Study No. 6

Report:	**CA 4.1.2(f)/06 (CA 8.2.7/01, previoulsy IIA 8.2.8-01)** Flatman, D. (2003) Lenacil technical; higher plant (*Lemna*) growth inhibition test Huntingdon Life Sciences Ltd. England Huntingdon Life Sciences project identity: ACD 039 DuPont Report No.: ACD 039/023827
Guidelines:	Not stated
GLP:	Yes

Matrices: aqueous media (diatom, OECD algal medium, dechlorinated tap water, lemna medium)

Description of the method:
Aqueous samples were extracted by liquid-liquid partitioning into dichloromethane. The combined organic extracts with triethylene glycol added were evaporated to dryness and the residues dissolved in sufficient acetonitrile/water (20/80 v/v) to bring the expected analyte concentration within the calibration range. The extract solutions were then analysed for the test substance by reverse-phase high-performance liquid chromatography using spectrophotometric detection at 270 nm. External calibration was used.

High concentrations (≥1 mg/L): Sub-samples were diluted first with acetonitrile and subsequently, where necessary, with acetonitrile/water (20/80 v/v) such that the expected analyte concentrations were within the calibration range. The final composition of the diluted sample solutions was acetonitrile/water (20/80 v/v). The diluted samples were then analysed for the test substance by reversed-phase high-performance liquid chromatography using spectrophotometric detection at 270 nm. External calibration was used.

Chromatographic conditions:
Column: YMC-pack, type AQ ODS, (ODS-A in study ACD 036/024694), 250 × 4.6 mm
Column temperature: ambient
Mobile phase: Acetonitrile/water (40/60, v/v)

Flow rate:	1 mL/min
Injection volume:	400 µL in study ACD 034/022511, 50 µL for high concentrations in study ACD 035/22512, 100 µL in study ACD 036/024694
Detection:	UV, 270 nm

Findings:

Specificity – absence of interferences:	Analysis of control samples resulted in no detectable apparent residues of lenacil; the response in the area of the lenacil peaks were always corresponded to less than 20% of the limit of determination. It can therefore, be concluded that few, if any, apparent residues or false positive values would arise. Chromatograms were provided for a calibration standard, a control and a test sample within each study report.
Linearity:	Calibration curves showed good linearity in the ranges of ~10 to ~300 µg/L (n = 6, r^2 = 0.9998) in study 034/022511 and ~0.4 to ~7.5 mg/L for high concentrations (n = 5, r^2 = 0.9998) in study 035/22512, ~50 to ~1300 µg/L (n = 5, r^2 = 1.000) in study 036/024694 and ~25 to ~700 µg/L (n = 6, r^2 = 0.9996). A typical calibration curve with the corresponding regression equation was provided within each study report. All calculations were performed using standards prepared in solvent (acetonitrile/water 20/80). The matrix effects were not assessed.
Recovery:	The fortification data reported in the method for lenacil residues in aqueous solutions are summarised in Table B.5.1.2.6-4 below. Within study ACD 034/022511, quality control samples (3.892 µg/L) were also analysed at 0 (n = 2) and 72 (n = 2) hours with recoveries between 98.5 and 114%. Within study ACD 035/22512, only the results of quality control samples at 2.008 mg/L were presented (see table below). Within study ACD 036/024694, quality control samples (5.21 ug/L, n = 2; 78.15 µg/L, n = 4; 625 µg/L, n = 2) were also analysed at days 0 and 3 and at 78.15 µg/L (n =2) without algae with recoveries between 90.1 and 108%. Within study ACD 039/023827, quality control samples 20.64 ug/L were also analysed at day 0 (fresh) (n = 2), day 2 (fresh) (n =2) and at 20.10 µg/L at day 2 (expired) (n = 2), day 5 (expired and fresh) (n = 4) and day 7 (expired) (n = 2) with recoveries between 95.2 and 109%. The test concentrations were also verified.
Repeatability:	Repeatability of the method is addressed by the data in Table B.5.1.2.6-4. Relative standard deviations of less than 20% were obtained for the tested fortifications. However, only the overall repeatability can mainly be determined due to an insufficient number

	of replicates at each fortification level tested. In each case where the overall RSD is re-calculated, it is below 20%.
Reproducibility:	The methods used to generate these data are not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	The LOQ is not mentioned within the different study reports but stated to be the lowest successfully validated level. Therefore, in the summary dossier for renewal, the notifier stated an LOQ of 0.2 µg/L.

Table B.5.1.2.6-4 : Validation data for analytical methods for the determination of lenacil in aqueous solutions (HPLC-UV)

Reference	Matrix	Number of tests	Fortification level (µg/L)	Average recovery (%)**	Standard deviation**	% Relative standard deviation**
Flatman, D. (2003b) ACD 034/022511	Diatom	2 2 2 Total = 6	0.2008 30.12 75.30	103 103 96 Overall = 101	- - -	- - - Overall = 4.7
	OECD Algal medium	2	2.510	104	-	-
	Dechlo-rinated tap water	2 2 2 Total = 6	1004 3012 6275	100 99 98 Overall = 99	- - -	- - - Overall = 0.7
Flatman D. (2003c) ACD 035/22512	Dechlori nated tap water	8* Total = 8	2008	99.3	3.0	3.1
Flatman, D. (2003d) ACD 036/024694	OECD Algal medium	2 2 Total = 4	2.166 1083	97 105 Overall = 101	- -	- - Overall = 4.3
Flatman, D. (2003e) ACD 039/023827	Lemna medium	2 2 2 Total = 6	1.011 10.11 1011	104 104 106 Overall= 104	- - -	- - - Overall = 2.6

[a] Fortifications were performed with analyte reference standard solutions

* Concurrent recoveries (quality control data). The number of samples is 8 by taking into consideration the samples at 0 h, 24h, 72 h and 96h.

A control has been analysed for each matrix where lenacil was not detected.

** Re-calculated by the notifier based on the results available within the study.

RMS Conclusion 2018 (renewal):

Validation of the method occurred in different water types. The method is not fully validated according to SANCO/3029/99 since the number of replicates at each fortification levels in the different water types tested was below the recommended number of 5. Specificity – absence of interferences and linearity were well demonstrated and despite the low number of replicates, the results indicated an accurate method with a good repeatability. Mean recoveries at each fortification levels (as well as overall mean recoveries) are within the acceptable limits of 70 – 110 % and overall % RSD (where available after re-calculation since number of replicates is insufficient to allow to determine the % RSD at each fortification level) was systematically below 20%. Analysis of quality control samples during the course of the studies confirmed that the method was working accurately and precisely. The LOQ was not stated within the study reports but stated as the lowest successfully validated level. The lowest level tested was 0.2 µg/L in diatom, 2.2 µg/L in OECD medium, 1 mg/L in dechlorinated tap water and 1.01 µg/L in

lemna medium. It seems therefore that the lowest achievable LOQ in aqueous media could be as low as 0.2 µg/L. Recoveries were acceptable for all of those levels but none can be considered as fully validated according to SANCO/3029/99 due to the limited number of replicates. However, the overall available data seems to indicate that although not fully validated according to SANCO/3029/99, the method worked correctly at the levels tested which covers the endpoints and test concentrations employed in the corresponding ecotoxicological studies. The method is therefore considered "fit for purpose".

Study No. 7

Report:	**CA 4.1.2(f)/07 (CA8.2.6.1/03, previously IIA 8.2.6-04 in the old dossier)** Jenkins, C.A. (2004a) IN-KE121 algal growth inhibition assay Huntingdon Life Sciences Ltd. England DuPont Report No.: ACD 064/042730
Guidelines:	Not stated
GLP:	Yes

Note: details of the analytical phase was reported in Appendix 3 to the study report.

Matrix: algal medium (OECD medium)

Description of the method:
Aqueous test samples were diluted with either acetonitrile:water (3:7 v/v) or acetonitrile to bring the expected analyte concentration within the calibration range. The diluted samples were analysed for IN-KE121 by reverse-phase high-performance liquid chromatography using spectrophotornetric detection at 254 nm. External calibration was used.

Chromatographic conditions:

Column:	Hypersil 5ODS, 250 × 4.6 mm
Column temperature:	45°C
Mobile phase:	Acetonitrile: pH 2.6 water (3:7 v/v), orthophosphoric acid used for pH adjustment.
Flow rate:	0.75 mL/min
Injection volume:	100 µL
Detection:	UV, 270 nm
Retention time:	~ 5.5 min.

Findings:

Specificity – absence of interferences: Analysis of control samples resulted in no detectable apparent residues of the analyte; the responses in the area of the analyte peak were always corresponded to less than 20% of the limit of determination. It can therefore, be concluded that few, if any, apparent residues or false positive values would arise.
Chromatograms were provided for a calibration standard solution, a control, a fortified sample and a test sample at days 0 and 3.

Linearity: Good linearity of calibration curve was observed in the range of 0 to ~2.1 mg/L (n = 5 without the origin point) for IN-KE121; the correlation coefficient r was equal to 0.9999. A typical calibration curve with the corresponding regression equation were provided within the study report. Calibration solutions were prepared in acetonitrile/water (3/7, v/v). The matrix effects were not assessed.

Recovery: The fortification data are summarised in tables B.5.1.2.6-5 and B.5.1.2.6-6 below. The number of replicates was only 2 replicates per fortification. However, there were five fortifications (total of 8 tests;

concentration range 0.81 – 129 mg/L) and the individual recoveries for IN-KE121 were within 94-102% (overall recovery was 97.1 and a RSD of 2.8). Furthermore, the percent (%) of measured concentrations of IN-KE121 from two freshly prepared samples (in different days) for the stability and algal growth tests at each nominal concentration of 1.94, 4.27, 9.39, 20.7, 45.5, 100 mg/L were 87%, 106%, 110%, 113%, 110%, and 110%, respectively (overall mean recovery = 106 %, RSD = 9 %, total number of tests = 12).

Repeatability: Overall repeatability of the method is presented in tables B.5.1.2.6-5 and B.5.1.2.6-6 below. Relative standard deviations of less than 20% were obtained. It is noted that due to the insufficient number of replicates per fortification level, only the overall repeatability can be determined.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: The LOQ was not stated within the study report. In the summary dossier, the notifier stated an LOQ of 0.8 mg/L.

Table B.5.1.2.6-5 : Validation data for analytical methods for the determination of IN-KE121 in aqueous solutions (HPLC-UV)

Reference	Matrix	Number of tests	Fortification level (mg/L)	Average recovery (%)	Standard deviation	% Relative standard deviation
Jenkins, C.A. (2004a) ACD 064/042730	OECD Algal medium	2 2 2 1 1 Total = 8	0.808 1.02 14.7 124 129	95 101 98 95 95 Overall = 97.1	- - - - -	- - - - - Overall = 2.8

Two control samples where IN-KE 121 was not detected were also analysed.

Table B.5.1.2.6-6 : Stability and algal growth tests of IN-KE121 in aqueous solutions - measured concentrations (HPLC-UV)

Reference	Matrix	Number of tests	Nominal level (mg/L)	Measured concentration (Arithmetic mean)	Average recovery (%)	Measured concentration (Geometric mean)	Average recovery (%)
Jenkins, C.A. (2004a) ACD 064/042730	OECD Algal medium	2 2 2 2 2 2 Total = 12	1.94 4.27 9.39 20.7 45.5 100	1.45 4.29 10.1 23.2 50.4 112	75 100 107 **112** **111** **112** Overall = 103 RSD 14%	1.36 4.26 10.1 23.2 50.4 111	70 100 107 **112** **111** **111** Overall = 102 RSD 16%

RMS Conclusion 2018 (renewal):
The method is not fully validated according to SANCO/3029/99 since the number of replicates at each fortification was below the recommended number of 5. Specificity – absence of interferences and linearity were well demonstrated and despite the low number of replicates, the results indicated an accurate method with a good repeatability. Mean recoveries at each fortification levels (as well as overall mean recoveries) are within the acceptable limits of 70 – 110 % and overall % RSD (since number of replicates is insufficient to allow to determine the % RSD at each fortification level) was systematically below 20%. Analysis of freshly prepared samples (in different days) for the stability and algal growth tests at each nominal concentration confirmed that the method was working accurately and precisely.
The LOQ was not stated within the study report. An LOQ of 0.8 mg/L was suggested in the summary dossier. This level showed acceptable recoveries but the repeatability cannot be determined due to the low number of replicates. As consequence, an LOQ of 0.8 mg/L, although more than likely achievable cannot be considered as fully validated. The level of 1 mg/L can be considered more supported based on the overall available results and still supports the endpoints determined within the study. Overall, the available data seems to indicate that although not fully validated according to SANCO/3029/99, the method worked correctly at the levels tested which covers the endpoints and test concentrations employed in the corresponding ecotoxicological study. The method is therefore considered "fit for purpose".

Study No. 8

Report:	**CA 4.1.2(f)/08 (CA8.2.6.1/04, previously IIA 8.2.6-05 in the old dossier)** Jenkins, C.A. (2004b) IN-KF313 algal growth inhibition assay *Selenastrum capricornutum* Huntingdon Life Sciences Ltd. England DuPont Report No.: ACD 066/042848
Guidelines:	Not stated
GLP:	Yes

Note: details of the analytical phase was reported in Appendix 3 to the study report.

Matrix: algal medium (OECD medium)

Description of the method
Aqueous samples were diluted to bring the expected analyte concentration within the calibration range, at the same time adjusting the solvent to a composition similar to that of the HPLC mobile phase. The extract solutions were then analysed for IN-KF313 by reverse-phase high-performance liquid chromatography using spectrophotornetric detection at 269 nm. External calibration was used.

Chromatographic conditions:

Column:	Hypersil 5ODS, 250 × 4.6 mm
Column temperature:	40°C
Mobile phase:	Acetonitrile: pH 2.6 water (3:7 v/v), orthophosphoric acid used for pH adjustment.
Flow rate:	0.5 mL/min
Injection volume:	100 µL
Detection:	UV, 269 nm
Retention time:	~ 11.5 min.

Findings:

Specificity – absence of interferences:	Analysis of control samples resulted in no detectable apparent residues of the analyte; the response in the area of the analyte peaks were always corresponded to less than 20% of the limit of determination. It can therefore, be concluded that few, if any, apparent residues or false positive values would arise. Chromatograms were provided for a calibration standard solution, a control, a fortified sample and a test sample at days 0 and 3.
Linearity:	Good linearity was observed for the calibration curve of IN-KF313 in the range of 0 to ~ 1 mg/L (n = 6 without the origin point) with a correlation coefficient r = 1.000. A typical calibration curve together

with the corresponding regression equation were provided within the study report. All calculations were performed using standards prepared in solvent (acetonitrile/water: 3/7, v/v). The matrix effects were not assessed.

Recovery: The fortification data reported in the method for IN-KF313 residues in aqueous solutions are summarised in tables B.5.1.2.6-7 and B.5.1.2.6-8 below. The analytical method principle is the same as with 064/042730, except that the analyte is IN-KF313. The number of replicates was only 2 replicates per fortification and there were three fortifications (total of 6 tests; concentration range 0.26 – 13 mg/L). However, the individual recoveries for IN-KF313 were within 95.4-101.7% (overall recovery was 97.4 and RSD of 2.5). Moreover, the percent (%) of measured concentrations of the analyte from two freshly prepared samples (in different days) for the stability and algal growth tests at each nominal concentration of 0.625, 1.25, 2.5, 5.0 and 10 mg/L were 96%, 101%, 101%, 103% and 109%, respectively (overall recovery of 102% and RSD of 5%; total number of tests =10).

Repeatability: Overall repeatability of the method is presented in tables B.5.1.2.6-7 and B.5.1.2.6-8. Relative standard deviations of less than 20% were obtained. It is noted that due to the insufficient number of replicates per fortification level, only the overall repeatability can be determined.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: The limit of quantification was not stated within the study report but defined as the lowest successfully validated level. Therefore, in the summary dossier, the LOQ was proposed to be 0.26 mg/L.

Table B.5.1.2.6-7 : Validation data for analytical methods for the determination of IN-KF313 in aqueous solutions (HPLC-UV)

Reference	Matrix	Number of tests	Fortification level (mg/L)	Average recovery (%)	% Relative standard deviation
Jenkins, C.A. (2004b) ACD 066/042848	OECD Algal medium	2 2 2 Total = 6	0.26 2.6 13	96 99 97 Overall = 97.4	- - - Overall = 2.5

Two control samples where IN-KE 121 was not detected were also analysed.

Table B.5.1.2.6-8 : Stability and algal growth tests of IN-KF313 in aqueous solutions - measured concentrations (HPLC-UV)

Reference	Matrix	Number of tests*	Fortification level (mg/L)	Average recovery (%)	% Relative standard deviation
Jenkins, C.A. (2004b) ACD 066/042848	OECD Algal medium	2 2 2 2 2 Total = 10	0.625 1.25 2.5 5.0 10	96 101 101 103 109 Overall = 102	- - - - - Overall = 5

* n = 2 taking into account the sampling on the two different days (days 0 and 3).

RMS Conclusion 2018 (renewal):
The method is not fully validated according to SANCO/3029/99 since the number of replicates at each fortification was below the recommended number of 5. Specificity – absence of interferences and linearity were well demonstrated and despite the low number of replicates, the results indicated an accurate method with a good repeatability. Mean recoveries at each fortification levels (as well as overall mean recoveries) are within the acceptable limits of 70 – 110 % and overall % RSD (since number of replicates is insufficient to allow to determine the % RSD at each fortification level) was systematically below 20%. Analysis of freshly prepared samples (in different days) for the stability and algal growth tests at each nominal concentration confirmed that the method was working accurately and precisely.
The LOQ was not stated within the study report. An LOQ of 0.26 mg/L was suggested in the summary dossier. This level showed acceptable recoveries but the repeatability cannot be determined due to the low number of replicates. Overall, the available data seems to indicate that although not fully validated according to SANCO/3029/99, the method worked correctly at the levels tested (including the LOQ level) which covers the endpoints and test concentrations employed in the corresponding ecotoxicological study. The method is therefore considered "fit for purpose".

Previous evaluation:	*Initial monograph November 2007*

The following ecotoxicity studies were considered for first approval but the details and assessment of the analytical method were not reported in the initial DAR, nor in the summary dossier for the renewal. Therefore, a consideration of the analytical methods by section 1 was requested by the ecotox. section. In order to avoid a change of all the cross-references in the ecotox. section, the following studies will be numbered as CA 4.1.2(f)/08.xx. (studies No. 8.xx)

Study No. 8.01

Report:	**CA 4.1.2(f)/08.01 (KCP 10.6.2/01)** Gossman a. and Meinerling M. (2006) Effects of Venzar 500 SC on Terrestrial (Non-Target) Plants: Seedling emergence and seedling Growth Test IBACON GmbH, Germany Project 26803086
Guidelines:	Not stated
GLP:	Yes

The analytical phase is reported in Appendix 2 of the report.

Matrix: stock solution

Description of the method
The samples were shaken to obtain homogeneous solutions. An aliquot of the sample was taken from the continuously stirred solution and diluted with methanol. The analysis of the active ingredient then occurred by HPLC-UV. External calibration.

Chromatographic conditions:

Column:	Synergie Polar RP80A, 150 × 3.0 mm
Oven temperature:	25°C
Mobile phase:	35% Acetonitrile/65% HPLC water with 0.1% H_3PO_4
Flow rate:	0.5 mL/min
Injection volume:	20 µL
Detection:	UV, 254 nm

Findings:

Specificity – absence of interferences:	Not demonstrated. The report only mentions that identity can be confirmed by comparison of the retention time with the retention time of a standard. No chromatograms were provided.

Linearity:	A good linearity was stated in the range of 5 - 50 mg a.s./L in methanol with a regression coefficient of 0.9995. The regression equation was provided. A typical calibration curve and the number of the different calibration solutions used were not reported.
Recovery:	Very limited data were reported. For results, please refer to Table B.5.1.2.6-8.01.
Repeatability:	Very limited data were reported. For results, please refer to Table B.5.1.2.6-8.01.
Reproducibility:	Not demonstrated.
Limit of quantification:	The limit of quantification was stated within the study report to be 2.4 mg test item/L (after dilution by a factor 40).

Table B.5.1.2.6-8.01 : Validation data for analytical methods for the determination of lenacil in aqueous solutions (HPLC-UV)

Reference	Matrix	Number of tests	Fortification level (g PPP/L)	Average recovery (%)	% standard deviation
Fortified samples					
Gossmann A. and Meinerling M. (2006) Project 26803086	Stock solution (spray solution)	6 4	2.4 24	105 101	4 3
Test samples during the test (spray mixture at the highest rate)					
Gossmann A. and Meinerling M. (2006) Project 26803086	Stock solution (spray solution)	2 2	22.8 (all species) 11.4 (Allium Cepa)	101 101	- -

RMS conclusion renewal (2018):
A full validation of the method is not presented in the study report. Only very limited results are summarized. The method seems to be linear but the calibration curve and the number of determination were not reported. Moreover, the calibration range seems to not cover the test concentrations. Nevertheless, in the sample preparation, it is indicated that samples are diluted but the dilution factor is not provided to control if the samples were within the calibration range. No chromatograms were provided. The mean recoveries seem acceptable since in the 70 – 110% range but details on individual mean recoveries were not provided. The LOQ is proposed to be 2.4 mg PPP/L (after dilution by a factor of 40) but is not substantiated by experimental data.
Based on the available data, it cannot be stated that the method is validated according to SANCO/3029/99. Although, the method seems more than likely to work accurately and precisely (based on the recovery, repeatability and linearity data) and to be "fit for purpose", this cannot be confirmed based on the available overall validation data.

Study No. 8.02

Report:	**CA 4.1.2(f)/08.02 (KCP 10.2.3/01)** Jenkins C.A. (2005) Lenacil (Venzar 80% WP) Effects on primary productivity and macrophyte biomass in field-based microcosms Huntingdon Life Sciences Ltd, UK

	Report ACD 072/043691
Guidelines:	Not stated
GLP:	Yes

Note: the HPLC-UV method was previously validated at Huntingdon Life Sciences, Report No. ACD 013/033850. The LC-MS/MS methods for determination of lenacil in water and sediment samples were validated within the study in appendix 3.

Matrix: (i) formulation (determination of the content of lenacil in the formulation), (ii) pond water, (iii) sediment

Description of the method (HPLC-UV for determination of a.s. in venzar 80 WP):
Samples of venzar were accurately weighed into separate 100 mL volumetric flasks. The samples were diluted to volume with THF. Aliquots (5 mL) of the resulting solution were transferred to separate 25 mL volumetric flasks and a portion (15 mL) of the 30% acetonitrile diluting solution was added. Each solution was then diluted to volume with THF and filtered. Analysis occurred the by HPLC-UV. External calibration.

Chromatographic conditions (HPLC-UV):
Column: Luna C18, 250 × 4.6 mm
Oven temperature: 45°C
Mobile phase: Acetonitrile/water: 32/68 v/v
Flow rate: 2.0 mL/min
Injection volume: 10 µL
Detection: UV, 254 nm

Findings:

Specificity – absence of interferences:	Not demonstrated. Chromatograms were provided for a standard sample and the test sample.
Linearity:	A good linearity was stated in the range of 107 - 639 mg/L in acetonitrile with r = 1.0 (n = 6 + origin point). The regression equation and the calibration curve were provided.
Recovery:	Not demonstrated within the study report. Only an assay of the test substance for the test has been made (in duplicate, content in % w/w was found to be 80.9 and 80.1).
Repeatability:	Not demonstrated within the study report.
Reproducibility:	Not demonstrated.
Limit of quantification:	Not stated.

RMS conclusion renewal (2018):
The validation of the HPLC-UV method for assaying venzar 80% WP didn't occur in the present study report and therefore, no validation data were reported. Nevertheless, the method would have been validated in a previous report No. ACD 013/033850. **The notifier is requested to submit the report ACD 013/03385 (if containing validation data) for the sake of completeness.**
Validated analytical methods however exist and were accepted (i.e. in the initial monograph and at zonal level) for the determination of lenacil in venzar 80 WP.

Description of the LC-MS/MS method for determination of a.s. in water:
Water samples were extracted using C18 solid phase extraction (SPE) cartridges. The cartridge was eluted with methanol which was removed by evaporation prior to reconstitution and dilution to volume with water:methanol (50:50 v/v). Quantitation was performed using LC-MS/MS. External calibration.

Chromatographic conditions (LC-MS/MS):
Column: Phenomenex Luna C8, 150 × 2.0 mm
Mobile phase:

Time (min)	A = water:methanol (90/10 v/v) + 0.01 M ammonium formate and 0.1% formic acid	B = 0.1 % formic acid in methanol
0	100	0
7	0	100
10	0	100
11	100	0
15	100	0

Flow rate: 0.2 mL/min
Injection volume: 20 µL
Ionisation mode: positive electrospray
Ion monitored: lenacil: MRM m/z 235.1 → 153.0; IN-KE 121: MRM m/z 249.2 → 153.0, IN-KE 313: MRM m/z 249.2 → 167.0.

Findings:

Specificity – absence of interferences: LC-MS/MS is known to be a highly specific method. However, only one m/z transition was followed here for each analyte.
From the provided chromatograms, no interferences were observed. Chromatograms were provided for calibration standards of lenacil and its both metabolites at the highest and lowest calibration levels, controls (untreated pond water) and fortified samples at 0.1 µg/L for each analyte.

Linearity: A good linearity was stated in the range of 1 - 50 ng/mL in water/methanol 50/50 v/v for the three analytes (n = 9 + origin point in each case). R^2 was found to be 0.9993 for lenacil, 0.9992 for IN-KE 121 and 0.9998 for IN-KE 313. Typical calibration curves and regression equations were provided for the three analytes. Matrix effects were not assessed.

Recovery: Fortification of water samples occurred at three different levels for the three analytes. The results are reported in Table B.5.1.2.6-8.02. Procedural recoveries were also determined an reported in Table B.5.1.2.6-8.02.

Repeatability: The results for the % RSD is presented in Table B.5.1.2.6-8.02 below.

Reproducibility: Not demonstrated.

Limit of quantification: 0.1 µg/L (LOD = 0.05 µg/L) which corresponds to the lowest fortification level with an acceptable recovery.

Table B.5.1.2.6-8.02 : Validation data for the determination of lenacil and its metabolite IN-KE 121 and IN-KE 313 in pond water by LC-MS/MS

Reference	Matrix	Number of tests	Fortification level (µg lenacil/L)	Average recovery (%) [range]	% Relative standard deviation
Lenacil					
Validation results					
Jenkins C.A. (2005), Report ACD 072/043691	Pond water	5 5 5 Overall (n =15)	0.1 10 200	77 [74 -85] 86 [84-88] 105 [102-108] 89	4.7 1.5 2.4 13.5

Procedural recoveries					
Jenkins C.A. (2005), Report ACD 072/043691	Pond water	9 11 11	0.5 25 100	[70-82] [83-98] [85-107]	nd nd nd
IN-KE 121					
Validation results					
Jenkins C.A. (2005), Report ACD 072/043691	Pond water	5 5 5 Overall (n =15)	0.1 10 200	97 [82-104] 96 [94-98] 103 [99-105] 99	9 1.6 2.5 6.1
Procedural recoveries					
Jenkins C.A. (2005), Report ACD 072/043691	Pond water	6 11 11	0.5 25 100	[88-110] [87-101] [89-110]	nd nd nd
IN-KE 313					
Validation results					
Jenkins C.A. (2005), Report ACD 072/043691	Pond water	5 5 5 Overall (n = 15)	0.1 10 200	105 [98-**113**] 93 [91-94] 106 [101-109] 101	6.0 1.2 3.1 7.1
Procedural recoveries					
Jenkins C.A. (2005), Report ACD 072/043691	Pond water	6 11 11	0.5 25 100	[70-76] [85-102] [91-110]	nd nd nd

Two control were also analysed for each validation set where no residues of lenacil, IN-KE 121 and IN-KE 313 were found.
Nd = not determined.

RMS conclusion renewal (2018):
The method is considered sufficiently validated according to SANCO/3029/99. All mean recoveries were within the 70 - 110% range and the % RSD were all < 20%. The proposed LOQ of 0.1 µg/L is supported by the experimental data. The method is considered "fit for purpose".

Description of the LC-MS/MS method for determination of a.s. in sediment:
Sediment samples were extracted with acetonitrile. The organic phase was removed by evaporation prior to reconstitution and dilution to volume with water:methanol (50:50 v/v). Quantitation was performed using LC-MS/MS. External calibration.

Chromatographic conditions (LC-MS/MS): see conditions for the determination in water

Findings:

Specificity – absence of interferences:	LC-MS/MS is known to be a highly specific method. However, only one m/z transition was followed here for each analyte. From the provided chromatograms, no interferences were observed. Chromatograms were provided for calibration standards of lenacil and its both metabolites at the highest and lowest calibration levels, controls (untreated sediment) and fortified samples at 10 µg/kg for each analyte.
Linearity:	A good linearity was stated in the range of 1 - 50 ng/mL in water/methanol 50/50 v/v for the three analytes (n = 9 + origin point

	in each case). R^2 was found to be 0.9993 for lenacil, 0.9992 for IN-KE 121 and 0.9998 for IN-KE 313. Typical calibration curves and regression equations were provided for the three analytes. Matrix effects were not assessed.
Recovery:	Fortification of water samples occurred at three different levels for the three analytes. The results are reported in Table B.5.1.2.6-8.03. Procedural recoveries were also determined an reported in Table B.5.1.2.6-8.03.
Repeatability:	The results for the % RSD is presented in Table B.5.1.2.6-8.02 below.
Reproducibility:	Not demonstrated.
Limit of quantification:	10 µg/kg (LOD = 5 µg/kg) which corresponds to the lowest fortification level with an acceptable recovery.

Table B.5.1.2.6-8.03 : Validation data for the determination of lenacil and its metabolites in sediment by LC-MS/MS

Reference	Matrix	Number of tests	Fortification level (µg lenacil/kg)	Average recovery (%) [range]	% Relative standard deviation
Lenacil					
Validation results					
Jenkins C.A. (2005), Report ACD 072/043691	sediment	5 5 5 Overall (n = 15)	10 100 1000	78 [73-81] 83 [79-86] 98 [91-104] 86	3.4 2.7 5.6 11.5
Procedural recoveries					
Jenkins C.A. (2005), Report ACD 072/043691	sediment	5 5 5	10 100 1000	[74-103] [74-90] [90-94]	nd nd nd
IN-KE 121					
Validation results					
Jenkins C.A. (2005), Report ACD 072/043691	sediment	5 5 5 Overall (n = 15)	10 100 1000	91 [82-97] 89 [87-91] 96 [87-104] 92	6.6 1.8 7.2 6.6
Procedural recoveries					
Jenkins C.A. (2005), Report ACD 072/043691	sediment	5 5 5	10 100 1000	[78-107] [75-93] [86-100]	nd nd nd
IN-KE 313					
Validation results					
Jenkins C.A. (2005), Report ACD 072/043691	sediment	5 5 5 Overall (n = 15)	10 100 1000	83 [79-86] 84 [83-85] 90 [84-93] 86	2.6 0.8 3.5 4.7

Procedural recoveries					
Jenkins C.A. (2005), Report ACD 072/043691	sediment	5 5 5	10 100 1000	[73-101] [72-93] [78-92]	nd nd nd

Two control were also analysed for each validation set where no residues of lenacil, IN-KE 121 and IN-KE 313 were found.
Nd = not determined.

RMS conclusion renewal (2018):
The method is considered sufficiently validated according to SANCO/3029/99. All mean recoveries were within the 70 - 110% range and the % RSD were all < 20%. The proposed LOQ of 10 µg/kg is supported by the experimental data.

Studies No. 8.03 to 8.08

Report:	**CA 4.1.2(f)/08.03 (KCA 8.2.1/03[IIA8.2.1-02])** [redacted] 1991b) Static, acute, 96-hour LC50 of DPX-B634-91 (lenacil) to fathead minnow (Pimephales promelas) [redacted] [redacted] Report No. 198-91 MR-4581-884
Guidelines:	Not stated
GLP:	Yes

Report:	**CA 4.1.2(f)/08.04 (KCA 8.2.1/02 [IIA 8.2.1-01])** [redacted] (1991a) Static, acute, 96-hour LC50 of DPX-B634-91 (lenacil) to rainbouw trout (Oncorhyncus mykiss) [redacted] [redacted] Report No. 199-91 MR-4581-884
Guidelines:	Not stated
GLP:	Yes

Report:	**CA 4.1.2(f)/08.05 (KCA 8.2.2.1/01)** [redacted] (1991c) Flow-through, 21-day toxicity of DPX-B634-91 (lenacil) to rainbow trout (Oncorhyncus mykiss) [redacted] [redacted] Report No. 200-91 MR-4581-884
Guidelines:	Not stated
GLP:	Yes

Report:	**CA 4.1.2(f)/08.06 (KCA 8.2.4.1/01)** Hutton D.G. (1989a) Static acute 48-hour EC50 of DPX-B634-84 to fed Daphnia magna Haskell Laboratory for Toxicology and Industrial Medicine, USA Haskell Laboratory Report No. 86-89

	MR-4581-675
Guidelines:	Not stated
GLP:	Yes

Report:	**CA 4.1.2(f)/08.07 (KCA 8.2.5.1/01)** Hutton D.G. (1989b) Chronic Toxicity of DPX-B634-84 (Lenacil) to Daphnia magna Haskell Laboratory for Toxicology and Industrial Medicine, USA Haskell Laboratory Report No. 130-89 MR-4581-675
Guidelines:	Not stated
GLP:	Yes

Report:	**CA 4.1.2(f)/08.08 (KCA 8.2.2.1/02)** [redacted] (1996) Early Life-Stage Toxicity of DPX-B634-91 (Lenacil) to Rainbow trout (Oncorhynchus mykiss) [redacted] [redacted] Report No. 235-96 MR-10351
Guidelines:	Not stated
GLP:	Yes

Matrix: Laboratory well water.

Description of the method:
Samples were analysed by HPLC-UV. External calibration.

Chromatographic conditions (HPLC-UV):

Column:	ODS-Hypersil, 60 × 4.6 mm
Column temperature:	40°C
Mobile phase:	Acetonitrile/ 3.1 mM H_3PO_4 : 35/65 (ACN/pH 2.6 water 35/65 in studies CA 8.2.4.1/01 and CA 8.2.5.1/01)
Flow rate:	1.5 mL/min
Injection volume:	25 µL (10 µL injected in study CA 8.2.4.1/01, 40 µL injected in study CA 8.2.5.1/01)
Detection:	UV, 254 nm

Chromatographic conditions (HPLC-UV) within study CA 8.2.2.1/02:

Column:	Zorbax RX-C8, 150 × 4.6 mm
Column temperature:	40°C
Mobile phase:	Acetonitrile/HPLC grade water adjusted to pH 3 : 38%/62%
Flow rate:	1.0 mL/min
Injection volume:	250 µL
Detection:	UV, 270 nm

In study CA 8.2.2.1/02, slightly amended chromatographic conditions were used to analyse samples after day 21 (involving a shorter retention time of the analyte):

Column:	Zorbax RX-C18, 150 × 2.1 mm
Column temperature:	40°C
Mobile phase:	Acetonitrile/3.1 mM H_3PO_4 (40%/60%)
Flow rate:	0.3 mL/min
Injection volume:	50 µL
Detection:	UV, 270 nm

Findings:

Specificity – absence of interferences:	Not discussed in the study report. Chromatograms were however provided for a standard sample, a control and a test sample. Based on these chromatograms, no interferences seem to occur.
Linearity:	**Study CA 8.2.1/03:** A good linearity was stated in the range of 0.1 - 25 mg/L in water. However, only a typical curve in the 1 – 5 mg/L range was provided (n = 5) but the correlation coefficient and the regression equation were not provided. **Studies CA 8.2.1/02, CA 8.2.2.1/01 and CA 8.2.5.1/01:** A good linearity was stated in the range of 0.1 - 5 mg/L in water. A typical curve in the 1 – 5 mg/L range was provided (n = 5 or 4) but the correlation coefficient and the regression equation were not provided. **Study CA 8.2.4.1/01:** A good linearity was stated in the range of 25 - 100 mg/L in methanol. A typical curve was provided (n = 3 + origin point) but the correlation coefficient and the regression equation were not provided. **Study CA 8.2.2.1/02:** A good linearity was stated in the range of ~ 16 - 2080 µg/L (duplicate injection). A typical curve together with regression equation were provided and r^2 was found to be 0.9996 (n = 6).
Recovery:	Not demonstrated. Only the measured concentrations in the test samples were reported (mean at Day 0 and 4).
Repeatability:	Not demonstrated.
Reproducibility:	Not demonstrated.
Limit of quantification:	0.1 mg/L in all studies except in CA 8.2.4.1/01 where an LOQ of 25 mg/L was stated and in CA 8.2.2.1/02 where an LOQ of 7.6 µg/L was stated.

RMS conclusion renewal (2018):
No validation of the HPLC-UV method occurred in these study reports. The analytical phases only report the results for the measured concentrations with some data on linearity and chromatograms. It is therefore difficult to state if the method was "fit for purpose".

The following studies (studies No. 9 to 25) were not evaluated at EU level for the 1st approval lenacil and have been submitted in the frame of the renewal. Details of the methods and validation data are summarised here below.

Previous evaluation:	*No, submitted for the purpose of renewal*

Study No. 9

Report:	**CA 4.1.2(f)/09 (KCA 4.1.2.16/01)** Nixon W. B., Kendall T. Z (2012) Analytical method verification for the determination of lenacil (DPX-B0634) technical in algal medium. Wildlife International, Ltd., USA Wildlife study No. 112C-192 DuPont Report No.: DuPont-35031

Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes

Matrix: 20 × AAP algal medium

Description of the method:
Samples were diluted with 20X AAP algal medium (as necessary such that samples fall within the calibration range) using volumetric flasks and gas-tight syringe. Concentrations of lenacil in the samples were determined by reversed-phase HPLC-MS/MS. External calibration.

Chromatographic conditions:
Column: Thermo Betasil C18, 50 mm × 2.1 mm, 5µm, Thermo Betasil C18 guard column, 10 mm × 2.1 mm.
Injection volume: 50 µL
Column temperature: 40°C
Mobile phase:

Time (min.)	Flow rate (µL/min)	0.1% formic acid	100% ACN
0.00	300	75	25
0.50	300	75	25
2.00	300	25	75
4.00	300	25	75
4.10	300	75	25
8.00	300	75	25

Retention time: 3.8 min.
Detection: MRM Turbo Ion Spray
235.2 → 153 amu (quantitation)
235.2 → 135.9 amu (confirmation)

Findings:

Specificity/absence of interference: Control samples and reagent blanks showed no interference at the characteristic retention time of lenacil. Chromatograms were provided for a calibration standards (2.00 and 20.0 µg/L), a reagent blank, a matrix blank sample and samples at nominal concentration of 4.00 µg/L). LC-MS/MS is highly specific by monitoring two mass transitions. However, in the present study, data were only generated on a unique m/z transition.
A product ion spectrum was not provided. Details to the chromatograms (i.e. considered m/z transition) were also not provided but it seems obvious that these are for the quantification transition since the recovery results are based on this transition.

Linearity: Calibration curves showed good linearity in the range of 2 to 20 µg/L (n = 5 with single to triplicate determination) for the standards prepared in 20X AAP algal medium with a correlation coefficient r = 0.9993. A typical calibration curve together with the corresponding regression equation was provided within the study report. The samples meet the calibration range.

Recovery: The fortification data reported in the method lenacil residues in aqueous samples are summarised in table B.5.1.2.6-9 below.

Repeatability:	Repeatability of the method is addressed by the data in table B.5.1.2.6-9. Relative standard deviations of less than 20% were obtained for the tested fortifications.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	2 µg/L based upon the product of the concentration of the lowest calibration standard and the dilution factor of the matrix blank samples (1.00).

Table B.5.1.2.6-9 : Validation data for analytical methods for the determination of lenacil in 20X AAP algal medium

Reference	Matrix	Number of tests	Fortification level (µg/L)	Average recovery (%) [range]	Standard deviation	% Relative standard deviation
Nixon W. B., Kendall T. Z (2012) DuPont-35031	20X AAP Algal medium	5 5 5 Total = 15	4 15 75	98.1 [95.8 – 100] 100 [98.7 – 101] 101 [97.3 – 105] Overall = 99.8	1.5 1.8 3.2	1.6 1.8 3.1 Overall = 2.5

Note: two reagent and two matrix blank samples were analysed.

RMS Conclusion 2018 (Renewal) :
The LC-MS/MS method is validated according to SANCO/3029/99. All mean recoveries and % RSD fall within the limits requested by the guidance (i.e. 70 – 110 % and < 20%, respectively). The LOQ of the method was set to 2.00 µg/L within the study report but was not substantiated by experimental data and therefore RMS thinks that the LOQ of 4.00 µg/L should be proposed based on the available validation results (see Table B.5.1.2.6-9).
However, the exact ecotoxicological studies in which the method has been used cannot be clearly identified. The notifier has been requested to clarify and answered the following:
"The method from Nixon and Kendall has not been used and/or referenced fully and exactly as such in any ecotox study. However, the method is validated according to SANCO/3029/99 rev. 4 and should be regarded as supportive data."

Study No. 10

Report:	**CA 4.1.2(f)/10 (KCA 4.1.2.16/02, but also under KCA 8.2.4.1/02)** Renner P., (2016a) Acute toxicity of Lenacil technical to *Daphnia magna* in a 48-hour static test BioChem agrar, Machern OT Gerichhain, Germany DuPont Report No.: 15 10 48 031 W Analytical phase (Verification of the concentration of the test item's active ingredient in the test solution) was part of the study report (appendix 3) and performed at BioChem agrar GmbH under project No. 15 10 35 2028 (Renner P. 2016), GLP, unpublished.
Guidelines:	SANCO/3029/99 rev. 4 (11/07/2000), SANCO/12571/2013 for the analytical phase
GLP:	Yes

Matrix: M4 medium (reconstituted water according to ISO 6341).

Description of the method:
Samples M4 medium were diluted and directly analysed by means of reversed phase HPLC-MS/MS (MRM). The detection was performed in the positive ESI ionisation mode monitoring the mass transition 235→153 (for quantitation) and 235→136 (for confirmation). External calibration.

Chromatographic conditions:

Column: Phenomenex Kinetex C18 Evo, 100 × 2.1 mm, 2.6 µm

Mobile phase:

Gradient	A: Water containing 5% acetonitrile, 0.1% (v/v) formic acid and 5 mM ammonium formiate	B: Acetonitrile containing 5 % water, 0.1% (v/v) formic acid and 5 mM ammonium formiate
0.5 min	100%	0%
1.0 min	75%	25%
5.0 min	0%	100%
6.0 min	0%	100%
7.0 min	100%	0%
8.0 min	100%	0%

Flow rate: 0.3 mL/min

Injection volume: 25 µL

Detection: ESI positive, MRM : m/z 235.1→ 153.1 and 235.1 → 136.1

Retention time: ~ 5.2 min.

Findings:

Specificity/absence of interference:

HPLC-MS/MS is a highly specific and self-confirmatory method. Validation results were only provided for the quantitation transition (m/z 235.1 → 153.1), however with no clear mention of that transition in the validation results table.

Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil (< 30% of the LOQ). It can therefore be concluded that few, if any, apparent residues or false positive values would arise.

Chromatograms (TIC and for each mass transition) were provided for calibration standards, blank validation sample (M4 medium), fortified samples at the lowest and highest fortification levels, untreated test solution and treated solution at the beginning and end of the test. Mass spectra were also provided within the study report.

Linearity:

The calibration curve for the six analytical standards of lenacil in M4 medium was linear in the range of 39 µg/L to 117 µg/L (corresponding to ~ 80% to the lowest concentration and to ~ 120% of the highest validation concentration taking into account the sample dilution). R^2 was >0.991. Calibration was prepared in M4-medium. The concentration of the samples meet the calibration range.

Recovery:

Test medium was spiked with test item at two fortification levels. Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.6-10.

Repeatability:

Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in the table above. The relative standard deviations of recovery data

	obtained are within the guideline of ≤20%. This method is adequate for determining lenacil residues in M4 medium.
Limit of quantification:	50.43 µg/L (corresponding to 50.43 mg/L before dilution) corresponding to the lowest concentration tested with acceptable recovery and RSD.

Table B.5.1.2.6-10 : Validation data for analytical methods for the determination of lenacil in M4 medium by HPLC-MS/MS

Reference	Matrix	Number of tests	Nominal fortification level (µg/L) [a]	Average recovery (%)**	% Relative standard deviation
Renner P. (2016a) 15 10 48 031 W	M4 medium	5 5	50.43* 100.87*	101.6 94.3	1.7 4.5

[a] Fortifications were performed with analyte reference standard solutions
* taking into account the sample dilution of 1000 (fortification concentrations before dilution: 50.43 and 100.87 mg/L).
** details on individual recoveries were not reported.
Two blank samples were analysed.

RMS Conclusion 2018 (renewal):
The HPLC-MS/MS method to determine lenacil in M4 medium is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recoveries at each fortification levels were within the recommended range of 70 - 110% and RSD was systematically below 20%. The LOQ is proposed to be 50.43 µg/L (taking into account the sample dilution) and is supported by the experimental data. Taking into the dilution factor and the measured concentration during the test, the working range covers the tested concentration as well as the endpoints derived from the study. The method is therefore considered "fit for purpose".

Study No. 11

Report:	**CA 4.1.2(f)/11 (KCA 4.1.2.16/03 but also under KCA 8.2.5.1/02)** Renner P., (2016b) Toxicity of Lenacil technical to *Daphnia magna* in a 21-day semi-static test BioChem agrar, Machern OT Gerichhain, Germany DuPont Report No.: 15 10 48 032 W Analytical phase (Verification of the concentration of the test item's active ingredient in the test solution) was part of the study report (appendix 5) and performed at BioChem agrar GmbH under project No. 15 10 35 2029 (Renner P. 2016), GLP, unpublished.
Guidelines:	SANCO/3029/99 rev. 4 (11/07/2000), SANCO/12571/2013 for the analytical phase
GLP:	Yes

Matrix: Elendt M4 medium (reconstituted to OECD 211).

Description of the method:
Samples of M4 medium were diluted and analysed by reversed phase HPLC with MS/MS detection. The detection was performed in the positive ESI ionisation mode monitoring the mass transitions 235→153 (quantification) and 235→136 (confirmation). External calibration.

Chromatographic conditions:
Column: Phenomenex Kinetex C18 Evo, 100 × 2.1 mm, 2.6 µm
Mobile phase:

Gradient	A: Water containing 5% acetonitrile, 0.1% (v/v) formic acid and 5 mM ammonium formiate	B: Acetonitrile containing 5 % water, 0.1% (v/v) formic acid and 5 mM ammonium formiate
0.5 min	100%	0%
1.0 min	75%	25%
5.0 min	0%	100%
6.0 min	0%	100%
7.0 min	100%	0%
8.0 min	100%	0%

Flow rate: 0.3 mL/min

Injection volume: 25 µL
Detection: ESI positive, MRM : m/z 235.1→ 153.1 and 235.1 → 136.1
Retention time: ~ 5.4 min.

Findings:

Specificity/absence of interference: HPLC-MS/MS is a highly specific and self-confirmatory method. Validation results were only provided for the quantitation transition (m/z 235.1 → 153.1), however with no clear mention of that transition in the validation results table.
Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil (< 30% of the LOQ). It can therefore be concluded that few, if any, apparent residues or false positive values would arise.
Chromatograms (TIC and for each mass transition) were provided for calibration standards, blank validation sample (M4 medium), fortified samples at the lowest and highest fortification levels, untreated test solution and treated solutions. Mass spectra were also provided within the study report.

Linearity: The calibration curve for the seven analytical standards of lenacil was linear in the range of ~ 4.5 µg/L to 142.8 µg/L (corresponding to ~ 65% to the lowest concentration and to ~ 120% of the highest validation concentration taking into account the sample dilution). R^2 was >0.997. Calibration was prepared in M4-medium. The concentration of the samples meet the calibration range.

Accuracy: Test medium was spiked with test item at two fortification levels. Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in

Table B.5.1.2.6-.

Repeatability: Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in

	the table below. The relative standard deviations of recovery data obtained are within the guideline of ≤20%.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	7.02 µg/L corresponding to the lowest fortification level with acceptable recovery and % RSD.

Table B.5.1.2.6-11 : Validation data for analytical methods for the determination of lenacil in M4 medium by HPLC-MS/MS

Reference	Matrix	Number of tests	Nominal fortification level (µg/L) [(a)]	Average recovery (%)**	% Relative standard deviation
Renner P. (2016b), 15 10 48 032 W	M4 medium	5 5	7.02* 120.6*	95.9 101.3	2.3 2.6

[a] Fortifications were performed with analyte reference standard solutions

* taking into account the sample dilution of 1 or 10 (fortification concentrations before dilution: 7.02 and 1206 µg/L).
** details on individual recoveries were not reported.
Two blank samples were analysed.

RMS Conclusion 2018 (renewal):
The HPLC-MS/MS method to determine lenacil in M4 medium is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification levels was within the recommended range of 70 - 110% and % RSD was systematically below 20%. The LOQ is proposed to be 7.02 µg/L and is supported by the experimental data. Taking into the dilution factors and the measured concentration during the test, the working range covers the tested concentrations (only the highest test concentration is only very slightly above the highest validated fortification level and therefore considered covered) as well as the endpoints derived from the study. The method is therefore considered "fit for purpose".

Study No. 12

Report:	**CA 4.1.2(f)/12 (KCA 4.1.2.16/04 but also under KCA 8.2.6.2/03)** Wenzel, A. (2014a) Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Synechococcus leopoliensis*. Fraunhofer (IME), DE DuPont Report No.: DPT 001/4 10/C Details of the analytical method and results are presented in chapter A.3 (Annex 3: Chemical analytical method and results)
Guidelines:	SANCO/3029/99
GLP:	Yes

Note: The analytical method for the determination of lenacil (CAS 2164-08-1) in water using LC-MS/MS was developed in experiments completed prior to this GLP ecotoxicity study (see DPT-001/4-80/C and DPT 001/4-80/D reported here below – studies No. 18 and 19).

Study No. 13

Report:	**CA 4.1.2(f)/13 (KCA 4.1.2.16/05 but also uner KCA 8.2.6.2/04)** Wenzel, A. (2014b) Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Anabaena flos-aquae*. Fraunhofer (IME), DE DuPont Report No.: DPT 001/4 10/E

	Details of the analytical method and results are presented in chapter A.3 (Annex 3: Chemical analytical method and results) (validation data are the same as in CA 4.1.2.16/04 [CA 8.2.6.2/03])
Guidelines:	SANCO/3029/99
GLP:	Yes

Study No. 14

Report:	**CA 4.1.2(f)/14 (KCA 4.1.2.16/06 but also under KCA 8.2.6.2/02)** Wenzel, A. (2014c) Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Ankistrodesmus falcatus*. Fraunhofer (IME), DE DuPont Report No.: DPT 001/4 10/F Details of the analytical method and results are presented in chapter A.3 (Annex 3: Chemical analytical method and results) (validation data are the same as in CA 4.1.2.16/04 [CA 8.2.6.2/03])
Guidelines:	SANCO/3029/99
GLP:	Yes

Study No. 15

Report:	**CA 4.1.2(f)/15 (KCA 4.1.2.16/07 but also uner KCA 8.2.6.2/05)** Wenzel, A. (2014d) Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Closterium cornu*. Fraunhofer (IME), DE DuPont Report No.: DPT 001/4 10/G Details of the analytical method and results are presented in chapter A.3 (Annex 3: Chemical analytical method and results) (validation data are the same as in CA 4.1.2.16/04 [CA 8.2.6.2/03])
Guidelines:	SANCO/3029/99
GLP:	Yes

Study No. 16

Report:	**CA 4.1.2(f)/16 (KCA 4.1.2.16/08 but also under KCA 8.2.6.2/06)** Wenzel, A. (2014e) Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Xanthonema debile*. Fraunhofer (IME), DE DuPont Report No.: DPT 001/4 10/I Details of the analytical method and results are presented in chapter A.3 (Annex 3: Chemical analytical method and results) (validation data are the same as in CA 4.1.2.16/04 [CA 8.2.6.2/03])
Guidelines:	SANCO/3029/99
GLP:	Yes

Study No. 17

Report:	**CA 4.1.2(f)/17 (KCA 4.1.2.16/09 but also under KCA 8.2.6.2/07)** Wenzel, A. (2014f)

	Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Nannochloropsis limnetica*. Fraunhofer (IME), DE DuPont Report No.: DPT 001/4 10/H Details of the analytical method and results are presented in chapter A.3 (Annex 3: Chemical analytical method and results) (validation data are the same as in CA 4.1.2.16/04 [CA 8.2.6.2/03])
Guidelines:	SANCO/3029/99
GLP:	Yes

Note: That study is not acceptable for risk assessment.

Matrix: OECD water.

Note: for the ecotoxicity test in CA 8.2.6.2/01, the following medium was used: sterilised synthetic medium according to Bringmann and Kühn (1980) (Bringmann, G. and Kühn, R. (1980). Comparison of the toxicity thresholds of water pollutants to bacteria. algae, and protozoa in the cell multiplication inhibition test. Water Research 14(3), 231-241)) which is more highly concentrated than the OECD medium recommended for green algae in OECD 201 and in CA 8.2.6.2/04, the Bacillariophycean medium (No. 11, SAG medium) was used as growth medium.

Description of the method:
Frozen aqueous samples were warmed up to room temperatures, ultrasonicated for 10 minutes and mixed thoroughly by hand. The algae in the samples were separated from the test media by centrifugation. An aliquot of each of the aqueous samples was taken, an internal standard solution of terbacil was added and each sample was analysed by LC-MS/MS.

Chromatographic conditions:
Column: BEH C18, 100 × 2.0 mm, 1.7 µm
Column temperature: 55°C
Mobile phase:

Gradient	A: Water/Methanol/formic acid (89.9+10+0.1 v,v)	B: Methanol/formic acid (99.9+0.1 v/v)
0.0 min	100%	0%
6.0 min	0%	100%
8.0 min	0%	100%
12.0 min	100%	0%

Flow rate: 0.3 mL/min

Injection volume: 10-50 µL
Detection: ESI positive, MRM : m/z 235.5→ 153.2 for lenacil and 161.2 → 144.2 for internal standard
Retention time: ~ 5.1 min for lenacil and ~ 4.7 min for internal standard

Findings:
Specificity/absence of interference: LC-MS/MS is known to be a highly specific and self-confirmatory method. Validation results were however only provided for one m/z transition (m/z 235.5 → 153.2).
Control samples showed none to insignificant interference (<30% of the LOQ) at the characteristic retention times of lenacil and the internal standard terbacil.
Chromatograms (m/z 235.2 → 153.2 for lenacil and m/z 161.2 → 144.2 for internal standard) were provided for two calibration standards, blank control sample (OECD medium), fortified samples at the lowest and highest fortification levels, control samples (growth

medium), test samples at the lowest and highest levels at the start and endo of the test. A mass spectra was not provided.

Linearity: The calibration curve showed good linearity in the range of 0.099 to 89.4 µg/L (3 series of calibration solutions were prepared covering respectively the 0.099 – 0.894 µg/L range, the 0.99 – 8.94 µg/L range and the 9.9 – 89.4 µg/L range, each with n = 9, calibration curve constructed on 27 calibration solutions) with $r^2 > 0.9997$. The regression equation was also provided. All standards were prepared in solvent (water) with terbacil as the internal standard. The concentration of the samples meet the calibration range. Samples exceeding the 89 µg/L level were diluted (0.1 mL sample + 0.8 mL water + 0.1 mL internal standard).

Recovery: Samples of OECD medium were fortified with lenacil at two levels. The fortification data reported for lenacil residues in aqueous samples are summarised in Table B.5.1.2.6- below; the mean recovery per fortification level was 70 % - 110 % with RSD of < 20 %.

Repeatability: Repeatability of the method is addressed by the data in Table B**.5.1.2.6-**. Relative standard deviations of less than 20% were obtained for the tested fortifications. Therefore, the repeatability of this method would seem to be adequate for the purposes of detecting lenacil residues in the formulation.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: 0.5 µg/L.

Table B.5.1.2.6-12 : Validation data for analytical methods for the determination of lenacil in aqueous solutions

Reference	Matrix	Number of tests	Fortification level (µg/L)	Average recovery (%) [range]	% Relative standard deviation
Wenzel, A. (2014a-f) DPT 001/4 10/C DPT 001/4 10/E DPT 001/4 10/F DPT 001/4 10/G DPT 001/4 10/I DPT 001/4 10/H	OECD matrix control water	5 5 Total = 10	0.50 4.97	106.2 [100.3 – 110.4] 103.9 [103.0 – 105.5] Overall = 105.1	3.5 0.9 Overall = 2.7

Two untreated control samples (blank samples) were analysed.

RMS Conclusion 2018 (Renewal):
The LC-MS/MS method to determine lenacil in algal medium (OECD medium) is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification levels was within the recommended range of 70 - 110% and % RSD was systematically below 20%.
The LOQ is proposed to be 0.5 µg/L and is supported by the experimental data.
The LOQ covers all the endpoints and the lowest test concentrations. Although dilution occurred, the measured concentrations seem however to fall outside the validated range of 0.5 – 5 µg/L but remain within the calibration range.

Study No. 18

Report:	**CA 4.1.2(f)/18 (KCA 4.1.2.16/10 but also under KCA 8.2.7/03)** Wenzel A., (2012a) Macrophytes, growth inhibition test – Effect of lenacil technical on the growth of *Elodea Canadensis* in the present of sediment Fraunhofer (IME), DE DuPont Report No.: DPT-001/4-80/C Details of the analytical method are presented in chapter A.3 (Annex 3: Analytical Report – Detaild of Method and Results)
Guidelines:	SANCO/3029/99 rev. 4 SANCO/825/00 rev. 7
GLP:	Yes

Note: The analytical method for the determination of lenacil (lenacil technical, CAS no. 2164-08-1) in water using LC-MS/MS was developed in experiments, which were completed prior to the current ecotoxicity studies.

Study No. 19

Report:	**CA 4.1.2(f)/19 (KCA 4.1.2.16/11 but also under KCA 8.2.7/02)** Wenzel, A. (2012b) Macrophytes, growth inhibition test – Effect of lenacil technical on the growth of *Chara globularis* in the present of sediment Fraunhofer (IME), DE DuPont Report No.: DPT-001/4-80/D Details of the analytical method are presented in chapter A.3 (Annex 3: Analytical Report – Detaild of Method and Results)
Guidelines:	SANCO/3029/99 rev. 4 SANCO/825/00 rev. 7
GLP:	Yes

Matrix: plant growth medium

Description of the method:
Frozen samples of aqueous test medium were thawed and ultrasonicated; sample aliquots were taken, diluted with methanol and mixed with the internal standard solution of terbacil in methanol. Sample analysis for lenacil was carried out by reverse-phase liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) using electrospray ionization (ESI) in the positive mode, monitoring the mass transition 235→153.

Chromatographic conditions:
Column: Waters Acquity UPLC BEH C18, 50 × 21.0 mm, 1.7 µm
Column temperature: 30°C
Mobile phase:

Gradient	A: Methanol with 2 mmol/L NH_4Ac	B: Purified water/methanol (90/10 v/v) with 2 mmol/L NH_4Ac
0.0 min	30%	70%
0.5 min	30%	70%
4.0 min	100%	0%
6.0 min	100%	0%
6.1 min	30%	70%
9.0 min	30%	70%

Flow rate: 0.2 mL/min

Injection volume: 25 µL

Detection:	ESI positive, MRM : m/z 235.17→ 153.06 for lenacil and 161.04 → 143.91 for internal standard
Retention time:	~ 4.7 min for lenacil and ~ 4.2 min for internal standard

Findings:

Specificity/absence of interference:	LC-MS/MS is known to be a highly specific and self-confirmatory method. Validation results were however only generated for one m/z transition (m/z 235.17 → 153.06). Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil or terbacil. It can therefore be concluded that few, if any, apparent residues or false positive values would arise. Chromatograms (lenacil and internal standard) were provided for calibration solutions, blank sample, control samples during the test, fortified samples at both levels and test samples at the start and end. No mass spectrum was provided but well in studies No. 12 to 17 by Wenzel (see above).
Linearity:	The calibration curve of the ten analytical standards of lenacil in plant growth medium in the range of 0.080 µg/L to 50.0 µg/L was linear. The regression equation (quadratic regression) was provided and R^2 was >0.999in CA 8.2.7/02 and $r^2 = 1$ in CA 8.2.7/01. Terbacil diluted in methanol was used as internal standard. The concentration of the samples meet the calibration range.
Recovery:	Samples of plant growth medium were fortified with lenacil at two levels. Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.6-13.
Repeatability:	Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in the table below. The relative standard deviations of recovery data obtained are within the guideline of ≤20%.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	0.1 µg/L corresponding to the lowest fortification level with acceptable recovery and % RSD.

Table B.5.1.2.6-13 : Validation data for analytical methods for the determination of lenacil in plant growth medium by LC-MS/MS

Reference	Matrix	Number of tests	Fortification level (µg/L) [a]	Average recovery (%) [range]	Standard deviation	% Relative standard deviation
Wenzel A. (2012a-b) DPT-001/4-80/C DPT-001/4-80/D	Plant growth medium	5 5 Total = 10	0.1 1	102 [100 – 105] 102 [100.4 – 107.1] Overall = 102	2.2 2.8 Overall = 2.4	2.1 2.7 Overall = 2.3

[a] Fortifications were performed with analyte reference standard solutions
Two untreated control samples (blank samples) were analysed.

RMS Conclusion 2018 (Renewal):

The LC-MS/MS method to determine lenacil in plant growth medium is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification level was within the recommended range of 70 - 110% and % RSD was systematically below 20%.
The LOQ is proposed to be 0.1 µg/L and is supported by the experimental data.
The LOQ covers all the endpoints and the lowest test concentrations. The validated working range does not fully cover all the test concentrations. Although dilution occurred, the measured concentrations fall outside the validated range of 0.1 – 1 µg/L but remain within the calibration range.

Study No. 20

Report:	**CA 4.1.2(f)/20 (KCA 4.1.2.16/12 but also under KCP10.6.2/02)** Stürz S., Knebel N. (2016) Lenacil 500 g/L SC: Effects on terrestrial (non-target) plants: Vegetative Vigour Test Ibacon GmbH, DE DuPont Report No.: 95231087
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes

Note: the stock solution from which the subsequent concentrations solutions were prepared by serial dilutions was checked analytically in the Analytical Phase (Appendix II). Validation data were also summarized under that section.

Matrix: aqueous test solution (deionized water).

Description of the method:
An aliquot of each aqueous test solution was diluted with acetonitrile:pure water (50:50, v:v) while solutions were stirring and then analysed by reverse-phase HPLC with UV detection at 268 nm. External calibration.

Chromatographic conditions:

Column:	US ES Pharm RP18, 150 × 3.0 mm, 5 µm
Oven temperature:	40°C
Mobile phase:	60% (95% acetonitrile/5% pure water) and 40% pure water + 0.3% H_3PO_4 (using 85% phosphoric acid)
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Detection:	UV-Vis at 268 nm

Findings:

Specificity/absence of interferences:	Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil. It can therefore be concluded that few, if any, apparent residues or false positive values would arise.
	Chromatograms were provided for a standard solution, solvent control, control solution, a fortified sample at the lowest fortification level and the stock solution.
	The identity of the analyte which was used for quantification of the test item concentration was confirmed by comparison of the retention time with the retention time of a standard solution prepared from the reference item.
Linearity:	The calibration curve was linear for the six analytical standards of lenacil in the range of ~ 20 mg/L to 80 mg a.i./L. The correlation coefficient R was equal to 0.9999. The typical calibration curve and linear regression calibration curve were provided within the report. Calibration standards were prepared in acetonitrile/pure water (50/50, v/v). The concentration of the samples meet the calibration range.

Recovery:	Samples were fortified at two levels with lenacil 500 g/L SC. Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.6-14.
Repeatability:	Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in Table . The relative standard deviations of recovery data obtained are within the guideline of ≤20%.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	9 g PPP/L diluted by a factor 100 and therefore corresponding to 90 mg PPP/L. This level corresponds to the lowest fortification level at which acceptable recovery and % RSD was obtained. LOD was estimated to be 0.05 mg a.i./L.

Table B.5.1.2.6-14: Validation data for analytical methods for the determination of lenacil in stock solution (water) by HPLC-UV

Reference	Matrix	Number of tests	Fortification level (PPP g/L) [(a)] (dilution by a factor 100)	Corresponding Fortification level (a.s. g/L) (dilution by a factor 100)	Average recovery (%) [range]	% Relative standard deviation
Stürz S., Knebel N. (2016) 95231087	Aqueous test solution	5 5 Total = 10	9 (90 mg/L) 13 (130 mg/L)	~ 4.1 (~ 41 mg/L) ~ 6.0 (~ 60 mg/L)	78 [72 – 84] 85 [79 – 90] Overall = 82	6 5 Overall = 7

[a] Fortifications were performed with analyte reference standard solutions

Two independent replicates of solvent control were also analysed as well as on replicate of control and one replicate of stock solution at 10.97 g PPP/L). For the solvent control and control, the concentration found was below the LOD and for the stock solutions, a recovery of 86% was obtained.

Note: the repeatability of the injection was also investigated with standard solution and found to be 0.2%

RMS Conclusion 2018 (Renewal):

The HPLC-UV method to determine lenacil in aqueous test solutions is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification level was within the recommended range of 70 - 110% and % RSD was systematically below 20%.

The LOQ is proposed to be 9 g PPP/L (corresponding to 90 mg PPP/L after applying the dilution factor of 100) and is supported by the experimental data. That level corresponds to ~ 4.1 g a.s./L (or ~ 41 mg a.s./L after applying the dilution factor of 100).

In the ecotoxicity study, only the stock solution of 10.97 g PPP/L (= ~ 5 g a.s./L) before dilution and from which the other solutions were prepared by subsequent dilutions was analysed. The level of the stock solution is covered by the LOQ and the validated working range. The method is considered “fit for purpose”.

The following ecotoxicity studies were not considered for first approval and have been also submitted in the renewal dossier. However, the details and assessment of the analytical method were not reported in the section 4 of the summary dossier for the renewal. Therefore, a consideration of the analytical methods by section 1 was requested by the ecotox. section. In order to avoid a change of all the cross-references in the ecotox. section, the following studies will be numbered as CA 4.1.2(f)/20.xx. (studies No. 20.xx).

Study No. 20.01

Report:	**CA 4.1.2(f)/20.01 (CP 10.2.1/01)** Pawlowski S. And Wydra V. (2006) Toxicity of Venzar 500 SC to Pseudokirchneriella subcapitata in an algal growth inhibition test Ibacon GmbH, DE Project No.: 26801210
Guidelines:	Not stated
GLP:	Yes

Matrix: algal medium (reconstituted water).

Study No. 20.02

Report:	**CA 4.1.2(f)/20.01 (CP 10.2.1/02)** Pawlowski S. And Wydra V. (2006) Toxicity of Venzar 500 SC to the aquatic plant Lemna gibba in a semi-static growth inhibition test. Ibacon GmbH, DE Project No.: 26802240
Guidelines:	Not stated
GLP:	Yes

Matrix: 20 × AAP medium.

Description of the method:
The method for determination is based on the method entitled "*Validation of a Monitoring Method for Determination of Lenacil Residues in Surface Water, Tap Water and Ground Water* – UCL GmbH (Wittig A. 2002)" (please refer to B. 5.1.2.7 – study No. 1) and has been adapted in the analytical laboratories of IBACON. The samples were enriched by means of solid phase extraction (SPE). Solid phase cartridges (LiChroLut EN) were conditioned with 5 mL HPLC-water and 5 mL methanol. Afterwards different amounts of the test samples (depending on the needed enrichment factor) were added to the cartridges, which were sucked to dryness. Elution was performed by application of 2 × 5 mL of a solution containing 50% acetonitrile/50% pure water on the cartridge with a pause of 5 min between the two elution steps. The technique used is LC-MS/MS. External calibration.

Chromatographic conditions:

Column:	Synergi 4µ Polar-RP 80A, 150 × 3.0 mm
Mobile phase:	80% acetonitrile/20% HPLC water/0.05% acetic acid
Flow rate:	0.3 mL/min
Injection volume:	20 µL
Ionization mode:	positive
Mass transitions :	235 → 153 amu

Findings:

Specificity/absence of interferences:	LC-MS/MS is known to be a highly selective method. However, only one mass transition was followed. Additional identification is possible by comparison of the retention time of the test item with the retention time of a standard solution of the test item. Based on the provided chromatograms, no interferences seem to occur. Chromatograms were provided for a standard solution, a control sample, a fortified sample at 10 µg/L and a test sample.
Linearity:	**In Study CP 10.2.1/01:**

The calibration curve was linear for the nine analytical standards in the range of 10 to 600 µg PPP/L in 50% acetonitrile/50% pure water. The regression coefficient was at least 0.9988. A typical calibration curve and the regression equation were provided within the report.

In Study CP 10.2.1/02:
The calibration curve was linear for the eight analytical standards in the range of 10 to 800 µg PPP/L in 50% acetonitrile/50% pure water. The regression coefficient was at least 0.9984. A typical calibration curve and the regression equation were provided within the report.

Recovery: Samples were fortified at three levels with venzar 500 SC. Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.6-14.01.

Repeatability: Repeatability of this method was demonstrated by the relative standard deviations of the recovery values given in Table B.5.1.2.6-14.01. The relative standard deviations of recovery data obtained are within the guideline of ≤20%.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: 1 µg PPP/L (corresponding to 0.417 µg a.s./L) (enriched by factor 25) in study CP 10.2.1/01 and 10 µg PPP/L (enriched by factor 5) in study CP 10.2.1/02.

Table B.5.1.2.6-14.01: Validation data for the determination of lenacil in reconstituted water and 20 × AAP medium by LC-MS/MS

Reference	Matrix	Number of tests	Fortification level PPP µg/L (a.s. ug/L)	Average recovery (%) [range]	% Relative standard deviation
Pawlowski S. And Wydra V. (2006)	Reconstituted water (algal medium)	4 4 4	1 (0.417)[a,*] 10 (4.167)[b] 100 (41.667)[c]	98 [79 – 110] 84 [79 – 92] 94 [89-100]	14 7 6
Pawlowski S. And Wydra V. (2006)	20 × AAP medium	6 5 6	3 (1.25)[b] 10 (4.167)[c] 100 (41.667)[c]	**122** [81-**182**]*** 94 [77-107**] 83 [75-89]	>> **20** 13.4 5.3

[a] Enrichment factor 25
b Enrichment factor 10
c Enrichment factor 5
* the first results at the fortification level of 1 µg/L gave a mean recovery of 179% with a % RSD of 30%. Therefore, this fortification level was prepared again and analysed a second time giving the results presented in the table here above.
** one result from the 6 replicates was removed (recovery of 480%).
*** 3 of the 6 replicates were above 120%.The average recovery and RSD for determination in 20 × AAP medium have re-calculated by RMS based on the available data.
Controls analysed during the test showed all no residue of lenacil (< LOQ).
Note: study CP 10.2.1/01: mean recovery in test samples in the 1 – 100 µg PPP/L range after 0 h is 88% (n=10, RSD = 10%) and after 72 h is 78% (n=6, RSD = 11%). Study CP 10.2.1/02: mean recovery in test samples is 102% (n =8, RSD = 16%) at 3 µg PPP/L, 107% (n = 7, RSD = 12%) at 10 µg PPP/L, 101% (n = 10, RSD = 19%) at 30 µg/L and 103% (n = 11, RSD = 13%) at 100 µg PPP/L.

RMS conclusion 2018 (renewal):
The LC-MS/MS method to determine lenacil residue in reconstituted water can be considered sufficiently validated according to SANCO/3029/99. The absence of interference and linearity were sufficiently demonstrated and the mean recoveries and % RSD were within the acceptable limits for each fortification levels. Only the number of replicates does not follow the recommendation of SANCO/3029/99 but RMS is of the opinion that the number of replicates is sufficient to show that the method worked accurately and precisely. The LOQ is proposed to be 1 µg PPP/L (enriched by a factor 25). This level is well supported by the experimental data and can be sufficiently validated.
The method is considered "fit for purpose" since the LOQ and the validated working range support the ErC50.

The LC-MS/MS method for determining lenacil residue in 20 × AAP medium can also be considered validated according to SANCO/3029/99 in the 10 – 100 µg PPP/L (absence of interference and linearity were demonstrated and the mean recoveries and % RSD were within the acceptable limits). The LOQ is proposed to be 10 µg PPP/L (enriched by factor 5) based on the accuracy experiment. This is acceptable and the level is considered sufficiently validated. The lowest level tested (3µg PPP/L) cannot be proposed for the LOQ based on the accuracy experiment results (that level cannot be considered as validated). The method has been considered "fit for purpose" (ErC50 > LOQ).

Feed

Previous evaluation:	*Initial monograph November 2007*

The following studies (studies No. 21 to 22) were already considered for the first approval of Lenacil. Nevertheless, the details and assessment of the analytical method were not reported in the initial DAR. Those are now summarized here below for the sake of completeness.

Study No. 21

Report:	**CA 4.1.2(f)/21 (CA8.1.1.2/01, previously IIA 8.1.2-01 in the old dossier)** [redacted]2004a) Lenacil technical – Dietary toxicity (LC_{50}) to the Bobwhite quail [redacted] [redacted] project identity: DPT/637 DuPont Report No.: DPT 637/033931
Guidelines:	Not stated
GLP:	Yes

Analytical phase is presented in appendix 4 of the study report. In this appendix, details and summary of the validation of the method were provided with a reference done to Dawe I.S. (2003) – analysis of Lenacil Technical in Chick Diet Formulation, Huntingdon Life Sciences Analytical Method ERC/FCH/M21/03 for validation and to Dupont report No. AMR-3747-96 – revision 1 for the analytical procedure which were not submitted within the renewal dossier.
Both reports were provided in the course of the assessment. ERC/FCH/M21/03 – Analysis of Lenacil in chick diet formulation (2003) only details the procedure to be used for the HPLC assay of lenacil in chick diet at concentrations in the 100 – 5000 mg/kg range with a range of 150 – 5000 mg/kg for validation. No validation results were generated within this report.
Report No. AMR-3747-96 was in fact already part of the submission dossier and concerns the analysis and certification of product ingredients for technical grade lenacil. It was not fully clear why reference to that report was made for the determination of lenacil in chick diet. The notifier answered that it was a typo and that reference should have been made to Report No. AMR 3419-95 (see Study No. 22). However, it is noted that the conditions in AMR 3419-95 are not identical to the conditions of the present method.

Matrix: avian diet formulations

Description of the method (ERC/FCH/M21/03):
Lenacil residues were extracted from test diet formulation by shaking in methanol; extract aliquots were filtered, diluted with methanol (expected concentration of lenacil between 10 and 20 µg/mL), and analysed by reverse phase HPLC with UV detection at 268 nm. External calibration was used.

Chromatographic conditions:
Column: Hypersil 5ODS, 100 × 4.6 mm (for homogeneity and stability analyses)
Hypersil 5ODS, 250 × 4.6 mm (for LC_{50} diet analysis)

Guard column:	C18 Security Guard, 4 × 3 mm, Phenomenex
Column temperature:	40°C
Mobile phase:	Acetonitrile/water (30/70, v/v) (for homogeneity and stability analyses)
	Acetonitrile/0.1M ammonium acetate (30/70, v/v), pH 5 (adjusted with acetic acid) (for LC_{50} diet analysis).
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Detection:	UV, 269 nm
Retention time:	~ 5.2 min or ~ 12.2 min depending on the chromatographic conditions.

Note: the column and the mobile phase were modified for the analysis of the LC_{50} diets in order to resolve an interfering diet co-extractive peak from the lenacil peak. The diet co-extractive co-eluted with the analyte peak in the chromatograms for the LC_{50} diets when using the initial conditions, although it was not present in the chromatograms for the homogeneity and stability diet formulations.

Findings:

Specificity – absence of interferences:	Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil. It can therefore be concluded that no apparent residues or false positive values would arise.
	Chromatograms were provided for a calibration standard solution, a control, an homogeneity sample and a fortified sample at the lowest and highest concentration levels but chromatograms were also provided for a calibration standard solution, a control, test samples and fortified samples at the lowest and highest concentration levels using the modified chromatographic conditions.
Linearity:	The calibration curve was linear for the five analytical standards of lenacil in the range of 5 µg/L to 25 µg/L (n = 5, triplicate analysis). The correlation coefficient R was equal to 0.99981. A typical calibration curve with the corresponding regression equation were provided within the study report. The calibration solutions were prepared in methanol. Matrix effects were not investigated.
Recovery:	Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.6-15.
Repeatability:	Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in

	the table here below. The relative standard deviations of recovery data obtained are within the guideline of ≤20%.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	LOQ was not stated within the study report (LOD stated to be 7.5 ppm). An LOQ of 156 mg/kg was stated in the summary dossier.

Table B.5.1.2.6-15 :Validation data for analytical methods for the determination of lenacil in bird feed (HPLC-UV)

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a]	Average recovery (%)	Standard deviation	% Relative standard deviation
Validation results						
[redacted] (2004) DPT 637/033931	Bird feed	6 2* Total = 8	156	101.1 100.4 Overall = 100.9	0.5 - -	0.5 - 0.6
		6 2* Total = 8	5000	99 99.9 Overall = 100	0.8 - Overall = 0.8	0.8 - Overall = 0.8
Homogeneity results						
[redacted] (2004) DPT 637/033931	Bird feed	2 (top) 2 (middel) 2 (bottom)	156	99.7** 100.3** 100.3** Overall = 100**	- - - -	- - - 1.2
		2 (top) 2 (middel) 2 (bottom)	5000	99.2** 100** 99.6** Overall = 99.6**	- - - -	- - - 0.5
Stability results (after 8 days at RT (nominally 21°C, 50% humidity with exposure to continuous 12 hour cycle of light and dark -						
[redacted] (2004) DPT 637/033931	Bird feed	1 1	156 5000	98.7** 98.8**	- -	- -
Procedural recoveries						
[redacted] (2004) DPT 637/033931	Bird feed	1	156	97.1	-	-
		1	313	97.2	-	-
		1	625	100.7	-	-
		1	1250	**110.7**	-	-
		1	2500	102.5	-	-
		1	5000	98.5	-	-

[a] Fortifications were performed with analyte reference standard solutions
* Reproducibility of the validation after 1 day
** each analysed in duplicate

Note: the system precision was evaluated by determining the precision of six replicate injections at 5 and 25 µg/mL and found to be < 1.5%.

Table B.5.1.2.6-16 : Dose verification in avian diet formulation (HPLC-UV)

Reference	Matrix	Analyte	Nominal Dose level [mg/mL]	Number of Replicates**	Mean analysed conc. In mg/mL (% of nominal dose level*)	RSD [%]
[redacted] (2004) DPT 637/033931	Bird feed	Lenacil	156	1	92.9*	-
			313	1	93.6*	-
			625	1	100.5*	-
			1250	1	100*	
			2500	1	103.2*	
			5000	1	99.6*	

* Re-calculated by RMS based on the results available in the report.
** Duplicate injection.
Note: the report mentions that analytical results were corrected for the appropriate procedural recoveries determined at analysis.

RMS Conclusion 2018 (renewal):
The HPLC-UV method to determine lenacil in avian diet formulation is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification level was within the recommended range of 70 - 110% and % RSD was systematically below 20%. The homogeneity of the diet formulation was demonstrated and the stability was estimated to be 8 days at ambient temperature.
The LOQ was not stated within the study report but is proposed to be 156 ppm corresponding to the lowest successfully validated concentration level as recommended in SANCO/3029/99. That LOQ level is fully supported by the validation data. The validated LOQ as well as the validated working range cover the determined endpoint and test concentrations used in the ecotoxicity study. The method, being fully validated, is "fit for purpose".

Study No. 22

Report:	**CA 4.1.2(f)/22 (CA8.1.1.3/01, previously IIA 8.1.3-01 in the old dossier)** [redacted] 1996) A reproduction study with the northern bobwhite (*Colinus virginianus*) [redacted] [redacted] project No. 112-416 DuPont Report No.: AMR 3419-95
Guidelines:	Not stated
GLP:	Yes

Analytical phase is presented in appendix XII of the study report (analytical report HA-96-006, GLP, Charlotte H. Lattin B.S. 1996). Samples were submitted to Haskell Laboratory for analysis.

Matrix: avian diet formulations

Description of the method:
Lenacil residues were extracted from the avian diet samples by sonicating in methanol. Extract aliquots were filtered and analysed by reverse-phase HPLC with UV detection at 269 nm. External calibration was used.

Chromatographic conditions:
Column: Zorbax RX-C18, 2.1 × 150 mm, 5 µ particle size
Column temperature: 40°C

Mobile phase:	40% Acetonitrile/60% 3.1 mM H_3PO_4 water
Flow rate:	0.3 mL/min
Injection volume:	2 µL
Detection:	UV, 269 nm
Retention time:	~ 2.4 min

Findings:

Specificity – absence of interferences:	Analysis of control samples resulted in no detectable apparent residues of lenacil; the response in the area of the lenacil peaks were always corresponded to less than 20% of the limit of determination. It can therefore, be concluded that few, if any, apparent residues or false positive values would arise. Chromatograms were provided for a calibration standard solution, a control diet sample and a fortified sample.
Linearity:	Calibration curve showed good linearity in the range of 0 mg/kg to ~ 120 mg/kg (n = 5, duplicate analysis) for lenacil with a correlation coefficient equal to 0.9990. A typical calibration curve with the corresponding regression equation were provided within the study report. Calibration solutions were prepared in methanol. Matrix effects were not investigated.
Recovery:	The fortification data reported in the study are summarised in tables B.5.1.2.6-17 and B.5.1.2.6-18 below. Recovery samples were prepared at two fortification levels (98.5 and 1024 ppm) by adding lenacil to control diet.
Repeatability:	Where possible, the repeatability was re-determined based on the available results (homogeneity) but repeatability cannot be determined based on the recovery tests during validation due to insufficient number of replicates at each fortification level.
Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	The LOQ was not stated within the study report.

Table B.5.1.2.6-17 : Validation data for analytical methods for the determination of lenacil in avian feed (HPLC-UV)

Reference	Matrix	Number of tests	Fortification level (mg/kg)	Average recovery (%)	Standard deviation***	% Relative standard deviation***
[redacted] (1996), AMR 3419-95	Avian diet	**Recovery test**				
		1	98.5	88.3	-	-
		1	1024	91.5	-	-
		Total = 2		Overall = 89.9	-	-
		Homogeneity test				
		6*	100****	92.7	11.5	12.4
		6*	320****	103.8	9.9	9.6
		6*	1024	104.5	5.2	5.0
		Total = 18		Overall = 100		Overall = 10.3
		Stability in avian diets for 7 days				
		1	100	96.9	-	-
		1**	320	113.1	-	-
		1	1024	112.3	-	-

* Two samples from the top + two samples from the middle and two samples from the left.
** Sample re-analysed in duplicate and mean therefore based on n = 3.
*** Re-calculated by the notifier based on the results available in the study report.
**** For one sample the recovery was above 120% and the sample were re-analysed in duplicate and the mean (n=3) of the results was taken into consideration.

Table B.5.1.2.6-18 : Dose verification (HPLC-UV)

Reference	Matrix	Analyte	Nominal Dose level [ppm]	Number of Replicates**	% of nominal dose level*	RSD [%]*
[redacted] 1996), AMR 3419-95	Avian diet	Lenacil	100	7	91*	6.6
			320	7	101.2*	4.8
			1024	7	102.7*	8.3

* Re-calculated by RMS based on the results available in the report.
** Samples were analysed at Day 0 from seven different weeks.

RMS Conclusion 2018 (renewal):
The validation data of the HPLC-UV method to determine lenacil in avian diet formulation does not answer to the current requirements according to SANCO/3029/99. The absence of interference and linearity were well demonstrated but the number of replicates at each fortification levels to demonstrate the accuracy was insufficient (n = 1 at each level) and the repeatability cannot be determined. The recovery obtained is however within the acceptable limit of 70 – 110 %. Although not sufficiently validated according to SANCO/3029/99, the method seems to correctly work by taking into consideration the overall available data (homogeneity, stability and dose verification).
The LOQ of the method was not stated within the study report but based on the overall data an LOQ of 100 ppm seems to be reasonably supported. That LOQ and the working range cover the determined endpoint and test concentrations used in the ecotoxicity study. RMS is of the opinion that the method, although not fully validated according to SANCO/3029/99, can be considered "fit for purpose".
The homogeneity of the diet formulation was demonstrated and the stability was estimated to be 7 days at ambient temperature.

Previous evaluation:	*No, submitted for the purpose of renewal*

The following studies (studies No.23 to 25) were not evaluated at EU level for the 1st approval lenacil. They are new and have been submitted in the frame of the renewal. Details of the methods and validation data are summarised here below.

Study No. 23

Report:	**CA 4.1.2(f)/23 (KCA 4.1.2.17/01 but also under KCP10.3.1.2/01)** Haupt S., Knebel N. (2016a) Lenacil 500 g/L SC: Chronic oral toxicity test on honey bee (*Apis mellifera* L.) in the laboratory Ibacon GmbH, DE DuPont Report No.: 95231136
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes

Note: the Analytical Phase is reported in Appendix II of the study report.

Matrix: sugar (sucrose) feeding solution.

Description of the method:
Feeding solution samples were diluted with acetonitrile:pure water (50:50, v:v) while solutions were stirring and then analysed by reverse phase HPLC with UV detection at 268 nm. External calibration.

Chromatographic conditions:

Column:	US ES Pharm RP18, 150 × 3.0 mm, 5 µm
Oven temperature:	40°C
Mobile phase:	60% (95% acetonitrile/5% pure water) and 40% pure water + 0.3% H_3PO_4 (using 85% phosphoric acid)
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Detection:	UV-Vis at 268 nm

Findings:

Specificity-absence of interferences: Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil.
Chromatograms were provided for a standard solution, a solvent control (50% aqueous sucrose solution), a fortified sample at the lowest concentration and feeding solutions at low and high concentration on day 0 and on day 4.
The identity of the analyte which was used for quantification of the test item concentration was confirmed by comparison of the retention time with the retention time of a standard solution prepared from the reference item.

Linearity: Calibration curve of the seven analytical standards of lenacil was linear in the range of 5 mg a.s./L to 80 mg a.s./L. A typical calibration curve and the corresponding linear regression equation were provided. The correlation coefficient R was equal to 0.9997. Calibration solutions were prepared in acetonitrile/pure water (50/50, v/v). The concentration of the samples meet the calibration range.

Recovery: The samples were fortified at two levels with the formulation (Lenacil 500 g/L SC). Dilution occurred by a factor 50 for the 1 g PPP/L level and by a factor 200 for the 20 g PPP/L. Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.6-19.

Repeatability: Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in

table B.5.1.2.6-19. The relative standard deviations of recovery data obtained are within the guideline of ≤20%. This method is adequate for determining lenacil concentration in feeding solution.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification: 1 g PPP /L (= ~ 0.45 g a.s./L) diluted by a factor 50 and therefore corresponding to 20 mg PPP/L (= ~ 9 mg a.s./L). That level corresponds to the lowest fortification level at which an acceptable recovery and % RSD were obtained. The LOD was set to 0.05 mg a.s./L.

Table B.5.1.2.6-19 :Validation data for analytical methods for the determination of lenacil in sucrose feeding solution by HPLC-UV

Reference	Matrix	Number of tests	Fortification level (g PPP/L) [level after dilution in mg/L] [(a)]	Corresponding Fortification level (a.s. g/L) [level after dilution in mg/L]	Average recovery (%) [range]	% Relative standard deviation
	Validation data					
Haupt S., Knebel N. (2016a) 95231136	Feeding solution	5 5 Total = 10	1 [20 mg/L] 20 [100 mg/L]	~ 0.45 [~ 9 mg/L] ~ 9.1 [~ 50 mg/L]	106 [103 – **111**] 92 [90 – 94] Overall = 99	3 2 Overall = 8
	Recovery rate in the feeding solutions					
	Feeding solution	2 2	1.075 17.24	0.49 7.86	95* – 105** 78* – 97**	- -

[a] Fortifications were performed with analyte reference standard solutions
* At day 4.
** At day 0.
Two independent replicates of solvent control were analysed (< LOD). Control = 50% w/v sucrose solution (500 g sucrose/L deionized water)
Note: the repeatability of the injection was also investigated with standard solution and found to be 0.3%

RMS Conclusion 2018 (Renewal):
The HPLC-UV method to determine lenacil in sugar feeding solutions is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification level was within the recommended range of 70 - 110% and % RSD was systematically below 20%.
The LOQ is proposed to be 1 g PPP/L (= ~ 0.45 g a.s./L) corresponding to 20 mg PPP/L (= ~ 9 mg a.s./L) after applying the dilution factor of 50 and is supported by the experimental data.
In the ecotoxicity study, the stock solution of 6874 mg a.s./kg (or 7859.7 mg a.s./L or 17.24 g PPP/L) and the lowest concentration of 430 mg a.s./kg (or 490 mg a.s./L or 1.075 g PPP/L) corresponding respectively ~ 34.4 mg a.s./kg (or ~ 39 mg a.s./L or ~ 86 mg PPP/L) and 8.6 mg a.s./kg (or ~ 9.8 mg a.s./L or ~ 21 mg PPP/L) after dilution were analysed. The level of those solutions is covered by the LOQ and the validated working range. The method is considered "fit for purpose".
It is noted that the method is the same as the one used in support of the ecotox study **CA 8.2.6/01** by Stürz S., Knebel N. (2016) - Lenacil 500 g/L SC: Effects on terrestrial (non-target) plants: Vegetative Vigour Test for determining lenacil in aqueous test solutions.

Study No. 24

Report:	**CA 4.1.2(f)/24 (KCA 4.1.2.17/02 but also under KCA 8.3.1.2_02)** Haupt S., Knebel N. (2016b) Lenacil 500 g/LG SC: Chronic oral toxicity test on bumble bee (*Bombus terrestris* L.) in the laboratory Ibacon GmbH, DE DuPont Report No.: 95231107
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes

Note: the Analytical Phase is reported in Appendix 3 of the study report.

Matrix: sugar (sucrose) feeding solution (50% w/v : 500g sucrose/L deionized water).

Description of the method:
Feeding solution samples were diluted with acetonitrile:pure water (50:50, v:v) and analysed by reversed phase HPLC with UV detection at 268 nm. External calibration.

Chromatographic conditions:

Column:	US ES RP18, 250 × 4.0 mm, 5 µm
Oven temperature:	40°C
Mobile phase:	60% (95% acetonitrile/5% pure water) and 40% pure water + 0.3% H_3PO_4 (using 85% phosphoric acid)
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Detection:	UV-Vis at 268 nm

Findings:

Specificity – absence of interference: Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil.
Chromatograms were provided for a standard solution, a solvent control, a fortified sample at the lowest concentration and a feeding solution on day 0.
The identity of the analyte which was used for quantification of the test item concentration was confirmed by comparison of the retention time with the retention time of a standard solution prepared from the reference item.

Linearity: Calibration curve of the six analytical standards of lenacil was linear in the range of 0.5 mg a.s./L to 15 mg a.s./L. A typical calibration curve and the corresponding linear regression equation were provided. The correlation coefficient R was 0.9997. Calibration solutions were prepared in acetonitrile/pure water (50/50, v/v). The concentration of the samples meet the calibration range.

Recovery: The samples were fortified at two levels with the formulation (Lenacil 500 g/L SC). Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are provided in table B.5.1.2.6-20.

Repeatability: Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in table B.5.1.2.6-20. The relative standard deviations of recovery data obtained are within the guideline of ≤20%. This method is adequate for determining lenacil concentration in feeding solution.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.

Limit of quantification:	0.04 g test item/L (diluted by a factor 20) corresponding to the lowest fortification level at which an acceptable recovery and % RSD were obtained. The LOD was set to 0.05 mg a.s./L.

Table B.5.1.2.6-20 : Validation data for analytical methods for the determination of lenacil in sucrose feeding solution by HPLC-UV

Reference	Matrix	Number of tests	Fortification level (g PPP/L) [level after dilution in mg/L] [a]	Corresponding Fortification level (a.s. mg/L) [level after dilution in mg/L]	Average recovery (%) [range]	% Relative standard deviation
Haupt S., Knebel N. (2016b) 95231107	**Validation data**					
	Sucrose feeding solution	5 5 Total = 10	0.04 [2 mg/L] 0.9 [18 mg/L]	~ 18.2 [~ 0.91 mg/L] ~ 410 [~ 8.2 mg/L]	86 [80 – 93] 105 [102 – **111**] Overall = 95	6 4 Overall = 11
	Recovery rate in the feeding solutions					
	Sucrose feeding solution	2 2	0.1044 1.671	~ 48 ~ 760	92* – 74** 99* – 69**	- -

[a] Fortifications were performed with the test item Lenacil 500 g/L SC
* At day 0.
** At day 3.
Two independent replicates of solvent control were analysed
Note: the repeatability of the injection was also investigated with standard solution and found to be 0.3%

RMS Conclusion 2018 (renewal):
The HPLC-UV method to determine lenacil in sugar feeding solutions is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification level was within the recommended range of 70 - 110% and % RSD was systematically below 20%.
The LOQ is proposed to be 0.04 g PPP/L (corresponding to 2 mg PPP/L after applying the dilution factor of 20) and is supported by the experimental data. That level corresponds to ~ 18.2 mg a.s./L (or ~ 0.91 mg a.s./L after applying the dilution factor of 20).
In the ecotoxicity study, the solution of 0.1044 g PPP/L (or ~ 48 mg a.s./L) and the concentration of 1.671 g PPP/L (or ~ 760 mg a.s./L) were analysed after dilution by a factor 20 and 50, respectively. The level of those solutions is covered by the LOQ but the validated working range 0.04 – 0.9 g PPP/L does not fully cover the highest level of 1.671 g PPP/L which is however of the same order of magnitude.
It is noted that the method is similar (i.e. other column parameters) to the one used in support of the ecotox study **CA 8.3.1.2/01** by Haupt S., Knebel N. (2016a) - Lenacil 500 g/L SC: Chronic oral toxicity test on honey bee (*Apis mellifera* L.) in the laboratory and validated in the 1 – 20 g PPP/L and the same method is validated also at higher levels in aqueous solutions (see CA 8.3.1.4-01 below).
The method can therefore be considered “fit for purpose”.

Study No. 25

Report:	**CA 4.1.2(f)/25 (KCA 4.1.2.17/03 but also under KCA 8.3.1.4-01)** Haupt S., Knebel N. (2016c) Lenacil 500 g/LG SC: Honey bees (*Apis mellifera* L.) larval toxicity test , repeated exposure Ibacon GmbH, DE DuPont Report No.: 95231032

Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes

Note: the Analytical Phase is reported in Appendix 2 of the study report.

Matrix: aqueous test solutions.

Description of the method:
Analysis of lenacil in aqueous test solutions.
Test solution samples were diluted with acetonitrile:pure water (50:50, v:v) and analysed by reverse phase HPLC with UV detection at 268 nm.

Chromatographic conditions:

Column:	US ES RP18, 250 × 4.0 mm, 5 µm (deviation from the study plan which provided 150 × 4.6 mm)
Oven temperature:	40°C
Mobile phase:	60% (95% acetonitrile/5% pure water) and 40% pure water + 0.3% H_3PO_4
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Detection:	UV-Vis at 268 nm

Findings:

Specificity – absence of interferences:	Analysis of control samples resulted in no detectable apparent residues at the characteristic retention time of lenacil. It can therefore be concluded that few, if any, apparent residues or false positive values would arise. Chromatograms were provided for a standard solution, a solvent control, a fortified sample at the lowest concentration and the stock solution on day 0. The identity of the analyte which was used for quantification of the test item concentration was confirmed by comparison of the retention time with the retention time of a standard solution prepared from the reference item.
Linearity:	The calibration curve of the six analytical standards of lenacil was linear in the range of 10 mg a.s./L to 50 mg a.s./L. A typical calibration curve and the corresponding linear regression equation were provided. The correlation coefficient R was equal to 0.9997. Calibration solutions were prepared in acetonitrile/pure water (50/50, v/v). The concentration of the samples meet the calibration range.
Recovery:	The samples were fortified at two levels with the formulation (Lenacil 500 g/L SC). Recovery results were within the respective guideline requirements (70-110%, RSD ≤20%). The results obtained are summarised in table B.5.1.2.6-21.
Repeatability:	Repeatability of this method was demonstrated by the standard deviations/relative standard deviations of the recovery values given in table B.5.1.2.6-21. The relative standard deviations of recovery data obtained are within the guideline of ≤20%. This method is adequate for determining lenacil concentration in aqueous test solution.

Reproducibility:	The method used to generate this data is not proposed as an enforcement method. Independent laboratory validations are not required for data collection methods.
Limit of quantification:	25 g PPP/L (diluted by a factor 500) corresponding to the lowest fortification level at which an acceptable recovery and % RSD were obtained. The LOD was set to 0.1 mg a.s./L.

Table B.5.1.2.6-21 : Validation data for analytical methods for the determination of lenacil in aqueous test solutions by HPLC-UV

Reference	Matrix	Number of tests	Fortification level (g PPP/L) [level after dilution in g/L] [a]	Corresponding Fortification level (a.s. g/L) [level after dilution in g/L]	Average recovery (%) [range]	% Relative standard deviation
Haupt S., Knebel N. (2016c) 95231032	**Validation data**					
	Aqueous test solution	5 5 Total = 10	25 [0.05 g/L] 35 [0.07 g/L]	~ 11.4 [~ 0.02 g/L] ~ 15.96 [~ 0.03 g/L]	83 [73 – 94] 74 [**68** – 81] Overall = 78	12 7 Overall = 11
	Recovery rate in the feeding solutions					
	Aqueous test solution	2	29.368	~ 13.38	95* – 93**	-

[a] Fortifications were performed with the test item Lenacil 500 g/L SC
* At day 3.
** At day 61;
Two independent replicates of solvent control were analysed
Note: the repeatability of the injection was also investigated with standard solution and found to be 0.2%

RMS Conclusion 2018 (renewal):
The HPLC-UV method to determine lenacil in aqueous solution is validated according to SANCO/3029/99. The absence of interference, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification level was within the recommended range of 70 - 110% and % RSD was systematically below 20%. The LOQ is proposed to be 25 g PPP/L (corresponding to 0.05 g PPP/L after applying the dilution factor of 500) and is supported by the experimental data. That level corresponds to ~ 11.4 g a.s./L (or ~ 0.02 g a.s./L after applying the dilution factor of 500).
In the ecotoxicity study, the stock solution of 29.368 g PPP/L (or ~ 13.38 g a.s./L) was analysed after dilution by a factor 500. The level of that solutions is covered by the LOQ and the validated working range 25 – 35 g PPP/L. The method is considered "fit for purpose".

B.5.1.2.7. Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Water

Previous evaluation:	*Initial monograph November 2007*

Study No. 1

Report:	**CA 4.1.2(g)/01 (IIA 4.2.3.1-01)** Wittig, A. (2002) Validation of a monitoring method for determination of lenacil residues in surface water, tap water and ground water UCL GmbH Köln, Germany

	DuPont Report No.: PR02/001
Guidelines:	SANCO/825/00 rev.6
GLP:	Yes

Matrix:
- Surface water from the river Ahr (Blankenheim, Germany, near spring); TOC: 1 mg/L, pH 7.6, total water hardness: 20°dH, filterable particles (2h): 0.1 mg/L;
- Tap water from Köln;
- Ground water: pH 6.8, oxygen content: 1.9 mg/L, conductivity: 496 µS/cm

Description of the method:
Samples were prepared by enrichment of lenacil onto an RP 18 SPE cartridge. After drying the cartridge, lenacil was eluted with acetonitrile/water; the eluate was reduced to dryness and was taken up in 1 mL of mobile phase (acetonitrile/water 80:20 v/v). This solution was directly analysed by HPLC (LiChrosphere 60, RP-select B, 250 mm x 4mm, 5µm) with DAD detection (UV wavelengths 200, 212 and 270 nm, the latter of which was used for quantification). External calibration was used.

Chromatographic conditions:	
Column:	LiChrosphere 60, RP-select B, 250 mm x 4mm, 5µm
Column temperature:	Room temperature
Mobile phase:	Water/acetonitrile : 20%/80%
Flow:	1 mL/min
Injected volume:	100 µL
Detector:	DAD, 200, 212 and 270 nm
Retention time:	~ 3.9 min

Findings:

Specificity - interferences: No interfering peaks occurred; No significant blank values (> 30 % LOQ) (control chromatograms showed no detectable peak at the characteristic retention time of lenacil). Chromatograms were provided for standard calibration solutions (at the lowest and highest concentration levels), control samples for the three types of water, and fortified samples at low and high levels.
Peak identification by DAD: in the report it was stated that the identity of lenacil residues in water is confirmed by comparison of the UV/Vis spectrum between the samples and the standard solutions. The characteristics UV/Vis spectrum of lenacil in the mobile phase was presented.

Linearity: Calibration curves showed good linearity in the range of 79 to 1188 ng/mL (n = 5x4 [4 injections]) for lenacil i.e., the linear regression line, R^2 = 0.9999; approximate corresponding residue concentration range: 0.08 – 1.19 µg/L. A typical calibration curve with the corresponding regression equation were provided within the study report. Calibration solutions were prepared in acetonitrile/water: 80/20.

Recovery: The fortification data reported in the method proposed for monitoring lenacil residues in water are summarised in Table below. The average recoveries and relative standard deviation are well within the criteria specified in the EU Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4).

Repeatability: Repeatability of the method is addressed by the data in Table . Relative standard deviations of less than 20% were obtained for the tested fortifications.

Reproducibility: The method used to generate this data is not proposed as an enforcement method. No ILV was presented (Independent laboratory validations were not required at the time where the method was developed).

Limit of quantification: 0.1 µg/L.

Table B.5.1.2.7-1 : Validation data for analytical methods for the determination of lenacil in water (drinking and surface water) (HPLC-UV)

Reference	Matrix	Number of tests	Fortification level (µg/L)	Average recovery (%) [range]	Standard deviation	% Relative standard deviation
Wittig, (2002) PR02/001	Tap water	5 5	0.1 1.0	90.9 [89 – 94] 101.2 [101 – 102] Overall = 96.1	1.8 0.6 Overall = 5.6	2.0 0.6 Overall = 5.8
	Surface water	5 5	0.1 1.0	94.1 [93 -96] 99.5 [96 -102] Overall = 96.8	1.3 2.8 Overall = 3.5	1.4 2.8 Overall = 3.6
	Ground water	5 5	0.1 1.0	87.0 [82 – 96] 96.7 [95 -99] Overall = 91.9	5.1 1.3 Overall = 6.2	5.9 1.3 Overall = 6.7

Two control for each water samples were analysed for interferences. No residue of lenacil were found.

RMS conclusion (initial monograph):
The HPLC-DAD method is suitable for determination of lenacil in tap, surface and ground water with a LOQ of 0.1 µg/L.

RMS Conclusion 2018 (renewal):
The method was considered as a valid method for monitoring of lenacil in water in the original monograph. However, in regards with the current requirements for a monitoring method in water, the method can no longer be considered as a fully validated monitoring method (absence of ILV in drinking water and absence of a confirmatory method). The method is however still considered for the renewal dossier in support of studies to generate data used for the physical and chemical properties tests and also in ecotoxicity tests. The method was used in study CEMS-2787 to determine the water solubility of lenacil.

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

Justification for analytes chosen
The proposed residue definition in plants is lenacil.

The proposed environmental residue definition in soil is lenacil.

The proposed residue definition in water is lenacil.

The proposed environmental residue definition in air for lenacil is parent only.

Table B.5.2-1: Proposed analytical methods for monitoring lenacil residues

Matrix	Reference and report	Separation / Quantitation	Limit of determination (mg/kg)	Comments
Rape seed	Witte, 2011, 10T02203-01-VMRA	HPLC/MS/MS	0.01	DFG multiresidue method S19
Lettuce	Witte, 2015, 14S07170-01-VMLE	HPLC/MS/MS	0.01	DFG multiresidue method S19
Wheat grain Lemon fruit	Witte, 2011, 11T02203-01-VMPL	HPLC/MS/MS	0.01	DFG multiresidue method S19
Rape seeds	Mende, P. (2011), S10-03652	HPLC/MS/MS	0.01	ILV to DFG multiresidue method S19
Wheat grain Lettuce	Stanislowski, 2015 P 3464 G	HPLC/MS/MS	0.01	ILV to DFG multiresidue method S19
Meat (Bovine) Fat (Bovine) Liver (Bovine) Milk Eggs	Wagner, 2016 15S07170-01-VMAT	QuEChERS HPLC/MS/MS	0.01	QuEChERS
Meat (Pig) Fat (Pig) Liver (Bovine) Milk Eggs	Bodsch, 2016 IF-16/03724646 +Amendment No.1	QuEChERS HPLC/MS/MS	0.01	ILV
Soil	Brodsky and Zietz, 1990 BE-A-11-90-10-BF	GC-MS	0.05	Analyses for lenacil
	Mende, 2003 20011048/E1-FSD	HPLC/MS/MS	0.02	Analyses for lenacil and its main environmental degradation product IN-KF313 (confirmatory method)
Water	Witte, 2009 09D2203-01-VMWA	HPLC-MS/MS	0.1µg/L	Analyses for lenacil
	Link, 2016, IF-15/03444276	HPLC-MS/MS	0.1µg/L	ILV
Air	Rawle, 2005 CEMS-2788	HPLC-MS/MS	0.1 mg/m^3	-
Body fluids and tissues				No monitoring method is needed (low acute toxicity, accumulation in tissues not expected).

B.5.2.1. Analytical methods for the determination of the active substance and/or metabolites in food of plant origin

Enforcement methods suitable for the European Union region
For the determination of lenacil in food of plant origin the multi-residue method DFG S19 using LC-MS/MS was successfully validated on four representative crop matrices (oil seed rape (high oil content), lettuce (high water content), wheat grain (high starch content) and lemon flesh (high acid content)) at the LOQ of 0.01 mg/kg. The method was successfully validated by an independent laboratory.

The method is successfully validated according to SANCO 825/00 rev.8.1 and suitable for the determination of lenacil in plant matrices. Therefore, the method DFG S19 using LC-MS/MS is proposed as the enforcement method for residues of lenacil in plants.

The following analytical methods on plant matrices have not been previously reviewed and are provided in support of this assessment.

Previous evaluation:	*No, submitted for the purpose of renewal* *Note: studies No.1, 3, 4 already considered at zonal level for Venzar 80WP*

Study No. 1

Report:	**CA 4.2(a)/01 [KCA 4.2.1/01]** Witte, A. (2011a) Validation of an analytical method for determination of residues of lenacil in rape seeds (oily matrix) CIP Chemisches Institut Pforzheim GmbH, Gemany CIP Study No.: 10T02203-01-VMRA
Guidelines:	SANCO/825/00 rev.7
GLP:	Yes

Study No. 2

Report:	**CA 4.2(a)/02 [KCA 4.2.1/02]** Witte, A. (2015) Validation of an analytical method for determination of residues of lenacil in a plant commodity with high water content (lettuce) CIP Chemisches Institut Pforzheim GmbH, Gemany CIP Study No.: 14S07170-01-VMLE
Guidelines:	SANCO/825/00 rev.8.1
GLP:	Yes

Study No. 3

Report:	**CA 4.2(a)/03 [KCA 4.2.1/03]** Witte, A. (2011b) Validation of an analytical method for determination of residues of lenacil in wheat grain (dry matrix) and lemon fruit (acidic matrix) CIP Chemisches Institut Pforzheim GmbH, Gemany CIP Study No.: 11T02203-01-VMPL
Guidelines:	SANCO/825/00 rev.7
GLP:	Yes

Study No. 4

Report:	**CA 4.2(a)/04 [KCA 4.2.1/04]** Mende, P. (2011) Independent Laboratory Validation (ILV) of an analytical method for determination of residues of lenacil in rape seeds Eurofins Agroscience GmbH, Germany Study No.: S10-03652

Guidelines:	SANCO/825/00 rev. 7
GLP:	Yes

Study No. 5

Report:	**CA 4.2(a)/05 [KCA 4.2.1/05]** Richter S. and Stanislowski, T. (2015) Independent Laboratory Validation (ILV) of the German DFG S19 Multi-Residue Method for the Determination of Lenacil in Two Crop Types, using LC/MS/MS PTRL Europe, Germany DuPont Report No.: P 3464 G
Guidelines:	SANCO/825/00 rev. 8.1
GLP:	Yes

Principle of the method(s):
Specimens were analysed for residues of lenacil with a method used in analogy to the Multi-Residue Method DFG S 19 (LC-MS/MS Module) with extraction module E 7 for rape seeds, extraction module E 1 for lettuce, extraction module E 2 for wheat (grain) and extraction module E 3 for lemon fruit (flesh), followed by further cleaned up by gel permeation chromatography (module GPC).

E 1: Cold extraction with acetone (200 mL after addition of water to adjust the water content) followed by sodium chloride and ethyl acetate/cyclohexane (1/1, v/v) for phase separation and partitioning of lenacil into the organic layer.
E 2: Cold extraction with acetone (200 mL) after wetting the dry sample matrix (to adjust the water content) followed by sodium chloride and ethyl acetate/cyclohexane (1/1, v/v) for phase separation and partitioning of lenacil into the organic layer
E 3: Cold extraction with acetone (200 mL) after adjusting the pH to 7 followed by sodium chloride and ethyl acetate/cyclohexane (1/1, v/v) for phase separation and partitioning of lenacil into the organic layer.
E 7: Cold extraction with acetonitrile/acetone (225 mL/25 mL) in the presence of Calflo E and Celite (extraction in the presence of high amounts of fat).

Sample extracts were analysed by LC MS/MS analysis. Two ion transitions (m/z) were monitored for lenacil: 235 → 153 (quantification) and 235 → 136 (confirmation) (MRM, Heated electrospray ionization, positive mode). External calibration.

Chromatographic conditions:
Column: Supelco Ascentis Express C18, 50 mm × 2.1 mm, 2.7 µm particle size (Part No. 53822-U).
Column temperature: 40°C
Injection volume: 20 µL
Retention time: ~ 4.6 min (in 10T02203-01-VMRA), ~ 4.8 min (in 14S07170-01-VMLE), ~ 4.7 min (in 11T02203-01-VMPL.
Mobile phase:

Time ([min]	% water	% Methanol	% 10% fromic acid in water	gradient
0.00	89	10	1	Linear
0.50	89	10	1	
4.50	0	99	1	
6.50	0	99	1	
6.51	89	10	1	
8.50	89	10	1	

Flow rate: 250 µL/min.

The ILV (described in study S10-03652 by Mende P., 2011) applies the same of extraction method of residues of lenacil in rape seeds (see above) and analysis occurs by LC-MS/MS with the following chromatographic parameters:

Column: Phenomenex Luna 3 µ C18(2), 50 mm × 3.0 mm, 3 µm particle size (Part No. 00B-4251-Y0), 4 mm C18 guard column.
Column temperature: 40°C
Injection volume: 20 µL
Retention time: 6.2 min
Mobile phase:

Time ([min]	% water + 0.1% formic acid	% Methanol + 0.1% formic acid	Flow rate (µL/min)	Gradient
0.00	90	10	250	-
0.50	90	10	250	-
4.50	0	100	250	linear
7.00	0	100	250	-
7.01	90	10	400	-
9.00	90	10	400	-

HPLC and mass detector conditions were optimized for the current hardware, maintaining the original conditions as closely as possible. The HPLC column is not identical but comparable to the column used in the original method validation.

The ILV (described in study P 3464G by Richter S. and Stanislowski, T., 2015) applies the same of extraction method of residues of lenacil from wheat grain and lettuce (see above) with only minor modifications to the conditions of the gel permeation chromatography and analysis occurs by LC-MS/MS with the following chromatographic parameters:

Column: Supelco Ascentis Express C18, 50 mm × 2.1 mm, 2.7 µm particle size, 4 mm Phenomenex C18 guard column.
Column temperature: 40°C
Injection volume: 20 µL
Retention time: 5.1 min
Mobile phase:

Time ([min]	% water + 0.1% formic acid	% Methanol + 0.1% formic acid	Flow rate (µL/min)	Gradient
0.00	90	10	300	-
0.50	90	10	300	-
2.50	0	100	300	linear
6.50	0	100	300	-
6.51	90	10	300	-
8.50	90	10	300	-

For LC-MS/MS analysis the gradient of the primary method was slightly modified due to different HPLC equipment. Since retention time, sensitivity and selectivity are comparable, it is stated in the report that the minor modifications do not have an important impact on the determination of lenacil in crops.

Findings:

Characteristics for the LC-MS/MS primary method (based on DFG S19) used for the quantitation of lenacil residue in plant commodities

Characteristics for the ILV method used for the quantitation of lenacil residue in plant commodities

	Lenacil
Method	***Primary method:*** DFG S19 - LC-MS/MS ***ILV:*** DFG S19 - LC-MS/MS

Characteristics for the LC-MS/MS primary method (based on DFG S19) used for the quantitation of lenacil residue in plant commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in plant commodities**	
	Lenacil
Specificity	***Primary method:*** Method is highly specific, monitoring of 2 mass transitions (no further confirmatory method is required) : Quantification : 235 → 153 Confirmation : 235 → 136 Method was validated on both mass transitions. Representative mass spectra were provided (full mass spectrum and product ion spectrum). No significant interferences (no residues of lenacil above 30% of the LOQ) from the specimen matrix were detected at the retention time corresponding to lenacil in any of the control specimens. Chromatograms were provided for calibration standards at different concentrations (matrix-matched or not depending on crop), blank controls for each crop specimen and fortified samples at the LOQ and 10 × LOQ levels, in each case for both mass transitions. ***ILVs:*** Method is highly specific, monitoring of 2 mass transitions (no further confirmatory method is required) : Quantification :235 → 153 Confirmation : 235 → 136 Method was validated on both mass transitions. A representative product ion spectrum was provided within study S10-03652; representative mass spectra were provided (full mass spectrum and product ion spectrum) within study P 3464G. No significant interference above 30% of LOQ was detected in any of the control specimen extracts of each matrix or the reagent blanks, so that a high level of selectivity was demonstrated. Chromatograms were provided for calibration solutions at different concentration (matrix-matched or not depending on crop), control specimen of rape seeds, lettuce and wheat grain, fortified specimens at LOQ and 10 ×LOQ for both mass transitions.

Characteristics for the LC-MS/MS primary method (based on DFG S19) used for the quantitation of lenacil residue in plant commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in plant commodities**	
	Lenacil
Linearity	***Primary method:*** Quantification 235 → 153 : Linear and r^2 = 0.9998 (rape seeds, in solvent) Linear and r^2 = 0.9997 (lettuce, in matrix-matched) Linear and r^2 = 0.9999 (wheat grain, in solvent) Linear and r^2 = 0.9980 (lemon fruit, in matrix-matched) Confirmation 235 → 136: Linear and r^2 = 0.9999 (rape seeds, in solvent) Linear and r^2 = 0.9997 (lettuce, in matrix-matched) Linear and r^2 = 0.9999 (wheat grain, in solvent) Linear and r^2 = 0.9977 (lemon fruit, in matrix-matched) Linear calibration curves (peak area vs. concentration) and corresponding regression equations were provided within the study reports for both mass transitions. These data are those related to the calibration performed in solvent (methanol/water: 30/70 v/v) for rape seeds and wheat grain and for matrix-matched samples for lettuce and lemon fruit. ***ILVs:*** Quantification 235 → 153 : Linear and r^2 = 0.9997 (rape seeds, in solvent) Linear and r = 0.9998 (wheat grain, in matrix-matched) Linear and r = 0.9999 (lettuce, in matrix-matched) Confirmation 235 → 136: Linear and r^2 = 0.9998 (rape seeds, in solvent) Linear and r = 0.9998 (wheat grain, in matrix-matched) Linear and r = 0.9999 (lettuce, in matrix-matched) Linear calibration curves (peak area vs. concentration) and corresponding regression equations were provided within the study reports for both mass transitions. These data are those related to the calibration performed in solvent (methanol/water: 30/70 v/v) for rape seeds.
Calibration	

Characteristics for the LC-MS/MS primary method (based on DFG S19) used for the quantitation of lenacil residue in plant commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in plant commodities**	
	Lenacil
Accepted calibration range in concentration units (e.g. in µg/ml or ng/µl)	***Primary method:*** 0.2 – 20 ng/mL(rape seed: calibration in solvent)) 0.5 – 30 ng/mL (wheat grain, calibration in solvent and lettuce and lemon fruit: matrix-matched calibration) No matrix-matched calibrations were used for rape seeds and wheat grain and matrix-matched calibrations were used for lettuce (although no matrix effects were observed) and lemon fruit (matrix effects > 10 % were observed). Calibration solutions (in solvent) were prepared in methanol/water (30:70, v/v). The concentration of the samples meet the calibration range. ***ILVs:*** 0.2 – 20 ng/mL (rape seed, study No. S10-03652: calibration in solvent) 0.5 – 30 ng/mL (wheat grain: calibration in solvent and lettuce: matrix-matched calibration, Study No. P 3464G) No matrix-matched calibrations were used for rape seeds and wheat grain and matrix-matched calibrations were used for lettuce. Calibration solutions (in solvent) were prepared in methanol/water (30:70, v/v). The concentration of the samples meet the calibration range.
Corresponding calibration range in mass ratio units for the sample (e.g.in mg/kg or µg/L)	***Primary method:*** ~ 0.001– 0.12 mg/kg in rape seeds ~ 0.002 – 0.14 mg/kg in lettuce ~ 0.002 – 0.13 mg/kg in wheat grain and lemon fruit ***ILVs:*** ~ 0.001– 0.12 mg/kg in rape seeds ~ 0.002 – 0.13 mg/kg in wheat grain and lemon fruit
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)? (yes/ no)	***Primary method:*** N = 8 for rape seeds, single injection, for both mass transitions N = 7 for lettuce, wheat grain and lemon fruit, single injection and for both m/z transitions ***ILVs:*** N = 8 for rape seeds, single injection, for both m/z transitions in study No. S10-03652 N = 7 for wheat grain and lettuce, single injection, for both mass transitions in study P 3464G

Characteristics for the LC-MS/MS primary method (based on DFG S19) used for the quantitation of lenacil residue in plant commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in plant commodities**	
	Lenacil
Assessment of matrix effects is presented (yes/no)	***Primary method:*** Matrix effects (i.e. response of analyte in calibration solutions in solvent [methanol/water] versus response in matrix matched calibration solutions) were not significant (no effects above 5%) for rape seeds, lettuce and wheat grain. Matrix effects above 10% were observed in lemon fruit. The matrix effects were, more than likely, demonstrated for both mass transitions in each matrix (at a concentration of 1 and 10 ng/mL for rape seeds, in the 0.5 – 30 ng/mL for lettuce and lemon fruit, at 2 and 20 ng/mL for wheat grain). Calibrations in solvent (methanol/water: 30/70 v/v) were therefore used except for lettuce where although no matrix effects were observed, matrix-matched calibrations were used, and for lemon fruit for which matrix effects were found to be above 10 % leading to recoveries below 70%. ***ILVs:*** Matrix effects (i.e. response of analyte in calibration solutions in solvent [methanol/water] versus response in matrix matched calibration solutions) were not significant for rape seeds (effects ≤ 3%, obtained by comparison of the concentration determined in the matrix-matched samples with the nominal concentration in solvent) and wheat grain (effects ≤ 6%). Significant matrix effects (suppression > 20%) were observed in lettuce. The matrix effects were demonstrated for both mass transitions in each matrix (at a concentration of 1 and 10 ng/mL for rape seeds, at 5 ng/mL for lettuce and wheat grain). Calibrations in solvent (methanol/water: 30/70 v/v) were therefore used for rape seeds and wheat grain while matrix-matched calibrations were used for lettuce.
Absence of interference >30% of LOQ in blank sample is demonstrated (yes/no)	***Primary method and ILV:*** Yes (no residues of analytes in the controls that interfered with the analysis at levels above 30% of the LOQ)
Chromatogram of sample spiked at LOQ demonstrates sufficient S/N ratio? (yes/no)	***Primary method:*** Yes based on the chromatograms provided in each matrix. ***ILV:*** Yes based on the chromatograms provided in each matrix.

Characteristics for the LC-MS/MS primary method (based on DFG S19) used for the quantitation of lenacil residue in plant commodities Characteristics for the ILV method used for the quantitation of lenacil residue in plant commodities	
	Lenacil
LOQ (mg/kg) (based on the the lowest analyte concentration in a sample at which the methodology has been validated, acceptable mean recovery and RSD)	***Primary method:*** 0.01 mg/kg in all matrices LOQ results from the lowest concentration level successfully tested within recovery experiments. The limit of detection (LOD) of the method was set to 30% of LOQ (LOD = 0.0003 mg/kg). ***ILV:*** 0.01 mg/kg in rape seeds (study No. S10-03652) 0.01 mg/kg in lettuce and wheat grain (study No. P 3464G) LOQ results from the lowest concentration level successfully tested within recovery experiments. The limit of detection (LOD) was set at 0.003 mg/kg (30% of the LOQ).

Recovery and repeatability:
Fortification experiments were performed at the LOQ level (0.01 mg/kg) and 10 × LOQ (0.1 mg/kg). The fortification data reported in the methods proposed for monitoring lenacil in high water, acidic, oily and dry crop samples are summarised in table B.5.2.1-1. The average recovery per fortification level specified in the decision-making criteria is 70 to 120%, with a standard deviation of ≤20%. Therefore, the recoveries of these methods are adequate for the purposes of residue data collection and enforcement of MRLs.

Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit and 10x the quantification limit for each matrix. Therefore, the repeatability of this method is adequate for the purposes of residue data collection and enforcement of MRLs.

Table B.5.2.1-1 : Validation data for analytical methods for the determination of lenacil in food of plant origin

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a, b]	Average recovery (%) [range]	% Relative standard deviation
Witte, A. (2011), 10T02203-01-VMRA	Lenacil MRM 235 → 153 (quantification)				
	Rape seed (high oil)	5 5 Total = 10	0.01 0.10	86 [78 – 92] 92 [86 – 99] Overall = 89	6.3 6.8 Overall = 7.2
	Lenacil MRM 235 → 136 (confirmation)				
	Rape seed (high oil)	5 5 Total = 10	0.01 0.10	94 [87 – 102] 95 [91 – 101] Overall = 95	6.3 4.0 Overall = 5.0
Witte, A. (2015), 14S07170-01-VMLE	Lenacil MRM 235 → 153 (quantification)				
	Lettuce (high water)	5 5 Total = 10	0.01 0.10	91 [88 – 95] 88 [83 – 93] Overall = 90	3.4 4.5 Overall = 4.0
	Lenacil MRM 235 → 136 (confirmation)				
	Lettuce (high water)	5 5 Total = 10	0.01 0.10	91 [86 – 95] 88 [83 – 93] Overall = 90	3.6 4.4 Overall = 4.1
Witte, A. (2015), 11T02203-01-VMPL	Lenacil MRM 235 → 153 (quantification)				
	Wheat grain (dry, high starch)	5 5 Total = 10	0.01 0.10	83 [81 – 86] 96 [93 – 97] Overall = 89	3.1 1.7 Overall = 7.7
	Lenacil MRM 235 → 136 (confirmation)				
	Wheat grain (dry, high starch)	5 5 Total = 10	0.01 0.10	85 [81 – 90] 94 [92 – 96] Overall = 90	5.0 1.7 Overall = 6.3
	Lenacil MRM 235 → 153 (quantification)				
	Lemon flesh (acidic)	5 5 Total = 10	0.01 0.10	73 [**68** – 79] 79 [77 – 83] Overall = 76	5.5 3.0 Overall = 5.9
	Lenacil MRM 235 → 136 (confirmation)				
	Lemon flesh (acidic)	5 5 Total = 10	0.01 0.10	79 [74 – 88] 80 [79 – 84] Overall = 80	6.8 2.7 Overall = 4.9

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Note: control specimens of each matrix were analysed each in duplicate.
All individual recoveries were between 70 – 120% except one in lemon fruit (flesh) at the LOQ level.

Table B.5.2.1-2 : Validation data for ILV analytical methods for the determination of lenacil in food of plant origin

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a, b]	Average recovery (%) [range]	% Relative standard deviation
Mende, P. 2011 S10-03652	Lenacil MRM 235 → 153 (quantification)				
	Rape seed (high oil)	5 5 Total = 10	0.01 0.10	97 [89 – 104] 94 [90 – 98] Overall = 96	7 4 Overall = 6
	Lenacil MRM 235 → 136 (confirmation)				
	Rape seed (high oil)	5 5 Total = 10	0.01 0.10	96 [86 – 107] 94 [91 – 98] Overall = 95	9 3 Overall = 6
Richter S. and Stanislowski, T. (2015) P 3464 G	Lenacil MRM 235 → 153 (quantification)				
	Wheat grain (dry, high starch)	5 5 Total = 10	0.01 0.10	81 [77 – 84] 81 [77 – 85] Overall = 81	3 4 Overall = 3
	Lenacil MRM 235 → 136 (confirmation)				
	Wheat grain (dry, high starch)	5 5 Total = 10	0.01 0.10	81 [78 – 85] 81 [77 – 84] Overall = 81	4 4 Overall = 4
	Lenacil MRM 235 → 153 (quantification)				
	Lettuce (high water)	5 5 Total = 10	0.01 0.10	85 [80 – 90] 82 [78 – 93] Overall = 84	4 8 Overall = 6
	Lenacil MRM 235 → 136 (confirmation)				
	Lettuce (high water)	5 5 Total = 10	0.01 0.10	84 [77 – 86] 82 [77 -91] Overall = 83	5 7 Overall = 6

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Note: control specimens of each matrix were analysed each in duplicate in both ILV studies. A reagent blank was also analysed in study P 3464G.
All individual recoveries were between 70 – 120%

Stability in final sample extract:
Within studies 10T02203-01-VMRA, 11T02203-01-VMPL and S10-03652 (ILV), the stability of lenacil had not be tested, since all samples were analysed within 24 hours after extraction.

Within study 14S07170-01-VMLE, the stability of the analyte in final extracts was considered as sufficiently proven since the recoveries during analysis of the samples extracts were within the acceptable range of 70 – 120%.

Within study P 3464G, lenacil was found to be stable when stored refrigerated as stock solution in methanol (10 ng/mL) and in fortification and calibration solutions (10 ng/mL) prepared in methanol/water (30/70, v/v) for at least 13 days (both mass transitions). Final extracts stored refrigerated for 5 days (for lettuce) or at least 9 days (for wheat grain) showed acceptable recoveries (both mass transitions) and no significant differences between samples before and after storage.

Extraction Efficiency

In the sugar beet metabolism study "Metabolism of Lenacil in Sugar beets" (KCA 6.2.1/01; Report No.: AMR 3649-95; see ***DRAR Vol.3, B.7.2.1***) it was shown that the extractability was quantitative with 94.4 to 99.6% of the

total radioactive residues (TRR) in sugar beet foliage being recovered in the acetonitrile:water (2:1 v/v) extract (at TRR values of 0.16 to 7.35 ppm).
For sugar beet root the extraction with acetonitrile:water (2:1 v/v) released between 67% and 80% of the TRR (at very low TRR levels, 0.01 – 0.03 ppm) and the unextracted radioactivity corresponded to less than 0.01 mg/kg (lenacil equivalent). For more details: see ***DRAR Vol.3, B.7.2.1.***

The solubility in organic solvents was tested in study AMR 2377-92 (KCA 2.6/01). In acetonitrile the solubility of lenacil is 230 ppm and in acetone 690 ppm. Taking the higher solubility of lenacil in acetone into account it can be concluded that the extraction with acetone results in the same, if not higher, lenacil residue level as the extraction with acetonitrile:water (2:1 v/v).

Furthermore, it has to be considered that according to SANCO 825/00 rev. 8.1 extraction procedures used in residue analytical methods for the determination of residues in plants, plant products, foodstuff (of plant and animal origin) and in feeding stuff should be verified for all matrix groups for which residues ≥ LOQ are expected.

The residue levels in sugar beet root are expected to be below the LOQ and no incurred residues in will be found (see Vol. 3 CA-B.7.3). Therefore, a further cross validation of the method is not necessary.

Until now no MRLs are set for sugar beet leaves. The enforcement methods are used to monitor possible misuses of plant protection products in plant matrices for which MRLs are set.

Therefore, a further cross validation of the method for sugar beet leaves is not necessary.

Furthermore, the validation of the method with mean recovery values between 76 and 95% for lenacil in plant matrices shows that the extraction of the residues is efficient.

RMS Conclusion 2018 (renewal):

The residue method for the determination of lenacil residues in the watery, acidic, oily and dry EU crop groups involves simple extraction, clean-up, and analysis by HPLC-MS/MS based on multi-residue method DFG S19. The method is fully validated according to SANCO/825/00 rev. 8.1 for specificity/absence of interferences, linearity, accuracy and repeatability. All mean recoveries at both fortification levels (0.01 and 0.1 mg/kg) were within the required 70- 120 % range with % RSD all below 20%. The limit of quantification of 0.01 mg/kg can be achieved consistently for the watery, acidic, oily and dry EU crop groups.
The method is self-confirmatory by monitoring two mass transitions and full and acceptable validation data were generated at both mass transitions.
The method has been successfully independently validated in rape seeds (oily), lettuce (high water) and wheat grain (dry, high starch) and the LOQ of 0.01 mg/kg in each matrix has been confirmed. An ILV in acidic matrix is not required according to SANCO/825/00 rev. 8.1, since the extraction of lenacil from acidic matrix occurs with adjustment of pH to 7 and therefore the results of the ILV in high water matrix covers the acidic matrix.
Consequently, the DFG S19 method is suitable for control purposes in the watery, acidic, oily and dry EU crop groups and can be recommended as monitoring method.

Regarding the extraction efficiency, metabolism studies were only performed on high water matrices and the solvent used for extraction in the metabolism studies (acetonitrile/water: 2/1) is different from the solvent used in the DFG S19 proposed for monitoring (acetone/water: 2/1) and a cross-validation is in principle required. Nevertheless, neither a radio-cross validation with samples from metabolism studies nor a cross-validation with samples from field trials was performed and instead the applicant provided a statement. Acetonitrile/water was shown to be a good solvent for extraction in the metabolism studies (> 70% of TRR extracted with lenacil > 50 % of TRR) and in the monitoring method acetonitrile is replaced by acetone in which the solubility of lenacil is higher compared to acetonitrile. Consequently, RMS agrees that it can be reasonably expected that extraction with acetone/water will also work correctly.
In the course of the assessment, the notifier mentioned that an extraction efficiency study using acetone as the extraction solvent is reported in the study AMR 4193-96 “Radiovalidation of the Trace Analytical Method for Lenacil Residues in sugar Beet Root and Top” by Anderson J.J., Hoesterev R.W. (1998) and that the study is available for submission. According to the applicant, incurred residues from radiolabelled samples were efficiently extracted using acetone with acceptable recoveries. **The study AMR 4193-96 should therefore be submitted.**

B.5.2.2. Analytical methods for the determination of the active substance and/or metabolites in food of animal origin

Enforcement methods suitable for the European Union region

For 1st approval, methods were not provided for monitoring of residues of lenacil in animal matrices. In the frame of renewal and for the determination of lenacil in food of animal origin, the multi-residue method QuEChERS method was validated on five representative animal foodstuff matrices (milk, eggs, meat, fat, liver) at the LOQ of 0.01 mg/kg. The method is successfully validated by an independent laboratory. Validation results are presented here below.

The method is found to be successfully validated according to SANCO 825/00 rev.8.1 and suitable for the determination of lenacil in animal matrices and therefore, proposed as the enforcement method for residues of lenacil in animal matrices.

Previous evaluation:	*No, submitted for the purpose of renewal*

Study No. 1

Report:	**CA 4.2(a)/06 [KCA 4.2.2/01]** Wagner, B. (2016) Validation of an analytical method for the determination of residues of lenacil in matrices of animal origin (milk, eggs, meat, fat, kidney/liver) CIP Chemisches Institut, Germany CIP Study code: 15S07170-01-VMAT DuPont Report No.: 15S07170-01-VMAT
Guidelines:	SANCO/825/00 rev. 8.1
GLP:	Yes

Study No. 2

Report:	**CA 4.2(a)/07 [KCA 4.2.2/02]** Bodsch, J. (2016) Study plan - Independent Laboratory Validation of analytical method 15S07170-01-VMAT for the determination of residues of Lenacil in matrices of animal origin (milk, eggs, meat, fat, kidney/liver) by LC-MS/MS SGS Institut Fresenius GmbH, Germany SGS study No.: IF-16/03724646 DuPont Report No.: IF-16/03724646
Guidelines:	SANCO/825/00 rev. 8.1 OECD ENV/JM/MONO (2007), 17 13/08/2013
GLP:	Yes

Study No. 3

Report:	**CA 4.2(a)/08 [KCA 4.2.2/03]** Bodsch, J. (2016) + amendment 2016 Final report - Independent Laboratory Validation of analytical method 15S07170-01-VMAT for the determination of residues of Lenacil in matrices of animal origin (milk, eggs, meat, fat, kidney/liver) by LC-MS/MS SGS study no.: IF-16/03724646 AR DuPont Report No.: IF-16/03724646 AR (IDD15272)
Guidelines:	SANCO/825/00 rev. 8.1 OECD ENV/JM/MONO (2007), 17 13/08/2013
GLP:	Yes

Description of the method:
Residues of lenacil in matrices of animal origin were extracted with a modified QuEChERS (DIN EN 15662:2009-02) method and analysed by high performance liquid chromatography coupled with tandem mass spectrometry. The specimens (10 g) of milk (cow), eggs (chicken), meat (pork), fat (pork) and liver (bovine) were extracted with acetonitrile (10 mL)/water (2.5 mL for eggs, meat, liver and 10 mL for fat) using a homogenizer. Thereafter, a buffer salt mixture was added and the samples were shaken and centrifuged for phase separation and partitioning of lenacil into the organic layer. An aliquot of each organic phase extract was purified by solid phase extraction using PSA-Kit-03 and analysed by HPLC-MS/MS. For lenacil, two ion transitions 235 → 153 and 235 → 136 were monitored and used for quantification and confirmation, respectively. External calibration.

For the ILV, the deviations compared to the original method are as follows:
Instead of the used of a split at the HPLC-MS/MS system the calibration range was adapted and therefore the samples were further diluted by factor 1:20 with acetonitrile/water (1/1, v/v).

Chromatographic conditions of the primary method (study 15S07170-01-VMAT):
Column: Supelco Ascentis Express C18, 50 mm × 2.1 mm, 2.7 µm (Part No. 53822-U)
Injection volume: 10 µL
Column temperature: 40°C
Mobile phase:

Time (min.)	Flow (µL/min.)	Water + 0.1% formic acid	Methanol + 0.1% formic acid
0.00	400	90	10
0.50	400	90	10
4.50	400	1	99
5.50	400	1	99
5.51	400	90	10
6.50	400	90	10

Linear gradient

Retention time: ~ 4.5 min (~ 3.9 min in ILV)
Detection: MRM, Turbo Ion Spray, ESI positive mode
m/z 235 → 153 (quantitation)
m/z 235 → 136 (confirmation)

Findings:

Characteristics for the LC-MS/MS primary method (modified QuEChERS) used for the quantitation of lenacil residue in animal commodities

Characteristics for the ILV method used for the quantitation of lenacil residue in animal commodities

	Lenacil
Method	***Primary method:*** LC-MS/MS (modified QuEChERS) ***ILV:*** LC-MS/MS (modified QuEChERS)

Characteristics for the LC-MS/MS primary method (modified QuEChERS) used for the quantitation of lenacil residue in animal commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in animal commodities**	
	Lenacil
Specificity	***Primary method:*** Method is highly specific, monitoring of 2 mass transitions (method is self-confirmatory) : Quantification : 235 → 153 Confirmation : 235 → 136 Method was validated on both mass transitions. Representative mass spectra were provided (full mass spectrum and product ion spectrum). No significant interferences (no residues of lenacil above 30% of the LOQ) from the specimen matrix were detected at the retention time corresponding to lenacil in any of the control specimens. Chromatograms were provided for calibration standards at 1 µg/L (lowest calibration level), 5 µg/L (corresponding to ~ LOQ) and 50 µg/L (corresponding to ~ 10 × LOQ), blank controls for each matrix and fortified samples at the LOQ and 10 × LOQ levels, in each case for both mass transitions. ***ILV:*** Method is highly specific, monitoring of 2 mass transitions (method is self-confirmatory) : Quantification :235 → 153 Confirmation : 235 → 136 Method was validated on both mass transitions. A representative mass spectrum (full scan) and a product ion spectrum were provided. No significant interference above 30% of LOQ was detected in any of the control specimen extracts of each matrix. Chromatograms were provided for a calibration solution at 0.25 ng/mL, a control specimen of each matrix, fortified specimens of each matrix at LOQ and 10 ×LOQ, in each case for both mass transitions.

Characteristics for the LC-MS/MS primary method (modified QuEChERS) used for the quantitation of lenacil residue in animal commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in animal commodities**	
	Lenacil
Linearity	***Primary method:*** Quantification 235 → 153 : Linear and r^2 = 0.9986 (milk) Linear and r^2 = 0.9988 (eggs) Linear and r^2 = 0.9998 (meat) Linear and r^2 = 0.9999 (fat) Linear and r^2 = 0.9993 (liver) Confirmation 235 → 136: Linear and r^2 = 0.9984 (milk) Linear and r^2 = 0.9989 (eggs) Linear and r^2 = 1.0000 (meat) Linear and r^2 = 0.9997 (fat) Linear and r^2 = 0.9985 (liver) Linear calibration curves (peak area vs. concentration) and corresponding regression equations were provided within the study report for both mass transitions and for all matrices (matrix-matched calibrations). ***ILV:*** Quantification 235 → 153 : Linear and r = 0.99998 (in solvent) Confirmation 235 → 136: Linear and r = 0.99995 (in solvent) Linear calibration curves (peak area vs. concentration) and corresponding regression equations were provided within the study report for both mass transitions.
Calibration	
Accepted calibration range in concentration units (e.g. in µg/ml or ng/µl)	***Primary method:*** 1.00 – 100 µg/L for all matrices (matrix-matched calibration) The concentration of the samples (~ 5 and 50 µg/L according to the method) meet the calibration range. ***ILV:*** 0.05 – 5.0 ng/mL (calibration in solvent, no matrix-matched calibration)

Characteristics for the LC-MS/MS primary method (modified QuEChERS) used for the quantitation of lenacil residue in animal commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in animal commodities**	
	Lenacil
Corresponding calibration range in mass ratio units for the sample (e.g.in mg/kg or µg/L)	***Primary method:*** ~ 0.002– 0.2 mg/kg in all matrices (matrix-matched calibration) ***ILV:*** ~ 0.002– 0.2 mg/kg in all matrices (no matrix-matched calibration)
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)? (yes/ no)	***Primary method:*** N = 7 (single injection) for each calibration set. ***ILV:*** N = 8 (single injection) for each calibration set.
Assessment of matrix effects is presented (yes/no)	***Primary method:*** All calibration standards were prepared in sample extracts. Matrix effects (i.e. response of analyte in calibration solutions in solvent [acetonitrile/water: 1/1] versus response in matrix matched calibration solutions) were investigated at two concentrations (5 and 50 µg/L) and, more than likely, for both mass transitions in each matrix. Significant matrix effects were observed (above 20%) for milk and liver. No significant matrix effects above 20 % were observed for eggs, meat and fat. Nevertheless, it was opted to use matrix-matched calibration for each matrix. ***ILV:*** Matrix effects were determined for both mass transitions by comparison of the peak area of a matrix-matched calibration standard for each of the concerned matrices at the concentration of ~ 2.5 ng/mL with a solvent calibration standard at the same concentration level. No significant matrix effects (all < 20%) were observed in any matrices and therefore, no matrix-matched calibration was used.
Absence of interference >30% of LOQ in blank sample is demonstrated (yes/no)	***Primary method and ILV:*** Yes (no residues of analytes in the controls that interfered with the analysis at levels above 30% of the LOQ)
Chromatogram of sample spiked at LOQ demonstrates sufficient S/N ratio? (yes/no)	***Primary method:*** Yes based on the chromatograms provided. ***ILV:*** Yes based on the chromatograms provided.

Characteristics for the LC-MS/MS primary method (modified QuEChERS) used for the quantitation of lenacil residue in animal commodities **Characteristics for the ILV method used for the quantitation of lenacil residue in animal commodities**	
	Lenacil
LOQ (mg/kg) (based on the the lowest analyte concentration in a sample at which the methodology has been validated, acceptable mean recovery and RSD)	***Primary method:*** 0.01 mg/kg in all matrices LOQ results from the lowest concentration level successfully tested within recovery experiments. The limit of detection (LOD) of the method was set to 30% of LOQ (LOD ≤ 0.003 mg/kg). ***ILV:*** 0.01 mg/kg in all matrices LOQ results from the lowest concentration level successfully tested within recovery experiments. The limit of detection (LOD) was set at 0.003 mg/kg (30% of the LOQ).

Recovery and repeatability:
Matrix samples were fortified with lenacil at two different levels. The fortification data reported in the method for lenacil residues in animal tissues, milk, and eggs are summarised in Table B.5.2.2-1. The average recovery per fortification level specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%. Therefore, the recovery of this method is adequate for the purposes of residue data collection and enforcement of MRLs.
The same analyst obtained these recovery data over the course of multiple days. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix, as well as at higher levels. Therefore, the repeatability of this method is adequate for the purposes of residue data collection and enforcement of MRLs.
Regarding the reproducibility, independent laboratory validations of the methods were successfully conducted and the results are presented in Table 5.2.2-2.

Table B.5.2.2-1 : Validation data for analytical methods for the determination of lenacil in food of animal origin

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a, b]	Average recovery (%) [range]	% Relative standard deviation
Wagner, B. (2016) 15S07170-01-VMAT	**Lenacil SRM 235 → 153 (quantification)**				
	Milk	5 5 Total = 10	0.010 0.10	84 [76 – 93] 83 [80 – 89] Overall = 84	7.6 4.5 Overall = 5.9
	Eggs	5 5 Total = 10	0.010 0.10	95 [86 – 99] 96 [89 – 97] Overall = 95	5.4 4.3 Overall = 4.7
	Meat	5 5 Total = 10	0.010 0.10	91 [89 – 92] 91 [88 – 92] Overall = 91	1.3 1.7 Overall = 1.4
	Fat	5 5 Total = 10	0.010 0.10	88 [85 – 89] 89 [85 – 91] Overall = 88	1.9 2.7 Overall = 2.3
	Liver	5 5 Total = 10	0.010 0.10	93 [91 – 94] 98 [96 – 99] Overall = 95	1.3 1.3 Overall = 2.9
	Lenacil SRM 235 → 136 (confirmation)				
	Milk	5 5 Total = 10	0.010 0.10	84 [76 – 92] 84 [80 – 89] Overall = 84	7.2 4.4 Overall = 5.6
	Eggs	5 5 Total = 10	0.010 0.10	95 [85 – 100] 96 [89 – 99] Overall = 95	6.4 4.3 Overall = 5.2
	Meat	5 5 Total = 10	0.010 0.10	91 [90 – 92] 90 [88 – 91] Overall = 90	0.9 1.4 Overall = 1.3
	Fat	5 5 Total = 10	0.010 0.10	87 [84 – 88] 89 [85 – 91] Overall = 88	2.0 2.7 Overall = 2.5
	Liver	5 5 Total = 10	0.010 0.10	92 [90 – 96] 96 [95 – 97] Overall = 94	2.7 0.9 Overall = 3.2

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Note: control specimens of each matrix were analysed in duplicate.

Table B.5.2.2-2 : Validation data for analytical methods for the determination of lenacil in food of animal origin

Reference	Matrix	Number of tests	Fortification level (mg/kg) [(a)]	Average recovery (%) [range]	% Relative standard deviation
Bodsch, J. (2016) IF-16/03724646	**Lenacil SRM 235 → 153 (quantification)**				
	Milk*	5 5 Total = 10	0.010 0.10	91.5 [88.3 – 92.8] 96.9 [95.0 – 98.4] Overall = 94.2	2.0 1.5 Overall = 3.5
	Eggs	5 5 Total = 10	0.010 0.10	91.3 [90.4 – 92.5] 95.5 [94.1 – 95.8] Overall = 93.4	1.0 1.5 Overall = 2.7
	Meat	5 5 Total = 10	0.010 0.10	88.2 [86.1 – 88.9] 87.8 [86.2 – 89.6] Overall = 88.0	1.3 1.6 Overall = 1.4
	Fat	5 5 Total = 10	0.010 0.10	86.6 [85.8 – 87.2] 90.1 [89.2 – 90.7] Overall = 88.3	0.6 0.7 Overall = 2.2
	Liver	5 5 Total = 10	0.010 0.10	89.8 [88.7 – 90.7] 90.6 [89.2 – 92.3] Overall = 90.2	1.0 1.9 Overall = 1.5
	Lenacil SRM 235 → 136 (confirmation)				
	Milk*	5 5 Total = 10	0.010 0.10	89.2 [83.6 – 92.9] 97.1 [94.4 – 98.9] Overall = 93.2	3.9 1.9 Overall = 5.3
	Eggs	5 5 Total = 10	0.010 0.10	93.1 [91.2 – 95-5] 95.6 [94.1 – 97.8] Overall = 94.3	2.2 1.5 Overall = 2.2
	Meat	5 5 Total = 10	0.010 0.10	90.5 [88.2 – 92.2] 87.7 [86 – 89.9] Overall = 89.1	1.7 1.7 Overall = 2.3
	Fat	5 5 Total = 10	0.010 0.10	86.7 [85.1 – 89.2] 90.3 [88.6 – 91.1] Overall = 88.5	1.9 1.2 Overall = 2.6
	Liver	5 5 Total = 10	0.010 0.10	92.8 [90.2 – 94.5] 90.9 [89.9 – 92.8] Overall = 91.8	2.0 1.4 Overall = 2.0

[a] Fortifications were performed with analyte reference standard solutions
* corrected (blank subtraction 3.8% and 4.4% of LOQ respectively)
Note: control specimens of each matrix were analysed in duplicate.

Stability of lenacil in final sample extract:
The samples were stored at < - 18°C in the dark for seven days. All recoveries remained within the 70 – 120 % range.

Since the stability of lenacil in the specimen extracts was performed in the validation of the primary method, the stability was not re-investigated in the ILV. However, the stability of the reference solutions prepared in acetonitrile/water (1/1, v/v) was proven by reanalysis of solutions prepared at the beginning of the study and comparing the results with solutions freshly prepared. The stability was sufficiently proven for a period of 20 days.

Extraction Efficiency
The applicant stated the following:

The solubility in organic solvents was tested in study AMR 2377-92 (KCA 2.6/01). In acetonitrile the solubility of lenacil is 230 ppm. Taking the high solubility of lenacil in acetonitrile into account it can be concluded that the extraction efficiency using acetonitrile/water is very good. The validation of the QuEChERS method with mean recovery values between 84 and 85% for lenacil in animal matrices shows that the extraction of the residues is efficient.

Furthermore, it has to be considered that according to SANCO 825/00 rev. 8.1 extraction procedures used in residue analytical methods for the determination of residues in plants, plant products, foodstuff (of plant and animal origin) and in feeding stuff should be verified for all matrix groups for which residues ≥ LOQ are expected.

The residue levels in animal matrices are expected to be below the respective LOQ and no incurred residues in will be found. Therefore, a further cross validation of the QuEChERS method is not necessary.

RMS Conclusion 2018 (Renewal):
The residue method based on the QuEChERS for the determination of lenacil in milk, meat (muscle), liver, fat and egg is fully validated according to SANCO/825/00 rev. 8.1. Specificity/absence of interferences, linearity, accuracy and repeatability have been demonstrated. Mean recovery in each matrix and at each fortification level was within the 70 – 120% range and % RSDs were systematically below 20% in accordance with the SANCO/825/00 rev. 8.1. The LOQ of 0.01 mg/kg in each matrix is substantiated by the validation data and corresponds to the lowest test concentration with acceptable recovery and repeatability (accuracy experiment).
The method is self-confirmatory since 2 mass transitions were followed and fully validated.
The method was successfully independently validated in all matrix types and the LOQ has been confirmed.
Extraction efficiency cannot be demonstrated and addressed at this stage since samples from radiolabeled animal metabolism or samples with incurred residues from feeding studies are not available (livestock metabolism or livestock feeding studies are not available). The statement of the notifier that the residue levels in animal matrices are expected to be below the respective LOQ and no incurred residues will be found cannot be confirmed by the RMS as no livestock metabolism studies or feeding studies were provided.
The method can be recommended for enforcement/monitoring purposes.

B.5.2.3. Analytical methods for the determination of the active substance and/or metabolites in soil

Enforcement methods suitable for the European Union region
Analytical methods (GC-MS and HPLC-MS/MS) for the determination of residues in soil are summarised in the Lenacil DAR, Volume 3, B.5, 2007. All analytical methods are active substance data and were provided in the EU review of lenacil and were considered adequate at that time. Summaries reflecting the Lenacil DAR, Volume 3, B.5.3.1, 2007 are given below for the sake of completeness.

During the 1st approval, the GC-MS was proposed to be the primary method for monitoring purposes and the HPLC-MS/MS was considered as the confirmatory method.

Previous evaluation:	*Initial monograph November 2007*

Study No. 1

Report:	**CA 4.2(b)/01 (IIA 4.2.2.1-01)** Brodsky J. and Zietz E. (1990) Determination of residues of lenacil in soil (treated with Venzar, Season 1989, Germany) Batelle Europe – Frankfurt Division DuPont Report No.: BE-A-11-90-10-BF
Guidelines:	Not stated

GLP:	Yes (GLP compliance stated)

Note: CoA of reference standard was not included in the study report and no expiry date was mentioned.

Description of the method:
Soil samples were extracted twice with acetone/dichloromethane by shaking at room temperature followed by centrifugation. The pooled extracts from each soil sample were evaporated to dryness, re-dissolved in acetone, and analysed by GC-MS (fused silica capillary column with DB-1301, 30m x 0.25mm, 0.25µm; mass fragment ion: m/z = 153). Validation of the method was performed using two kinds of soil: Standard soil 2.2 (86.8% sand, 8.8% silt, 4.2% clay) and clay loam (19.2% sand, 8.8% silt, 32.0% clay). External calibration was used.

Chromatographic conditions:

Column:	fused silica capillary column with DB-1301, 30m x 0.25mm, 0.25µm (J&W Scientific)
Column head pressure:	69 kPa
Injector:	250°C, splitless mode with 1 min. purge off time, split flow: 25.4 mL.min, purge flow: 4.0 mL/min
Oven temperature:	60°C (2 min.) 60°C → 250°C (20°C/min.) 250°C → 260°C (5°C/min.) 260°C → 280°C (20°C/min) 280°C (3 min.)
Injection volume:	2 µL
Ionization:	electron impact ionistation, 70eV
m/z fragment ion:	153
Retention time:	12.5 min.

Characteristics of soils:

Soil type	Sand	Silt	Clay
Soil 2.2 (standard soil Speyer)	86.8%	8.8%	4.2%
Soil TL (clay loam)	19.2%	48.8%	32.0%

Findings:

Specificity - interferences: No significant interferences (< 30% LOQ) were detected at the characteristic retention time of lenacil in any of the control (untreated) samples. Chromatograms were provided for a calibration standard, a control sample (untreated soil from field trials), a fortified sample at 0.05 mg/kg and a sample from the field trials. No mass spectrum was however provided. The notifier mentioned that the GC-MS spectrum will be provided as a supplement to the report. However, if the HPLC-MS/MS is fully re-validated to be recommended as the monitoring method, then further data on the mass spectrum for GC-MS will not be necessary.
Validation data were generated only for one fragment ion.

Linearity: Good linearity of calibration curves (based on the typical curve peak height vs. pg injected for Soil 2.2) was observed in the range of 100 – 1000 ng/mL (n=5) for lenacil; approximate corresponding residue concentration is 0.02 – 0.2 mg/kg, but the correlation coefficient was not provided. All calibration standards were prepared in sample extracts. Where required, the extracts were diluted appropriately with acetone to meet the calibration range.

Recovery: The fortification data reported in the method for lenacil residues in soil are summarised in Table B.5.2.3-1 below. The average recovery per fortification level specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%. Fortification of untreated samples of field trials were also performed at 0.1 and 1 mg/kg with all recoveries within the 70 – 120 % range.

Repeatability: Relative standard deviations of less than 20% were obtained for the fortifications made at the quantification limit, as well as at higher levels.

Therefore, the repeatability of this method is adequate for the purposes of residue data collection and enforcement of MRLs.

Limit of quantification: The limit of quantification of the method for lenacil in soil is 0.05 mg/kg.

Table B.5.2.3-1 : Validation data for analytical method GC-MS for the determination of lenacil in soil

Reference	Matrix	Number of tests per soil type	Fortification level (mg/kg) [a, b]	Average recovery (%) [range]	Standard deviation	% Relative standard deviation
Brodsky, J., Zietz, E. (1990), BE-A-11-90-10-BF	Soil (results combined for Soil 2.2 and Soil TL)	2 (Soil 2.2)	0.05	82.0 [80 – 84]	-	-
		3 (Soil TL)		90.0 [86 – 94]	4.0	4.4
		Total = 5		Overall = 86.8	5.4	Overall = 6.2
		2 (Soil 2.2)	0.10	97.0 [96 – 98]	-	-
		3 (Soil TL)		83.7 [81 – 86]	2.5	3.0
		Total = 5		Overall = 89.0	7.5	Overall = 8.5
		3 (Soil 2.2)	0.51*	73.3 [73 – 74]	0.6	0.8
		2 (Soil TL)		72.0 [**66** – 78]	-	-
		Total = 5		Overall = 72.8	4.3	Overall = 5.9
		2 (Soil TL)	2.55*	71.0 [**69** – 73]	-	-
		Overall = 17		Overall = 81.5	Overall = 9.5	Overall = 11.7

* appropriate dilution of the final extract was carried out in order to obtain responses within the calibration range

Extraction Efficiency

In the study "[^{14}C]-Lenacil: Aerobic Soil Metabolism and Transformation" (KCA 7.1.2.1.1/01; Report No.: 8247509) it was shown that the extractability was quantitative with amounts of applied radioactivity in the acetonitrile/acetonitrile:water (3:1 v/v) extract at mean levels of 99.7 to 100.5%.

The solubility in organic solvents was tested in study AMR 2377-92 (KCA 2.6/01). In acetonitrile the solubility of lenacil is 230 ppm, in acetone 690 ppm and in dichloromethane 2000 ppm. Taking the high solubility of lenacil in acetone and dichloromethane into account it can concluded that the extraction with acetone/dichloromethane is to be at the same level as the extraction with acetonitrile/acetonitrile: water (3:1 v/v).

RMS comment on extraction efficiency:
The statement of the notifier for extraction efficiency of solvents used in method GC-MS for determination of lenacil in soil seems reasonable to the RMS. Nevertheless, it is noted that SANCO/825/00 rev. 8.1 does not require to demonstrate the extraction efficiency from soil.

RMS Conclusion (initial monograph):
The GC-MS method appears to be suitable for the determination of lenacil in soil with a LOQ of 0.05 mg/kg. The method is however not considered to be highly specific (only 1 fragment ion was used for MS detection) and therefore, a confirmatory method is still required for enforcement purposes. It is noted that the HPLC-MS/MS method validated by Mende (2003) (*vide infra*) can serve as confirmatory method.

RMS Conclusion 2018 (Renewal):
The proposed GC-MS method can be considered as sufficiently validated for SANCO/3029/99 (absence of interferences, linearity, accuracy within 70 – 110% and repeatability < 20%) and the number of replicates is considered sufficient if the results of the different soils are combined. The LOQ of 0.05 mg/kg is sufficiently substantiated by experimental data.

As mentioned for 1st approval, the method cannot be considered sufficiently validated according to SANCO/825/00 rev. 8.1 to recommend it for monitoring since no confirmation occurred. The HPLC-MS/MS method validated by Mende (2003) (*vide infra – Study No. 2*) and which was also already considered for 1st approval was proposed and considered as an acceptable confirmatory of the GC-MS method.

However, in the frame of the renewal, the notifier initially proposed the HPLC-MS/MS method (more specific than the GC-MS) validated on soil with an LOQ of 0.02 mg/kg as the recommended monitoring method and proposed to consider the previous GC-MS as the confirmatory method of the HPLC-MS/MS. This was in

consideration that detection by HPLC/MS/MS is more specific than by GC/MS and that methods by HPLC/MS/MS and GC/MS are independent analytical techniques in accordance with the guideline SANCO/825/00 rev. 8.1.

RMS could however not fully agree with this proposal since the GC-MS (proposed as confirmatory method) was only validated at the LOQ of 0.05 mg/kg whereas the validated LOQ of the HPLC-MS/MS according to the data is 0.02 mg/kg.

It is noted that the HPLC-MS/MS method has been tested within the 0.02 – 0.5 mg/kg (validated in the 0.02 -0.2 mg/kg) where the level of 0.05 mg/kg was also investigated but with only one replicate and a limit recovery (71 % , % RSD cannot be determined). Although, it can be reasonably expected that the 0.05 mg/kg level is validated for the HPLC-MS/MS (by considering the validation data at 0.02, 0.05 and 0.2 mg/kg), strict validation at that level and ten times higher (0.5 mg/kg) was not performed and RMS has some reservation to fully agree with the proposal to consider again the GC-MS as the primary monitoring method and to consider the HPLC-MS/MS as the confirmatory method.

Consequently, RMS was and remains of the opinion that, in order to be fully in agreement with the SANCO/825/00 rev. 8.1 and to use a more state of the art technique, a full re-validation of the HPLC-MS/MS method (described here below) should be performed by monitoring and validation of two mass transitions.

In the course of the assessment and following the above discussed point, the notifier informed the RMS to his wish to reconsider the GC-MS as the primary method recommended for enforcement/monitoring method and to consider the HPLC-MS/MS method again as the confirmatory method.
Nevertheless, the notifier informed the RMS that a re-validation of the soil method 20011048/E1-FSD (HPLC-MS/MS) with a confirmatory method will be conducted immediately to satisfy new method requirements (two mass transitions will be monitored).

Study No. 2

Report:	**CA 4.2(b)/02** (IIA 4.2.2.1-02) Mende (2003) Analytical Report – Venzar 80% WP (containing 80% lenacil) related soil dissipation on bare soil, four sites in Europe, 2001 GAB Biotechnologie GmbH and IFU Umweltanalytik GmbH, Germany DuPont Report No.: 20011048/E1-FSD
Guidelines:	Not stated.
GLP:	Yes (GLP compliance stated)

Description of the method:
Lenacil and its metabolite IN-KF313 are extracted from the sample with acetonitrile/water. The extract is saturated with sodium chloride and an aliquot was evaporated to dryness. The residue is reconstituted in methanol/water and analysed by HPLC (ThermoHypersil HyPurity C8, 150mm x 3mm, 5µm) with MS/MS detection (m/z 233 → m/z 151 for lenacil; m/z247 → m/z 165 for IN-KF 313). Validation of the method was carried out using soil samples from field trials performed in Spain, Germany and France in the period 2001 to 2002. External calibration was used.

Chromatographic conditions:
Column: ThermoHypersil HyPurity C8, 150mm x 3mm, 5µm
Mobile phase:

Time (min)	Flow (mL/min)	% MeOH/H_2O: 20/80 v/v	% ACN/ H_2O: 50/50 v/v	% ACN	Gradient
0.00	0.5	100	0	0	-
5.00	0.5	0	100	0	Linear
10.00	0.5	0	50	50	Linear
10.01	0.5	0	0	100	-
12.00	0.5	0	0	100	-
13.00	0.5	100	0	0	Linear

Column temperature:	40°C
Detection:	ThermoFinningan LCQ Duo, ESI in negative mode
m/z mass transitions:	Lenacil: 233 → 151 m/z Metabolite KF-313: 247 → 165 m/z

Findings:

Specificity - interferences:

No interfering peaks were detected (< 30% LOQ) in control (untreated samples) at the characteristic retention times of lenacil and IN-KF313.
HPLC-MS/MS is a highly specific technique but validation was only performed on one mass transition. The method is principle self-confirmatory but the GC-MS method by Brodsky J. and Zietz E. (1990) is proposed as confirmatory method.
Chromatograms were provided for a calibration standard, a control sample, a fortified samples at the LOQ and a treated sample, in each case for both analytes lenacil and metabolite IN-KF313.
No product ion spectra were provided but a spectrum can be found in report 20011048/E1-FPSB Analytical (same conditions) (Mende 2002 – *Analytical Final Report – Generation of Samples for the determination of Residues of Venzar 80 % WP (containing 80 % Lenacil) in sugar Beets* – reported under B.5.1.2.5.1 – Study No. 1.

Linearity:

Good linearity of calibration curves (peak area vs. concentration) was observed for lenacil and IN-KF313.
Lenacil: calibration range 20 – 500 ng/mL (n=8); linear regression line (R^2 = 0.9993); approximate corresponding residue concentration range: 0.008 – 0.2 mg/kg (depending on final volume)
IN-KF313: calibration range 20 to 200 ng/mL (n=5), quadratic regression line (R^2 = 0.9984); approximate corresponding residue concentration range: 0.008 – 0.08 mg/kg (depending on final volume.
Quantification was performed by means of a calibration curve prepared in methanol/water: 20/80 or in extracts from control soils depending on the presence of matrix effects. Results on matrix effects were however not reported within the study report.

Recovery:

The fortification data reported in the method for lenacil residues in soil are summarised in Table B.5.2.3-2 below. The average recovery per fortification level specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%.

Repeatability:

See Table B.5.2.3-2 below. All % RSD were below 20%.

Limit of quantification:

The limit of quantification of the method for lenacil in soil is 0.02 mg/kg.

Table B.5.2.3-2 : Validation data for analytical method for the determination of lenacil and the metabolite IN-KF313 in soil

Reference	Matrix	Number of tests	Fortification level (mg/kg) [a]	Average recovery (%) [range]	Standard deviation	% Relative standard deviation
Mende, P. (2003) 20011048/E1-FSD	**Lenacil in soil (m/z 233 → 151)**					
	Elmton Soil	10	0.02	90 [84 – 100]	5	6
		1	0.05	71 [-]	-	-
		10	0.20	89 [80 – 95]	4	5
		2	0.50	95 [91 – 98]	-	-
		Total = 23		Overall = 89		Overall = 7
	IN-KF313 in soil (m/z 247 → 165)					
	Elmton Soil	10	0.02	78 [71 – 88]	6	7
		1	0.05	**68** [-]	-	-
		8	0.20	84 [75 – 96]	7	8
		2	0.50	87 [84 – 90]	-	-
		Total = 21		Overall = 80		Overall = 9

[a] Fortifications were performed with analyte reference standard solutions

Stability of extracts
The storage stability of lenacil in deep-frozen soil samples was verified by repeated analysis of treated soil samples and comparison of lenacil residues with the values obtained from earlier analyses. Each sample was analyzed in duplicate in February 2002 and November 2002. The results indicate that lenacil is stable in deep-frozen soil samples for at least 8.5 months (87 to 112 % of the initial value).

Extraction Efficiency
In the study "[^{14}C]-Lenacil: Aerobic Soil Metabolism and Transformation" (KCA 7.1.2.1.1/01; Report No.: 8247509) it was shown that the extractability was quantitative with amounts of applied radioactivity in the acetonitrile/acetonitrile:water (3:1 v/v) extract at mean levels of 99.7 to 100.5%. This shows that the extraction efficiency is very high using extraction with acetonitrile/water.

RMS comment on extraction efficiency:
The statement of the notifier for extraction efficiency of solvents used in LC-MS/MS method for determination of lenacil in soil seems reasonable to the RMS. Nevertheless, it is noted that SANCO/825/00 rev. 8.1 does not require to demonstrate the extraction efficiency from soil.

RMS Conclusion (initial monograph):
The HPLC-MS/MS method is suitable for the determination of residues of lenacil and metabolite KF-313 in soil with a LOQ of 0.02 mg/kg.

RMS Conclusion 2018 (Renewal):
The HPLC-MS/MS method seems suitable for the determination of residues of lenacil and metabolite KF-313 in soil with a LOQ of 0.02 mg/kg (corresponding to the lowest fortification level with an acceptable recovery and % RSD).
The HPLC-MS/MS method is validated for absence of interferences, linearity, accuracy and repeatability. Mean recoveries at each fortification level was within the 70 -120 % range and the % RSD is below the recommended 20%. The method is in principle highly specific and self-confirmatory. However, validation data were only generated for one mass transition and SANCO/825/00 rev.8.1 requires that the method is confirmed.
The notifier informed the RMS that a re-validation of the soil method 20011048/E1-FSD (HPLC-MS/MS) with a confirmatory method will be conducted immediately to satisfy new method requirements (two mass transitions will be monitored) (please refer to the RMS conclusion here above under Study No. 1: CA 4.2(b)/01, Brodsky J. and Zietz E. (1990)).

B.5.2.4. Analytical methods for the determination of the active substance and/or metabolites in water

Enforcement methods suitable for the European Union region
For the first approval of lenacil, the monitoring method that was proposed was the method by Wittig A. 2002 (IIA 4.2.3.1-01) summarised under point B.5.1.2.7 above. However, although the method a self-confirmatory method HPLC-MS/MS, no validation data were generated for a second mass transition and no ILV was developed.

Therefore, in the frame of renewal, a new HPLC-MS/MS method was developed and validated on drinking and surface waters at a LOQ of 0.1 µg/L for the determination of lenacil in water. The method is successfully validated according to SANCO 825/00 rev.8.1 and suitable for the determination of lenacil in water and is therefore proposed as the enforcement method for residues of lenacil in water. The method is successfully validated by an independent laboratory.

Previous evaluation:	*No, submitted for the purpose of renewal* *Note: Study No. 1 has already been assessed at zonal level for venzar 80WP*

Study No. 1

Report:	**CA 4.2(b)/03 [KCA 4.2.4/01]** Witte, A., (2009) Development and validation of an analytical method for the determination of residues of lenacil in drinking and surface water CIP Chemisches Institut, Germany CIP Study code: 09D2203-01-VMWA DuPont Report No.: 09D2203-01-VMWA
Guidelines:	SANCO/825/00 rev.7
GLP:	Yes

Study No. 2

Report:	**CA 4.2(b)/04 [KCA 4.2.4/02]** Link, Th. (2016) Independent Laboratory Validation of analytical method 09D02203-01-VMWA for the determination of residues of Lenacil in tap water by LC-MS/MS SGS Institut Fresenius GmbH, Germany SGS Study No. IF-15/03444276 DuPont Report No.: DuPont-IF-15/03444276
Guidelines:	SANCO/825/00 rev. 8.1 OECD ENV/JM/MONO (2007), 17 13/08/2013 Consensus Document of the Working Group on GLP 'The application of OECD Principles of GLP to the Organisation and Management of Multi-site studies ENV/JM/MONO (2002)9 No.13
GLP:	Yes

Description of the method:
Water samples were injected after dilution with HPLC water (1:5 v/v) into the HPLC-MS/MS system. Quantification is performed by using external standard in water. External calibration.

For the ILV, water specimens were diluted with ultra-pure water (1:4; v/v) and injected directly into the HPLC-MS/MS system. External calibration.

Chromatographic conditions of the primary method (study 09D2203-01-VMWA):
Column: Supelco Ascentis Express C18, 50 mm × 2.1 mm, 2.7 µm particle size (Part. No. 53822-U)
Injection volume: 100 µL
Column temperature: 40°C
Mobile phase:

Time (min.)	Flow rate (µL/min)	Water	Methanol	0.5% acetic acid	gradient
0.00	250	89	10	1	Linear
0.50	250	89	10	1	
4.50	250	0	99	1	
6.50	250	0	99	1	
6.51	250	89	10	1	
8.50	250	89	10	1	

Retention time: ~ 4.7 min.
Detection: MRM, Heated Electrospray Ionisation, positive mode
m/z: 235 → 153 (quantitation)
m/z: 235 → 136 (confirmation)

Chromatographic conditions of the ILV (study IF-15/03444276):
The tertiary gradient from the primary method was replaced with a binary gradient due to given HPLC hardware. The acetic acid was pre-mixed with the methanol to obtain the same ratio of components.

Column: Supelco Ascentis Express C18, 50 mm × 2.1 mm, 2.7 µm particle size (Part. No. 53822-U)
Injection volume: 15 µL
Column temperature: 40°C
Mobile phase:

Time (min.)	Flow rate (µL/min)	0.005% acetic acid in water	0.005% acetic acid in methanol
0.1	250	90	10
0.5	250	90	10
4.5	250	1	99
6.0	250	1	99
6.51	250	90	10
8.5	250	90	10

Retention time: ~ 4.05 min.
Detection: MRM, Heated Electrospray Ionisation, positive mode
m/z: 235 → 153 (quantitation)
m/z: 235 → 136 (confirmation)

Characteristics of the water samples:
Drinking water:

pH	8.20
conductivity	261 µS/cm
TOC	1.4 mg/L
Total hardness	4.0 °dH

°dH is defined as 10 mg of CaO/L of water. This is equivalent to 17.848 mg of $CaCO_3$/L of water

Surface water:

pH	7.19
Conductivity	156 µS/cm
DOC	2.3 mg/L
Total hardness	2.0 °dH
Suspended solids	< 10 mg/L

°dH is defined as 10 mg of CaO/L of water. This is equivalent to 17.848 mg of $CaCO_3$/L of water

Characteristics of the water samples in ILV:
Drinking water (tap water):

pH	7.90
conductivity	592 µS/cm
TOC	1.24 mg/L
Total hardness	16.1 °dH

°dH is defined as 10 mg of CaO/L of water. This is equivalent to 17.848 mg of $CaCO_3$/L of water

Findings:

Specificity - interferences:

Primary method:

The HPLC-MS/MS is a highly specific technique by monitoring two mass transitions. (method is self-confirmatory). Monitoring two mass transitions ensured unambiguous identification.

No significant interferences from the specimen (no residues above 30% of the LOQ) matrix were detected at the retention time corresponding to lenacil in any of the control specimens.

Chromatograms were provided for calibration standards at 0.02 µg/L and 0.2 µg/L, control samples, fortified samples at LOQ and 10 × LOQ, in each case for both water types and both mass transitions.

A product ion spectrum was also presented in the study report.

ILV:

The HPLC-MS/MS is a highly specific technique by monitoring two mass transitions. (method is self-confirmatory). Monitoring two mass transitions ensured unambiguous identification.

No significant interferences from the specimen (no residues above 30% of the LOQ) matrix were detected at the retention time corresponding to lenacil in any of the control specimens.

Chromatograms were provided for calibration standards at 0.02 ng/mL (corresponding to the LOQ level), control samples, fortified samples at LOQ for both mass transitions.

A full mass spectrum and a product ion spectrum were also presented in the study report.

Linearity:

Primary method:

Good linearity was observed in the range of 0.005 ng/mL to 0.5 ng/mL (n = 7, single injection, corresponding to ~ 0.025 – 2.5 µg/L) for lenacil with r^2 = 1.0000 at both mass transitions. All calculations were performed using standards prepared in solvent (water). Matrix-matched standards were prepared for each water matrix under investigation at two concentration levels corresponding to the LOQ and 10 × LOQ (0.02 and 0.2 µg/L). Water samples were diluted 1:5 with HPLC water to minimize matrix effects and therefore the matrix effect was tested in drinking water and surface water diluted 1:5 in HPLC water. No significant matrix effects (> 5%) were observed.

ILV:

Good linearity was observed in the range of 0.006 ng/mL to 0.3 ng/mL (n = 8, single injection, corresponding to ~ 0.03 – 1.5 µg/L according to the method) for lenacil with r = 0.99983 and r = 0.99988 at m/z 235 → 153 and 235 → 136, respectively. All calculations were performed using standards prepared in solvent (water). A matrix-matched calibration standard (tap water) was prepared at ~ 0.2 ng/mL. The peak areas of this matrix-matched standard was compared to the peak area of a solvent calibration standard at the same concentration. No matrix effects (≤ - 2%) were observed for both mass transitions. Consequently, no matrix-matched calibration was used. All the samples meet the calibration range.

Recovery:

Primary method and ILV:

Samples of surface and drinking water were fortified with lenacil at two different levels. Fortification data reported in the method proposed for monitoring lenacil residues in water are summarised in Table 5.2.4-1 below.

Repeatability:

Primary method and ILV:

Repeatability of the method is addressed by the data in Table 5.2.4-1. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit and 10x the quantification limit for each matrix. Therefore, the repeatability of this method would seem to be adequate for the purposes of detecting lenacil residues in water.

Limit of quantification:

Primary method and ILV:

0.1 µg/L (0.0001 mg/L) in both water types. The EU guidelines for drinking water methods specify that methods must be capable of measuring levels at or above 0.1 µg/L, which is the maximum allowable level of any crop protection chemical in drinking water. LOD was set to 30% of the LOQ (i.e. 0.03 µg/L).

Table B.5.2.4-1 : Validation data for analytical methods for the determination of lenacil in water

Reference	Matrix	Number of tests	Fortification level (µg/L) [a, b]	Average recovery (%) [range]	% Relative standard deviation
Witte (2009), DuPont-09D02203-01-VMWA	**Lenacil MRM 235 → 153 (quantification)**				
	Drinking Water	5 5 Total = 10	0.1 1.0	100 [98 – 102] 98 [96 – 99] Overall = 99	1.8 1.2 Overall = 2.1
	Surface Water	5 5 Total = 10	0.1 1.0	102 [97 – 107] 98 [98 – 99] Overall = 100	3.9 0.6 Overall = 3.3
	Lenacil MRM 235 → 136 (confirmation)				
	Drinking Water	5 5 Total = 10	0.1 1.0	99 [92 – 103] 95 [90 – 100] Overall = 97	5.1 3.9 Overall = 4.7
	Surface Water	5 5 Total = 10	0.1 1.0	104 [94 – **111**] 98 [97 – 99] Overall = 101	6.2 0.9 Overall = 5.3

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Note: Control specimens of surface and drinking water were analysed each in duplicate.

Table B.5.2.4-2 : Validation data for the ILV for the determination of lenacil in water

Reference	Matrix	Number of tests	Fortification level (µg/L) [a, b]	Average recovery (%) [range]	% Relative standard deviation
Link (2016), IF-15/03444276	**Lenacil MRM 235 → 153 (quantitation)**				
	Tap Water	5 5 Total = 10	0.1 1.0	86 [83 – 88] 99 [98 – 100] Overall = 92	2.6 0.9 Overall = 7.3
	Lenacil MRM 235 → 136 (confirmation)				
	Tap Water	5 5 Total = 10	0.1 1.0	86 [81 – 89] 98 [97 – 99] Overall = 92	3.5 1.0 Overall = 7.3

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level.

Note: Control specimens of tap water were analysed each in duplicate.

Stability of lenacil in final samples:
Analysis of lenacil in water was performed within 24 hours after preparation of the samples and therefore, no separate stability check was performed. In the ILV, a stability test with the reference solutions was however performed and showed acceptable %.

RMS Conclusion 2018 (Renewal):
The LC-MS/MS method for the determination of lenacil in tap water is fully validated according to SANCO/825/00 rev. 8.1. The specificity/absence of interferences, linearity, accuracy and repeatability have been demonstrated. The mean recovery at each fortification level is systematically within the 70 – 120 % range with % RSD below 20%.
The validated LOQ of 0.1 µg/L is substantiated by the experimental data and corresponds to the lowest tested concentration showing an acceptable recovery with an acceptable % RSD (lowest fortification level). The method is self-confirmatory by monitoring two mass transitions (m/z 235 → 153 and m/z 235 → 136) and both mass transition have been validated. The method has also been successfully independently validated and the LOQ of 0.1 µg/L has been confirmed.
The method can be recommended for enforcement/monitoring purposes. The LOQ complies with the trigger limit of 0.1 µg/L set in SANCO/825/00 rev. 8.1 for the drinking and surface water and that LOQ for surface water complies with the lowest effect concentration of 3.78 µg a.s./L (ErC50 as measured for *Elodea canadensis*).

B.5.2.5. Analytical methods for the determination of the active substance and/or metabolites in air

Enforcement methods suitable for the European Union region

In PRAPeR Expert Meeting 66 (April 2009), EFSA identified a data gap regarding the acceptability of the lenacil in air method (open point: 1.11, see EFSA 2009, Conclusion on the peer review of the pesticide risk assessment of the active substance lenacil). There is an existing method validation report available that was reviewed in the Lenacil DAR, Volume 3, B.5, 2007, which gives an LOQ of 0.1 mg/m³ for lenacil in air (Rawle, 2005). However, the meeting concluded that a new method was required that must achieve an LOQ of at least 0.048 mg/m³. The derivation of the LOQ of 0.048 mg/m³ has been evaluated, and it is believed that the data gap is no longer justified. The rationale is presented below.

The LOQ (concentration) is calculated according to SANCO/825/00 rev 8.1:

concentration c = AOEL × 0.1 (safety factor) × 60 kg (bodyweight) / 20 m³/day (air intake)

Based on this calculation, it appears that the LOQ of 0.048 mg/m³ has been derived using an AOEL value of 0.16 mg/kg bw/d. This AOEL value was set in the Lenacil DAR (2007), based on the 90-day oral toxicity study in mice. Following the EU review of lenacil, the AOEL was revised to 0.4 mg/kg bw/d, based on the 90-day rat study and supported by the 90-day dog study (EFSA Journal 2009; 7(10):1326). Using the EU agreed AOEL value of 0.4 mg/kg bw/d, the calculated LOQ value is 0.12 mg/m³.

On the basis of the revised LOQ of 0.12 mg/m³, a new air method study is no longer required. In the existing method validation report (Rawle, 2005), the LOQ of the method was 0.036 mg lenacil per tube, equivalent to 0.1 mg/m³. As this value is lower than the calculated LOQ, the existing study may be considered valid and the development of a new method with a LOQ of at least 0.048 mg/m^3 is not necessary. The data gap can be considered closed.

An analytical method for the determination of residues in air is described in the Lenacil DAR, Volume 3, B.5, 2007. All analytical methods are active substance data and were provided in the EU review of lenacil and were considered adequate. A summary reflecting the Lenacil DAR, Volume 3, B.5.3.3, 2007 is given below.

Previous evaluation:	*Initial monograph November 2007*

Study No. 1

Report:	**CA 4.2(c)/01 (IIA 4.2.4.1-01)** Rawle (2005) Validation of an analytical method for the determination of lenacil in air

	CEMAS UK DuPont Report No.: CEMS-2788
Guidelines:	SANCO/825/00 rev.7
GLP:	Yes

Description of the method:
Residues of lenacil are extracted from air by passage through XAD-2 OVS adsorbent tubes for 6 hours at a flow rate of 1 L/min. Lenacil residues are eluted from the sorbent by sonication and shanking with tetrahydrofuran (THF). The extract is diluted with acetonitrile: water (50:50 v/v) and residues of lenacil are determined by HPLC (Synergi 4µ Polar-RP 80A, 150mm x 4mm) with tandem mass spectrometric determination (MS/MS, mass transition m/z 235 → m/z 153). External calibration was used.

Chromatographic conditions:
Column: Synergi 4µ Polar-RP 80A, 150 × 4.6 mm
Mobile phase:

Tim (min)	Water	Acetonitrile
0	60	40
5	20	80
6	20	80
7	60	40
10	60	40

Flow rate: 1.0 mL/min

Injection volume: 10 µL
Ion source and polarity: Turbospray, positive
Mass transitions monitored: 235.16 → 153.00

Findings:
Specificity - interferences: A comparison of chromatograms and spectra produced for reference items and control and fortified specimens demonstrates that the method, based on LC-MS/MS, is highly specific for the analysis of lenacil extracted from XAD-2 air-monitoring tubes. Apparent residue of lenacil in the control tubes was less than 30% of the LOQ. Validation data were generated only for one mass transition. A product ion spectrum was not provided.

Linearity: Good linearity was observed in the range of 0.0036 – 0.18 µg/mL (n = 6) with a correlation coefficient r = 0.9999. A typical calibration curve with the corresponding regression equation were provided within the study report. All calculations were performed using standards prepared in solvent (acetonitrile/water: 50/50).

Recovery: The fortification data reported in the method proposed for monitoring lenacil residues in air are summarised in tables B.5.2.5-2 below.

Repeatability: Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit and 10x the quantification limit for each matrix. Therefore, the repeatability of this method would seem to be adequate for the purposes of detecting lenacil residues in air.

Limit of quantification: The limit of quantification of the method for air is 0.036 mg/tube, corresponding to 0.1 mg/m^3.

Breakthrough: For breakthrough testing, sets of tubes, fortified at 0.036, 0.36 and 3.6 mg, were each connected to a pump and air was drawn through at 1.0 L/min for 6 hours with the section of fortified adsorbent facing the air flow. For one set of tubes the test was carried out in a laboratory at normal temperature and humidity (21 °C, relative humidity 73%). For a second set of tubes, this was carried out in a room with an air temperature of 35 °C and a relative humidity

set to approximately 80%. At the end of the 6-hour period, the front and rear sections of adsorbent were extracted and analysed separately.
The breakthrough results show that mean recoveries from the air-sampling tubes are within the range 70% to 110% at both sets of atmospheric conditions. In all cases breakthrough into the rear section of the tube is within acceptable limits (<10%) (see results in Table B.5.2.5.-2).

Table B.5.2.5-1 : Extraction efficiency (HPLC-MS/MS)

Reference	Matrix	Number of tests	Fortification level (mg/tube) [(a)]	Fortification level (mg/m^3) [(a)]	Average recovery (%) [range]	% Relative standard deviation
Rawle (2005), CEMS-2788	Air	5 5 5 Total = 15	0.036 0.36 3.6	0.1 1.0 10.0	92 [87 – 96] 81 [75 – 87] 81 [73 – 85] Overall = 85	3.9 5.9 5.8 Overall = ~~5.8~~ 8.2

[a] Limit of quantification: 0.036 mg/tube = 0.1 mg/m^3 air
The lenacil content in the control extracts was less than 30% of the LOQ

Table B.5.2.5-2: Breakthrough at room temperature and humidity

Analyte	Fortification level (mg)	Recovery (%)	
		Front segment	Rear segment
At 21°C and 73 % relative humidity			
Lenacil	0.036	103 101 107 98 103 Overall = 102%, RSD = 3.2%	0 0 0 0 0
	0.36	90 96 96 98 95 Overall = 95%, RSD = 3.2%	0 0 0 0 0
	3.6	90 95 89 89 89 Overall = 90%, RSD = 2.9%	0 0 0 0 0
At elevated temperature (35°C) and 82 % relative humidity			
Lenacil	0.036	98 104 110 105 110 Overall = 105%, RSD = 4.7%	0 0 0 0 0
	0.36	97 96 99 101 97 Overall = 98%, RSD = 2.0%	0 0 0 0 0
	3.6	89 98 96 89 92 Overall = 93%, RSD = 4.4 %	0 0 0 0 0

Storage stability

Three sets of eight adsorbent tubes were used. One set was stored in the dark at room temperature, one in a cold room set to +4°C and the latest at less than – 18°C. Six tubes in each set were fortified at the mid-level of 0.36 mg, the other two were unfortified. Following fortification, the tubes were capped and stored in the dark at the relevant

temperature. Then three fortified tubes and one unfortified tube from each storage condition were extracted at 4 and 7 days after fortification.
Lenacil residues were found to be stable on the air tubes when stored for up 7 days at room temperature, 4°C or -18°C (the recovery ranges were within 88 – 104%, all tests considered).

Extract stability
The stability of lenacil in the final extracts was measured by the reanalysis of selected extracts from the extractability test after storage at room temperature or + 4°C for up to 10 days.
Lenacil was found to be stable in the final extracts for up to 10 days when stored at room temperature or in a cold room temperature at 4°C (the recovery ranges were within 93 – 119%, all tests considered).

RMS Conclusion 2018 (renewal):
The method is successfully validated according to SANCO 825/00 rev.8.1: absence of interferences, linearity, accuracy and repeatability were successfully validated. Three fortification levels were tested with a sufficient number of replicates at each level. No breakthrough occurred. The method is suitable for the determination of lenacil in air with a LOQ of 0.1 mg/m^3.

B.5.2.6. Analytical methods for the determination of the active substance and/or metabolites in body fluids and tissues

A monitoring method for determination of residues in body tissues is covered by the validated method for monitoring residues of lenacil in animal matrices described in section B.5.2.2 in this document. (Report No.: 15S07170-01-VMAT). The LOQ of the method is 0.01 mg/kg.

No monitoring method was available for the determination of the residues of lenacil in body fluids in the renewal dossier. The notifier stated at that time that due to the low toxicity, accumulation in tissues is not expected and no monitoring method is therefore required. However, the notifier mentioned in the course of the assessment that an analytical method for lenacil in body fluids has been developed and validated: S. Gaag (2017), *Validation of an Analytical Method for the Determination of Residues of Lenacil in Body Fluids*, FMC Study Report No. 17S07170-01-VMBF and that the study is available upon request.

Since Reg. 283/2013 requires a monitoring method in body fluids independently of the toxicity of the active substance, the monitoring method developed by FMC for body fluids has to be provided **(data gap)** and the notifier should also consider what will be the most relevant analyte for monitoring in body fluids.

B.5.3. REFERENCES RELIED ON

Data Point according to reg. (EC) 283/2013 [data point as initially referred by notifier in CADDY] Ref. in Vol.3 CA-B.5	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
~~CA 4.1.1(a)/01 (IIA 1.11/02) Vol.3 CA-B.5.1.1.1 Study No. 1~~	~~Hansen, S.W.~~	~~1998~~	~~Technical grade lenacil (DPX-B0634). Analysis and certification of product ingredients E.I. Du Pont De Nemours and Company, Delaware DuPont Report No.: AMR 3747-96 GLP, Unpublished~~	~~N~~	~~N~~		~~FMC~~	~~Initial monograph November 2007~~
~~CA 4.1.1(a)/02 (IIA 1.11/01) Vol.3 CA-B.5.1.1.1 Study No. 2~~	~~Wittig~~	~~2000~~	~~Lenacil technical determination of purity and content in five batches UCL Umwelt Control Labor GmbH, Köln, Germany DuPont Report No.: PR00/015 GLP, Unpublished~~	~~N~~	~~N~~		~~FMC~~	~~Initial monograph November 2007~~
CA 4.1.1(a)/03 [CKA 4.1.1/04]	Nagaraj K.	2012	Validation of the analytical method for determination of lenacil (DPX-B0634) in technical grade lenacil and lenacil end-use products Syngene International Limited DuPont-34533 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted.	FMC	No, submitted for the purpose of the renewal

Vol.3 CA-B.5.1.1.1 – Study No. 3						The study was already considered for the zonal dossier of Venzar 80 WP		The study was already considered for the zonal dossier of Venzar 80 WP
CA 4.1.1(a)/04 [KCA 4.1.1/05] Vol.3 CA-B.5.1.1.1 – Study No 4	Anonymous	2016	Lenacil (DPX-B0634); Determination of DPX-B0634; Reversed-Phase High Performance Liquid Chromatographic (RPLC) and/or Ultra Performance Liquid Chromatography Assay Method B0634.220.03.ES Not GLP: Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of the renewal
CA 4.1.1(a)/05 [KCA, 4.1.1/06] Vol.3 CA-B.5.1.1.1 – Study No. 5	Repp, M. C.	2015	Validation of the analytical method for determination of lenacil (DPX-B0634) in technical grade lenacil and lenacil end-use products DuPont-44061 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted and contains information on composition of the active substance.	FMC	No, submitted for the purpose of the renewal
CA 4.1.2(a)/01 [IIA 7.1.2/04] Vol.3 CA-B.5.1.2.1 – Study No. 1	Kane, T.	2004	IN-KE 121, Adsorption/Desorption on soil, Huntingdon Life Sciences Ltd., ACD Report No. 063/042264 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 The study is a fate study but the analytical method and validation data were provided

								within that study.
CA 4.1.2(a)/02 [KCA 4.1.2/01, also in KCA 7.1.3.1.2/01] Vol.3 CA-B.5.1.2.1 – Study No. 2	Wright, D., Gilbert, J., Heslop, D.	2011	IN-KF313: Adsorption/ desorption study in three soil. Covance Laboratories Ltd Report No. :8224952 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal. The study is a fate study but the analytical method and validation data were provided within that study.
CA 4.1.2(a)/03 [KCA 4.1.2.2/01] Vol.3 CA-B.5.1.2.1 – Study No. 3	Jooß, S.	2015	Development and validation of an LC/MS/MS method for the determination of the lenacil metabolite IN-KF 313 in surface water PTRL Europe, Germany P3463G GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal
CA 4.1.2(c)/01 [IIA 5.3.1.1-01] Vol.3 CA-B.5.1.2.3 – Study No. 1	[redacted]	2002	Lenacil technical: preliminary study by dietary administration to Han Wistar rats for 4 weeks DuPont Report No.: ACD 001/010098 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 The study is a toxicological study but the analytical method and validation data were

								provided within that study.
CA 4.1.2(c)/02 [IIA 5.3.2.2-01] Vol.3 CA-B.5.1.2.3 – Study No. 2	[redacted]	1991	Subchronic oral toxicity: 90 day study with DPX-B634-91 (Lenacil) feeding study in mice DuPont Report No.: HLR293-91 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 The study is a toxicological study but the analytical method and validation data were provided within that study.
CA 4.1.2(c)/03 [IIA 5.5.2-01] Vol.3 CA-B.5.1.2.3 – Study No. 3	[redacted]	1994	Oncogenicity study with DPX-B634-91 (Lenacil) eighteen-month feeding study in mice DuPont Report No.: HLR336-93 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 The study is a toxicological study but the analytical method and validation data were provided within that study.
CA 4.1.2(c)/04	[redacted]	2003a	Lenacil technical: Study of effects on reproductive performance in Han Wistar Rats	Y	N		FMC	Initial monograph

[IIA 5.6.1.2-01] Vol.3 CA-B.5.1.2.3 – Study No. 4			treated continuously through two successive generations by dietary administration DuPont Report No.: ACD 020/023865 GLP, Unpublished					November 2007 The study is a toxicological study but the analytical method and validation data were provided within that study.
CA 4.1.2(c)/05 [IIA 5.6.2.1-04] Vol.3 CA-B.5.1.2.3 – Study No. 5	[redacted]	2003 b	Lenacil technical: Study of effects on embryo-fetal development in CD rats treated continuously by gavage administration DuPont Report No.: ACD 058/032316 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 The study is a toxicological study but the analytical method and validation data were provided within that study.
CA 4.1.2(e)/01 [IIA 4.2.1.1-01 and IIA 6.3-02b]	Mende	2002	Analytical Final Report – Generation of Samples for the determination of Residues of Venzar 80 % WP (containing 80 % Lenacil) in Sugar Beets. Five Sites in Europe, 2001 GAB Biotechnology GmbH, Germany DuPont Report No.: 20011048/E1-FPSB GLP, Unpublished	N	N		FMC	Initial monograph November 2007

Vol.3 CA-B.5.1.2.5.1 – Study No. 1								
CA 4.1.2(e)/02 [IIA 4.2.1.1-02] Vol.3 CA-B.5.1.2.5.1 – Study No. 2	Turnbull	2003	Validation of Analytical Methodology for the Determination of Lenacil in Sugar Beet Pesticides and Veterinary Medicines Team D, Central Science Laboratory (CSL), UK DuPont Report No.: PDG-107 GLP, Unpublished	N	N		FMC	Initial monograph November 2007
CA 4.1.2(e)/03 [IIA 6.3-03b] Vol.3 CA-B.5.1.2.5.1 – Study No. 3	Hamberger, R	2002	Analytical Report – Generation of samples for the determination of residues of Venzar (80% WP (containing 08% lenacil) in sugar beets, one site in Europe, 2002. GAB Biotechnology GmbH, Germany DuPont Report No.: 20011048/E2-FPSB GLP, Unpublished	N	N		FMC	Initial monograph November 2007 The study is a residue study considered for the results of the procedural recoveries
CA 4.1.2(e)/04 [IIA 4.2.1.1-03] Vol.3 CA-B.5.1.2.5.1 – Study No. 4	Tillkes M.	1998	Magnitude of residue of lenacil and trisulfuron methyl in sugar beet grown in France following application of Venzar and DPX-MX843-1 – Season 1995 DuPont Report No.: F-95-001-RES → Analytical Part: Gas-Chromatographic Determination of Pesticides Residues after Clean-up by Gel-Permeation Chromatography and Mini-Silica Gel-Column Chromatography. 6. Communication: Replacement of dichloromethane by ethyl acetate/cyclohexane in liquid-liquid partition and simplified conditions for	N	N		FMC	Initial monograph November 2007 The study is a residue study but the analytical method and validation were provided

			extraction and liquid – liquid partition (Specht *et al.*, 1995 – *Fresenius J. Anal. Chem.* 353, 183-190) Dr. Specht & Partner, Germany Specht & Partner No. DUP-9701/az. 56301/97 Report No. F-95-001-RES GLP, Unpublished					within that study.
CA 4.1.2(e)/05 [IIA 4.2.1.1-04 and IIA 6.3-04] Vol.3 CA-B.5.1.2.5.1 – Study No. 5	Anderson I., Kakkonen J.E. Witte A. for the analytical part	2006	Decline of lenacil residues in sugar beet (root and tuber vegetables) following a single application of Venzar® 80WP (Lenacil)-Southern Europe, Season 2005 Charles River Laboratories, UK DuPont Report No.: CRL 688479	N	N		FMC	Initial monograph November 2007 The study is a residue study but the analytical method and validation were provided within that study.
CA 4.1.2(f)/01 [CA 8.1.1.1/01, previously IIA 8.1.1-01] Vol.3 CA-B.5.1.2.6 – Study No. 1	[redacted]	2002	Lenacil technical Acute oral toxicity (LD_{50}) to the mallard duck [redacted] DuPont Report No.: ACD 048/022425 GLP, unpublished	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/02 [CA 8.1.1.1/02,	[redacted]	2002	Lenacil Technical: Acute Oral Toxicity (LD_{50}) to the Bobwhite Quail [redacted] DuPont Report No.: ACD 049/022426 GLP, unpublished	Y	N		FMC	Initial monograph November 2007

previously IIA 8.1.1-02] Vol.3 CA-B.5.1.2.6 – Study No. 2								Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/03 [CA 8.2.6.1/01, previously IIA 8.2.6-02] Vol.3 CA-B.5.1.2.6 – Study No. 3	Flatman, D.	2003	Leancil technical; algal growth inhibition assay *Selenastrum capricornutum* Huntingdon Life Sciences, UK DuPont Report No.: ACD 034/022511 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/04 [CA 8.2.1/01, previously IIA 8.2.1-03] Vol.3 CA-B.5.1.2.6 – Study No. 4	[redacted]	2003	Lenacil technical – Acute toxicity to fish (*Cyprinus carpio*) [redacted] DuPont Report No.: ACD 035/22512 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/05 [CA 8.2.6.2/01, previously IIA 8.2.6-01]	Flatman, D.	2003	Lenacil technical; algal growth inhibition assay *Navicula pelliculosa* Huntingdon Life Sciences, UK DuPont Report No.: ACD 036/024694 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were

Vol.3 CA-B.5.1.2.6 – Study No. 5								part of the ecotox. study
CA 4.1.2(f)/06 [CA 8.2.7/01, previously IIA 8.2.8-01] Vol.3 CA-B.5.1.2.6 – Study No. 6	Flatman, D.	2003	Lenacil technical; higher plant (*Lemna)* growth inhibition test Huntingdon Life Sciences, UK DuPont Report No.: ACD 039/023827 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/07 [CA 8.2.6.1/03, previously IIA 8.2.6-04] Vol.3 CA-B.5.1.2.6 – Study No. 7	Jenkins, C.A.	2004	IN-KE 121 algal growth inhibition assay Huntingdon Life Sciences, UK DuPont Report No.: ACD 064/042730 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/08 [CA 8.2.6.1/04, previously IIA 8.2.6-05] Vol.3 CA-B.5.1.2.6 – Study No. 8	Jenkins, C.A.	2004	IN-KF 313 algal growth inhibition assay *Selenastrum capricornutum* Huntingdon Life Sciences, UK DuPont Report No.: ACD 066/042848 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study

CA 4.1.2(f)/08.01 (KCP 10.6.2/01) Vol.3 CA-B.5.1.2.6 – Study No. 8.01	Gossman a. and Meinerling M.	2006	Effects of Venzar 500 SC on Terrestrial (Non-Target) Plants: Seedling emergence and seedling Growth Test IBACON GmbH, Germany Project 26803086 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/08.02 (KCP 10.2.3/01) Vol.3 CA-B.5.1.2.6 – Study No. 8.02	Jenkins C.A.	2005	Lenacil (Venzar 80% WP) Effects on primary productivity and macrophyte biomass in field-based microcosms Huntingdon Life Sciences Ltd, UK Report ACD 072/043691 GLP, Unpublished	N	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/08.03 (KCA 8.2.1/03[IIA 8.2.1-02]) Vol.3 CA-B.5.1.2.6 – Study No. 8.03	[redacted]	1991 b	Static, acute, 96-hour LC50 of DPX-B634-91 (lenacil) to fathead minnow (Pimephales promelas) [redacted] [redacted]Report No. 198-91 MR-4581-884 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/08.04 (KCA 8.2.1/02 [IIA 8.2.1-01])	[redacted]	1991a	Static, acute, 96-hour LC50 of DPX-B634-91 (lenacil) to rainbouw trout (Oncorhyncus mykiss) [redacted]	Y	N		FMC	Initial monograph November 2007

Vol.3 CA-B.5.1.2.6 – Study No. 8.04			[redacted] Report No. 199-91 MR-4581-884 GLP, Unpublished					Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/08.05 (KCA 8.2.2.1/01) Vol.3 CA-B.5.1.2.6 – Study No. 8.05	[redacted]	1991c	Flow-through, 21-day toxicity of DPX-B634-91 (lenacil) to rainbow trout (Oncorhyncus mykiss) [redacted] [redacted] Report No. 200-91 MR-4581-884 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/08.06 (KCA 8.2.4.1/01) Vol.3 CA-B.5.1.2.6 – Study No. 8.06	Hutton D.G.	1989a	Static acute 48-hour EC50 of DPX-B634-84 to fed Daphnia magna Haskell Laboratory for Toxicology and Industrial Medicine, USA Haskell Laboratory Report No. 86-89 MR-4581-675 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/08.07 (KCA 8.2.5.1/01) Vol.3 CA-B.5.1.2.6 – Study No. 8.07	Hutton D.G.	1989 b	Chronic Toxicity of DPX-B634-84 (Lenacil) to Daphnia magna Haskell Laboratory for Toxicology and Industrial Medicine, USA Haskell Laboratory Report No. 130-89 MR-4581-675 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were

								part of the ecotox. study
CA 4.1.2(f)/08.08 (KCA 8.2.2.1/02) Vol.3 CA-B.5.1.2.6 – Study No. 8.08	[redacted]	1996	Early Life-Stage Toxicity of DPX-B634-91 (Lenacil) to Rainbow trout (Oncorhynchus mykiss) [redacted] Report No. 235-96 MR-10351 GLP, Unpublished	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/09 [KCA 4.1.2.16/01] Vol.3 CA-B.5.1.2.6 – Study No. 9	Nixon W. B., Kendall T. Z	2012	Analytical method verification for the determination of lenacil (DPX-B0634) technical in algal medium Wildlife International Ltd., USA Wildlife No. 112C-192 DuPont-35031 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal
CA 4.1.2(f)/10 [KCA 4.1.2.16/02 and KCA 8.2.4.1/02] Vol.3 CA-B.5.1.2.6 – Study No. 10	Renner P	2016a	Acute toxicity of Lenacil technical to *Daphnia magna* in a 48-hour static test. BioChem agrar, Machern OT Gerichhain, Germany Report n°: 15 10 48 031 W, GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/11 [KCA 4.1.2.16/03	Renner P	2016 b	Toxicity of Lenacil technical to *Daphnia magna* in a 21-day semi-static test. BioChem agrar, Machern OT Gerichhain, Germany	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal

and KCA 8.2.5.1/02] Vol.3 CA-B.5.1.2.6 – Study No. 11			Report n°: 15 10 48 032 W, GLP, Unpublished					Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/12 [KCA 4.1.2.16/04 and KCA 8.2.6.2/03] Vol.3 CA-B.5.1.2.6 – Study No. 12	Wenzel, A.	2014 a	Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Synechococcus leopoliensis*. Fraunhofer (IME) DPT 001/4-10/C GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/13 [KCA 4.1.2.16/05 and KCA 8.2.6.2/04] Vol.3 CA-B.5.1.2.6 – Study No. 13	Wenzel, A.	2014 b	Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Anabaena flos-aquae*. DPT-001/4-10/E. GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/14 [KCA 4.1.2.16/06 and KCA 8.2.6.2/02]	Wenzel, A.	2014c	Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Ankistrodesmus falcatus*. DPT-001/4-10/F. GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were

Vol.3 CA-B.5.1.2.6 – Study No. 14								part of the ecotox. study
CA 4.1.2(f)/15 [KCA 4.1.2.16/07 and KCA 8.2.6.2/05] Vol.3 CA-B.5.1.2.6 – Study No. 15	Wenzel, A.	2014 d	Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Closterium cornu.* DPT-001/4-10/G. GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/16 [KCA 4.1.2.16/08 and KCA 8.2.6.2/06] Vol.3 CA-B.5.1.2.6 – Study No. 16	Wenzel, A.	2014e	Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Xanthonema debile.* DPT-001/4-10/I. GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
~~CA 4.1.2(f)/17 [KCA 4.1.2.16/09 and KCA 8.2.6.2/07] Vol.3 CA-B.5.1.2.6 – Study No. 17~~	~~Wenzel, A.~~	~~2014f~~	~~Freshwater Alga and Cyanobacteria, Growth Inhibition Test: Effect of Lenacil technical on the growth of *Nannochloropsis limnetica.* DPT-001/4-10/H. GLP, Unpublished~~	~~N~~	~~Y~~	~~The study is necessary for the regulatory decision and has not previously been protected or submitted~~	~~FMC~~	~~No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study~~

CA 4.1.2(f)/18 [KCA 4.1.2.16/10 and KCA 8.2.7/03] Vol.3 CA-B.5.1.2.6 – Study No. 18	Wenzel, A.	2012	Macrophytes, growth inhibition test. Effect of Lenacil technical on the growth of *Elodea canadensis* in the presence of sediment DPT-001/4-80/C. GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/19 [KCA 4.1.2.16/11 and KCA 8.2.7/02] Vol.3 CA-B.5.1.2.6 – Study No. 19	Wenzel, A.	2012	Macrophytes, growth inhibition test. Effect of Lenacil technical on the growth of *Chara globularis* in the presence of sediment DPT-001/4-80/D. GLP, Unpublished	N	Y	The study is necessary for the regulatory decision and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/20 [KCA 4.1.2.16/12 and KCP 10.6.2/02] Vol.3 CA-B.5.1.2.6 – Study No. 20	Stürz S., Knebel N.	2016	Lenacil 500 g/LG SC: Effects on terrestrial (non-target) plants: Vegetative Vigour Test ibacon GmbH, Germany 95231087 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/20.01 (CP 10.2.1/01)	Pawlowski S. And Wydra V.	2006	Toxicity of Venzar 500 SC to Pseudokirchneriella subcapitata in an algal growth inhibition test Ibacon GmbH, DE	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and	FMC	No, submitted for the purpose of renewal

Vol.3 CA-B.5.1.2.6 – Study No. 20.01			Project No.: 26801210 GLP, Unpublished			has not previously been protected or submitted		Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/20.02 (KCP 10.2.1/02) Vol.3 CA-B.5.1.2.6 – Study No. 20.02	Pawlowski S. And Wydra V.	2006	Toxicity of Venzar 500 SC to the aquatic plant Lemna gibba in a semi-static growth inhibition test. Ibacon GmbH, DE Project No.: 26802240 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/21 [CA 8.1.1.2/01, previously IIA 8.1.2-01] Vol.3 CA-B.5.1.2.6 – Study No. 21	[redacted]	2004	Lenacil technical – Dietary toxicity (LC_{50}) to the Bobwhite quail [redacted] project identity: DPT/637 DuPont Report No.: DPT 637/033931	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/22 [CA 8.1.1.3/01, previously IIA 8.1.3-01] Vol.3 CA-B.5.1.2.6 – Study No. 22	[redacted]	1996	A reproduction study with the northern bobwhite (*Colinus virginianus*) [redacted] DuPont Report No.: AMR 3419-95	Y	N		FMC	Initial monograph November 2007 Description and data on the analytical method were

								part of the ecotox. study
CA 4.1.2(f)/23 [KCA 4.1.2.17/01 and KCP 10.3.1.2/01] Vol.3 CA-B.5.1.2.6 – Study No. 23	Haupt S., Knebel N.	2016	Lenacil 500 g/LG SC: Chronic oral toxicity test on honey bee (Apis mellifera L.) in the laboratory ibacon GmbH, Germany Report No.: 95231136 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/24 [KCA 4.1.2.17/02 and KCA 8.3.1.2-02] Vol.3 CA-B.5.1.2.6 – Study No. 24	Haupt S., Knebel N.	2016	Lenacil 500 g/LG SC: Chronic oral toxicity test on bumble bee (Bombus terrestris L.) in the laboratory ibacon GmbH, Germany Report No.: 95231107 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(f)/25 [KCA 4.1.2.17/03 and KCA 8.3.1.4-01] Vol.3 CA-B.5.1.2.6 – Study No. 25	Haupt S., Knebel N.	2016	Lenacil 500 g/LG SC: Honey bees (Apis mellifera L.) larval toxicity test , repeated exposure ibacon GmbH, Germany Report No.: 95231032 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal Description and data on the analytical method were part of the ecotox. study
CA 4.1.2(g)/01	Wittig, A.	2002	Validation of a Monitoring Method for Determination of Lenacil Residues in Surface Water, Tap Water and Ground Water	N	N		FMC	Initial monograph

[IIA 4.2.3.1-01] Vol.3 CA-B.5.1.2.7 – Study No. 1			UCL GmbH Köln, Germany DuPont Report No.: PR02/001 GLP, Unpublished					November 2007
CA 4.2(a)/01 [KCA 4.2.1/01] Vol.3 CA-B.5.2.1 – Study No. 1	Witte, A.	2011	Validation of an analytical method for determination of residues of lenacil in rape seeds (oily matrix) Chemisches Institut Pforzheim GmbH 10T02203-01-VMRA GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted RMS: Study has already been considered and protected at zonal level (Venzar 80 WP and Venzar 500 SC)	FMC	No, submitted for the purpose of renewal but study already considered at zonal level (Venzar 80 WP and Venzar 500 SC)
CA 4.2(a)/02 [KCA 4.2.1/02] Vol.3 CA-B.5.2.1 – Study No. 2	Witte, A.	2015	Validation of an analytical method for determination of residues of lenacil in a plant commodity with high water content (lettuce) Chemisches Institut Pforzheim GmbH 14S07170-01-VMLE GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal
CA 4.2(a)/03 [KCA 4.2.1/03] Vol.3 CA-B.5.2.1 – Study No. 3	Witte, A.	2011	Validation of an analytical method for determination of residues of lenacil in wheat grain (dry matrix) and lemon fruit (acidic matrix) Chemisches Institut Pforzheim GmbH 11T02203-01-VMPL GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted. RMS: Study has already been considered and protected at zonal	FMC	No, submitted for the purpose of renewal but study already considered at zonal level (Venzar 80 WP and

						level (Venzar 80 WP and Venzar 500 SC)		Venzar 500 SC)
CA 4.2(a)/04 [KCA 4.2.1/04] Vol.3 CA-B.5.2.1 – Study No. 4	Mende, P.	2011	Independent Laboratory Validation (ILV) of an analytical method for determination of residues of lenacil in rape seeds Eurofins Agroscience Services GmbH S10-03652 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted. RMS: Study has already been considered and protected at zonal level (Venzar 80 WP and Venzar 500 SC)	FMC	No, submitted for the purpose of renewal but study already considered at zonal level (Venzar 80 WP and Venzar 500 SC)
CA 4.2(a)/05 [KCA 4.2.1/05] Vol.3 CA-B.5.2.1 – Study No. 5	Richter S., Stanislowski, T.	2015	Independent Laboratory Validation (ILV) of the German DFG S19 Multi-Residue Method for the Determination of Lenacil in Two Crop Types, using LC/MS/MS PTRL Europe P 3464 G GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal
CA 4.2(a)/06 [KCA 4.2.2/01] Vol.3 CA-B.5.2.2 – Study No. 1	Wagner, B.	2016	Validation of an analytical method for the determination of residues of lenacil in matrices of animal origin (milk, eggs, meat, fat, kidney/liver) CIP Chemisches Institut Pforzheim GmbH 15S07170-01-VMAT GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal
CA 4.2(a)/07 [KCA 4.2.2/02] Vol.3 CA-B.5.2.2 – Study No. 2	Bodsch, J.	2016	Study plan - Independent Laboratory Validation of analytical method 15S07170-01-VMAT for the determination of residues of Lenacil in matrices of animal origin (milk, eggs, meat, fat, kidney/liver) by LC-MS/MS SGS INSTITUT FRESENIUS GmbH IF-16/03724646 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal

CA 4.2(a)/08 [KCA 4.2.2/03] Vol.3 CA-B.5.2.2 – Study No. 3	Bodsch, J.	2016	Amendment No.1 to Final Report: Independent Laboratory Validation of analytical method 15S07170-01-VMAT for the determination of residues of Lenacil in matrices of animal origin (milk, eggs, meat, fat, kidney/liver) by LC-MS/MS SGS INSTITUT FRESENIUS GmbH IF-16/03724646 AR GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal
CA 4.2(b)/01 [IIA 4.2.2.1-01] Vol.3 CA-B.5.2.3 – Study No. 1	Brodsky J. and Zietz E.	1990	Determination of residues of lenacil in soil (treated with Venzar, Season 1989, Germany) Batelle Europe – Frankfurt Division DuPont Report No.: BE-A-11-90-10-BF GLP, Unpublished	N	N		FMC	Initial monograph – November 2007
CA 4.2(b)/02 [IIA 4.2.2.1/02] Vol.3 CA-B.5.2.3 – Study No. 2	Mende P.	2003	Analytical Report – Venzar 80% WP (containing 80% lenacil) related soil dissipation on bare soil, four sites in Europe, 2001 GAB Biotechnologie GmbH and IFU Umweltanalytik GmbH, Germany DuPont Report No.: 20011048/E1-FSD GLP, Unpublished	N	N		FMC	Initial monograph – November 2007
CA 4.2(b)/03 [KCA 4.2.4/01] Vol.3 CA-B.5.2.4 – Study No. 1	Witte, A.,	2009	Development and validation of an analytical method for the determination of residues of lenacil in drinking and surface water CIP Chemisches Institut Pforzheim GmbH 09D02203-01-VMWA GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	FMC	No, submitted for the purpose of renewal
CA 4.2(b)/04 [KCA 4.2.4/02]	Link, T.H.	2016	Independent Laboratory Validation of analytical method 09D02203-01-VMWA for the determination of residues of Lenacil in tap water by LC-MS/MS SGS INSTITUT FRESENIUS GmbH	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and	FMC	No, submitted for the purpose of renewal

Vol.3 CA-B.5.2.4 – Study No. 2			IF-15/03444276 GLP, Unpublished			has not previously been protected or submitted		
CA 4.2(c)/01 [IIA 4.2.4.1-01] Vol.3 CA-B.5.2.5 – Study No. 1	Rawle, N.	2005	Validation of an analytical method for the determination of residues of lenacil in air CEM Analytical Services Ltd. (CEMAS) CEMS-2788 GLP,Unpublished	N	N		FMC	Initial monograph – November 2007

Note:

- Several of the above mentioned studies were owned by DuPond and Schirm GmbH. DuPont confirmed (letter dated from 20.06.2016) that for all lenacil active substance and lenacil product data sponsored by Schirm GmbH or by both companies, E.I. Du Pont de Nemours and Company (DuPont) and Schirm GmbH, which were submitted or referred to for the renewal of approval of lenacil, DuPont is the sole data owner.
- Following of a transfer of the responsibility of EU approval activities of certain active substances owned by E.I. duPont de Nemours and Company, the data applicant for lenacil is FMC Agricultural Solution A/S and the data owner is FMC International Switzerland Sarl (FISSarl) (letter from FMC dated from 05 December 2018).